A QUALITATIVE AND QUANTITATIVE EVALUATION OF FREEZING STRESS IN WHEAT

by

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CHAPTER 4

ASSESSMENT OF FROST STRESS TOLERANCE IN SOUTH AFRICAN WINTER, INTERMEDIATE AND SPRING WHEAT

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INTRODUCTION

Agronomy is defined as "the science of manipulating the crop/environment continuum with the dual aim of improving agricultural productivity and gaining a deeper understanding of the processes involved" (Norman, 1980). To place wheat agronomy in perspective, it is useful to take a broader view by examining the general features of wheat production in the world today.

The area allocated to wheat production exceeded 215.5 Mha during the 2003-growing season and the yield was 55,348,627 Mt. This means that the average world wheat yield was 2.665 t ha^{-1} at the end of the mentioned season which is 3.38% lower than the average wheat yield in the five year period 1999 – 2003 (2.755 t ha^{-1}), but the same as that of the previous 10 year average of 2.652 t ha^{-1} (FAO, 2004). During the past four decades the average wheat yield increased from a mere 0.66 t ha^{-1} to 2.42 t ha^{-1} in South Africa. The area devoted to wheat production in 1965 was 1,360 Mha but decreased to 0.959 Mha in 2001 (National Department of Agriculture, 2002). During the 2003-growing season the area decreased to 0.900 Mha and the average yield was 1.778 t ha^{-1} (FAO, 2004). This meant that the average South African yield was still approximately 0.9 t ha^{-1} lower than the world average of the 2003-growing season. Bearing this in mind the role of the agronomist and breeder to assist farmers in gaining higher yields through improved agronomic practices and genetic manipulation, respectively and collectively, becomes inevitable. Continuous arable cropping occurs in areas between semi-arid rainfed conditions, where wheat commonly follows a long (1 year) fallow, and humid or irrigated areas and this offers a major contrast to the agronomist that demands skillful adaptation in terms of management (Fisher, 1981).

In South Africa wheat is subjected to adverse weather conditions (drought, waterlogged, heat, freezing, etc.) during most stages of its growth period. During winter and spring, low

temperature injury can be particularly destructive and injury usually occurs whenever low temperatures coincide with sensitive plant growth stages (Warrick & Miller, 1999). The damage sustained may be severe or confined to only a few fields or parts of fields. It is most severe under irrigation conditions, along river bottoms, valleys and depressions in fields where cold settles (Figure 1) (Afanasiev, 1966; Shroyer, Mikesell & Paulsen, 1995).

Figure 1.1 Frost stress in the flowering stage of wheat in a low-lying area
(The yellow brown area illustrates the severity of the frost damage encountered and as the field rises towards the hill (facing in a northerly direction) the damage declined to a zero factor – Central South Africa, 5 October 2002)

Freezing stress, commonly known as frost damage, is a reality in the central South African wheat production areas. The occurrence of frost stress is predominant in specific regions of South Africa due to the application of certain planting techniques and management practices, but it is not confined to these regions. The soil, plant (crop) and atmosphere continuum plays an integral role in the occurrence of freezing and/or frost damage in wheat.

Winter wheat undergoes a complex process of hardening during autumn that increases its tolerance to frost injury during winter (Levitt, 1980). However, cold hardiness is quickly lost when growth accelerates during spring. Wheat is most sensitive to frost injury during reproductive growth that includes the flag-leaf, pollination and heading stages (Peel, 1998).

During September through mid-October most of the early maturing wheat has developed to these growth stages. Early-maturing wheat is also more likely to be damaged by frost than late maturing wheat. Susceptibility to frost (freezing) temperatures steadily increases as maturity of wheat advances during spring. Temperatures that are below freezing can severely damage wheat at these stages and greatly reduce grain yields (Warrick & Miller, 1999).

Environmental and plant factors as well as human intervention, that is management decisions, play a major role in the occurrence and degree of frost stress. Wheat growers can decide to alter the recommended planting dates for the different wheat cultivars (spring, intermediate and winter types) which might lead to severe frost damage in spring wheat types (early maturing) when planted earlier than the recommended date. With regard to environmental and plant factors the degree of low temperature as well as the duration of exposure to these low temperatures influence the degree of frost damage. Prolonged exposure to freezing at a specific temperature causes a higher degree of damage than a brief exposure at that specific temperature. Plant factors, of which the growth stage as well as the plant's physical (water content) and physiological condition plays a major role on the extend of frost damage during spring, makes it difficult to predict the degree of injury. Further, the interaction between the mentioned factors and the topography among and within wheat fields intensify the complexity and difficulty to predict frost damage (Warrick & Miller, 1999).

Insurance companies allow the insurance of wheat crops against frost injury with certain prerequisites of which the most important is planting date. A specific and realistic planting date has been set as standard to force the wheat growers not to plant too early whereby the risk of encountering frost damage is lowered to a minimum. The usual reason for wheat growers planting early is to prolong the growing season whereby the grain filling period is slightly prolonged in an attempt to increase the yield. However, this might lead to early flowering during the beginning of spring when late frost may still occur, thus increasing the risk of frost damage.

All these environmental, plant and human factors involved with frost damage in wheat prompted this study. The main vacuum in current knowledge of frost damage in wheat is related to the following frequently asked questions by South African wheat producers: 1) What are the visible symptoms associated with frost injury?, 2) which growth stages are the most sensitive?, 3) are there cultivar or variety differences? and 4) what effect does frost injury have on the expected yield and quality of commercially produced wheat? These questions as well as the request from a leading insurance company in South Africa, who financially supported this study, supplied the rationale for this investigation.

Answers to these questions, from an agronomic perspective, are regarded as essential for the wheat growing industry and insurance companies. During the growing season preceding this study (2002) enormous losses were encountered due to late frost in early spring. Further, over the past decade 544 394 ha of wheat were insured against frost damage of which 47 062 ha were damaged (8.6%) (Willemse, 1999). According to the author an average loss of R 6.7 million was encountered annually and the need to investigate the effect of frost (freezing) stress on wheat became inevitable. The mentioned figures are not a true reflection of the real problem because it only indicates the insured fraction of frost injury and not the actual figures for the whole region or country. These high levels of frost damage indicated that wheat cultivars, their growth stages, visual symptoms and reaction as a result to frost damage needed to be investigated and verified for South African conditions.

The main objectives of this study were to:

- evaluate the quantitative characteristics of three different growth types (spring, intermediate and winter wheat) for tolerance to frost (freezing) during the tillering, flag leaf, flowering and hard dough stages at 0, –3, –6, –9 and –12°C;
- evaluate the qualitative characteristics of three different growth types (spring, intermediate and winter wheat) for tolerance to frost (freezing) during the tillering, flag leaf, flowering and hard dough stages at 0, –3, –6, –9 and –12°C;
- evaluate two different growth types for frost tolerance during early, full and late flag leaf stages for quantitative and qualitative characteristics;

- evaluate two different growth types for frost tolerance at 0, 50 and 100% flowering stages;
- compile a guide to illustrate and identify frost symptoms for use by wheat growers and other relevant role players in the wheat production industry of Southern Africa.

In pursuit of meeting all the above-mentioned objectives visual, morphological and physiological methods were applied in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Wheat

2.1.1 Production in South Africa

In South Africa wheat is produced in areas where neither the climate nor the soil is favourable in comparison to that found in the wheat producing areas of North America or Europe. Each of the wheat-producing areas in South Africa has its own unique problems, so that cultivation practices, planting date, cultivars and harvesting have to be adjusted accordingly. The South African wheat grower has to cultivate the soil as effectively as possible to achieve a reasonable yield and grade. South Africa's wheat production tonnage per hectare therefore compares unfavourable with that of the rest of the world.

Wheat-producing areas in South Africa can mainly be divided into two regions, that is the winter and summer rainfall regions that include the irrigation areas (Figure 2.1). The following descriptions represent the concentrated wheat producing areas only.

Winter-rainfall region – Soils in the winter rainfall region is generally shallow, very stony and lacks soil fertility. Due to the stoniness these soils do not retain water. Production is, to a large extent, dependent upon reliable and well-dispersed rainfall. The annual precipitation for this area is between 400 and 600 mm.

Summer-rainfall region – Western Free State: The agricultural soil in this region is generally deep, varying from sandy to sandy loam. Predominantly red and yellow soils are to be found in this area. The average precipitation varies between 425 and 600 mm per annum and the majority of the precipitation occurs during the summer months. This means that wheat can only be produced successfully after a fallow period of at least 11 months (Fisher, 1981). Wheat is well adapted for the long and cold winters but planting dates are adapted to avoid the occurrence of late frost.

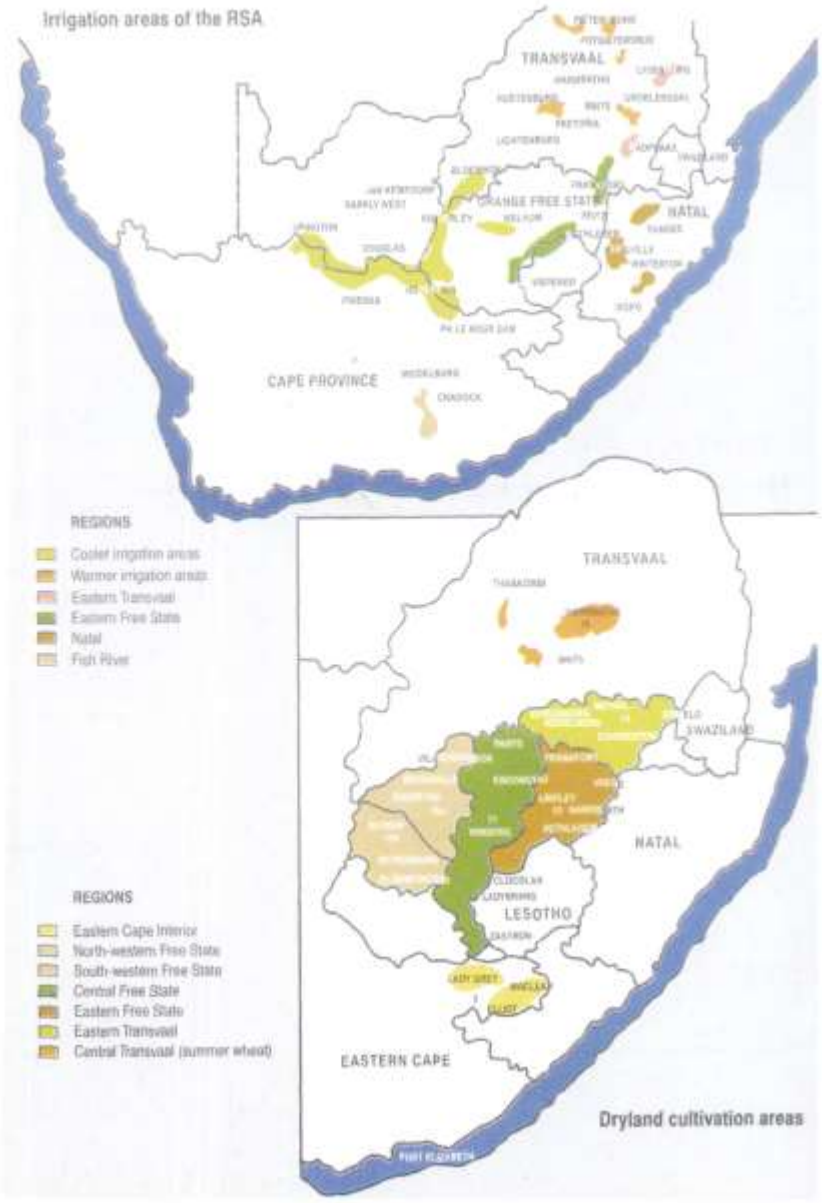


Figure 2.1 Summer wheat production areas of South Africa (ARC-Small Grain Institute, 2000).

Eastern Free State: The soil in this region is, on average, shallower than that of the Western Free State with a clay content varying from sandy loam to sandy clay loam. Mostly yellow and also clayey soils are found in this area. The average rainfall varies between 600 and 725 mm per annum and compensates for the relatively low water retention capacity of the

soil. The eastern and western Free State are responsible for 37 to 45% of the total wheat production of South Africa (National Department of Agriculture, 2002). The Free State together with the Western Cape province produces approximately 70% of the total wheat crop (2001/2002 growing season). Eight of the nine South African provinces produce wheat of which the former two contributes to the main yield. The Northern Cape (production under irrigation) and North West Provinces produce respectively 11.5% and 7.1% of the total wheat yield respectively, followed by smaller productions in Mpumalanga (4.5%), Northern Province (2.5%), Kwazulu Natal (2.3%), Gauteng (0.6%) and the Eastern Cape (0.4%). Wheat production varies considerably in South Africa due to harsh and erratic environmental conditions such as the occurrence of heat waves, erratic rainfall, hail and frosts.

2.1.2 Occurrence of frost

The occurrence of damaging frost is one of the limiting factors with regard to crop production in large areas of South Africa. The earliest and latest dates that damaging frost occur determines the length of the growing season in a specific area. The length of the growing season, on the other hand is an indication of the ability of a specific crop to complete its life cycle from the last date of frost in spring until the first date of frost in autumn (Kotzé, 1980). This is of utmost importance to summer crops.

Although wheat is a cool season crop, its cultivation is concentrated between latitudes 30 to 60°N and between 27 to 40°S (Briggle & Curtis, 1987). According to the authors wheat is also cultivated within the Arctic circle and up to the equator with the prerequisite that cultivation occurs at locations with a high elevation.

The adaptability of a wheat cultivar in a specific area of cultivation is influenced by the cold requirements of wheat which is directly involved with early and late cultivation (Aitken, 1965). According to Cook and Veseth (1991) temperature stress inhibits the growth, development and yield of wheat in three ways:

- The development from emergence through tillering, stem elongation, flowering and grain fill is driven by growing degree-days or accumulated heat units.

- To proceed from seed to seed wheat requires a certain minimum time period within a favourable temperature range while the optimum temperature for growth and development is between 10 and 24°C. Large, well tillered plants with wide leaves and large ears are the result of accumulated growing degree-days within this temperature range, provided that no limiting factors such as too much or too little water or light influence the normal plant development.
- Wheat plants are sensitive to temperature extremes during critical stages of development and these extremes include frozen roots or leaves, winterkill, frost damage to the internodes and florets and heat damage.

The minimum, optimum and maximum temperature requirements for normal growth and development of wheat is 3 to 4°C, 25°C and 30 to 32°C respectively (Briggle, 1980).

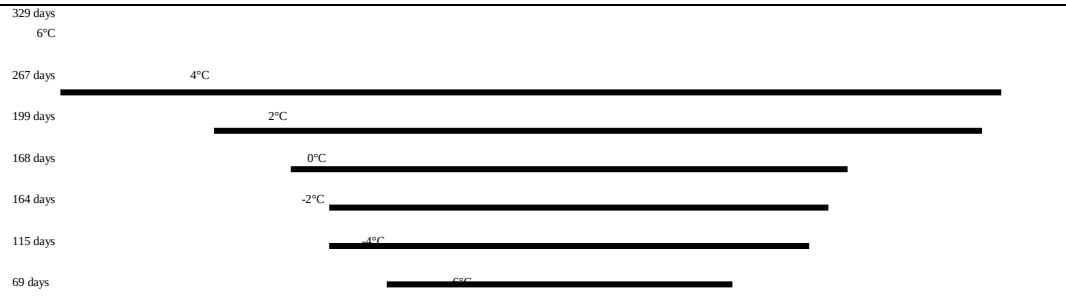
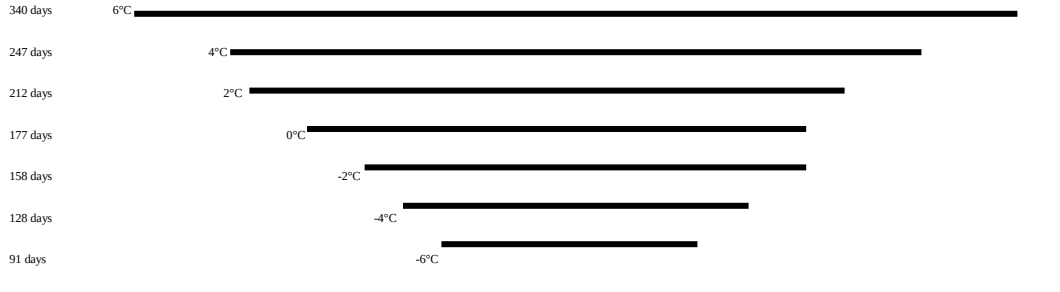
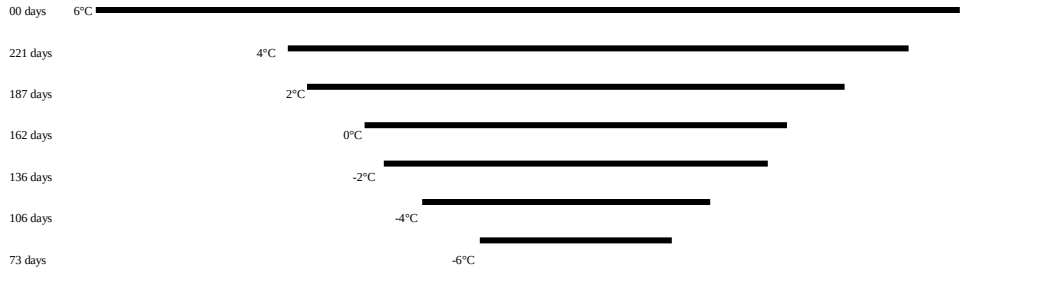
The last date of frost at the beginning of spring is of importance to winter crops, in this case wheat. In South Africa the occurrence of frost during the beginning of spring could be as late as the first or even the second week of October (Kotzé, 1980). This could have a detrimental effect on the growth and development of wheat due to the fact that wheat is usually in the flag leaf or flowering stage (Cook & Veseth, 1991). Marcellos and Single (1984) also indicated that the emerging ear from the flag leaf is highly susceptible to damage by frost radiation. Different mechanisms as well as managing practices exist to avoid frost of which planting date is the most important and common mean. In practice wheat growers tend to plant too early with the main objective to extend the growing season to enhance yields. This practice also enhances the risk of frost damage to wheat.

Various factors influence and determine the occurrence of frost, for example climatic conditions, height above sea level, topography, slope, direction of the slope, soil coverage, soil type, air movement or circulation, etc. Apart from natural conditions that promote the occurrence of frost, certain farming practices also contribute to frost (Kotzé, 1980). The probability of frost incidences and number of days of frost for the eastern and western Free State is depicted in Table 2.1 (Bethlehem - east and Bloemfontein - west). Only the 10% and 30% frost probability factors are indicated due to the fact that only these two factors include temperatures of –2°C and lower during periods when sensitive growth stages of the wheat crop occur. For example, for both Bethlehem and Bloemfontein temperatures of –2°C even as late as the first week of October and temperatures of –4°C during the first two weeks of September and the third week of September for Bloemfontein and Bethlehem respectively, has been recorded (Kotzé, 1980).

2.2 Chilling and Freezing

There are two types of injuries a plant can sustain through exposure to low temperature and that is chilling injury that occurs between 0 to 20°C and freezing injury that occurs when the external temperature drops below the freezing point of water (Stushnoff, Fowler & Brule-Babel, 1984). Furthermore, plants assume the temperature of their immediate environment and this means that plants are poikilotherms. Historically, small climatic changes on plants have rather been accepted than addressed. An example is that the production of rice could be reduced by 40% if the world temperature would decrease by 1°C. Alternatively a 2°C increase in frost hardiness of citrus, deciduous fruit tree blossoms, potatoes, tender vegetables and winter cereals could increase world yields. Therefore, not only could yields be increased but also the production areas of wheat when a 2°C increase in hardiness could be obtained. This would also increase production of wheat to areas currently only under spring wheat (Hale & Orcutt, 1987).

Table 2.1 The probability of frost incidences and number of days of frost for Bloemfontein and Bethlehem (Kotzé, 1980)

Frost probability	***BLOEMFONTEIN* (1422m above see level, average data of 29 years)**
10 %	
30 %	
Frost probability	***BETHLEHEM* (1631m above see level, average data of 16 years)**
10 %	
30 %	

2.2.1 Chilling

Chilling injury can be observed in many plants of tropical and subtropical origin when they are exposed to low temperatures, in their chilling range, which is usually from 25 to 10°C (Raison & Lyons, 1986). Temperatures in the range of 15 to 0°C apply for plants of temperate origin. The chilling effect is manifested by both physiological and cytological changes. These changes can be reversible or irreversible depending on the time and temperatures of exposure. Hardening of chilling sensitive plants enable these plants to adapt to chilling if they are hardened for a specific period of time at temperatures slightly above their critical temperatures (Hudák & Salaj, 1999). The temperatures at which membrane lipids undergo a two-dimensional phase transition from a disordered state to a more ordered state with a drop in temperature correspond with the critical temperature. This will also affect the conformation of enzymatic active proteins within the membrane and therefore alter the kinetics of reactions catalyzed by membrane associated enzymes.

2.2.2 Freezing

According to Luyet (1966) freezing injury in plants generally coincides with the conversion of liquids in cells to a solid state. Vitrification (solidification of the cellular content into a noncrystalline state) and crystallization (arrangement of liquid molecules into orderly structures) are the two types of freezing that occur in plant cells and tissues. Vitrification of the cell volume is a result of rapid freezing (more than 3°C/min) of plant tissue to a very low temperature. Although vitrification does not occur in nature, the significance to researchers is of high importance as it enables plants to survive temperatures close to absolute zero (Alden & Herman, 1971).

A common phenomenon in nature is the formation of ice or crystallization. Crystallization of ice may occur either within or outside the cells, but the process depends on the speed of cooling. Both internal nucleation or by penetration of external crystals into the cells can lead to the formation of ice inside the cells (Mazur, 1969). This type of freezing, also called intracellular freezing, is in both cases lethal because of the immediate disruption of the cells. Only cells that exhibit deep supercooling may be an exception to this rule (Asworth, 1984).

If crystals that form during freezing are very fine, cooling is usually rapid and these crystals melt before they reach a harmful size and plant cells may survive intracellular ice formation (Sakai & Otsuka, 1967).

Freezing stress mainly targets biomembranes and as a result, the plasma membrane has attracted the attention of researchers in this field. A loss of semi-permeability (1), a loss of active transport ions (2), a degradation of phospholipids (3), a redistribution of proteins due to lateral displacement (4) and a dehydration induced phase transition in biological membranes are typically related to freezing or frost injury (Hällgren & Öquist, 1990). In the past different hypotheses and theories have been used to fit experimental data and to study the mechanisms of freezing damage (Levitt, 1980). The status of the plant after a freeze/thaw cycle is of importance and that is why both freezing and thawing has to be considered to understand freezing injury. Furthermore, in freezing injury it is not just the low temperature that is of importance but to a greater extend also the secondary stress caused by dehydration of extracellular water (Hällgren & Öquist, 1990).

Ice formation in the intercellular spaces is termed extracellular freezing (Levitt, 1980). Intercellular ice formation could commence at the high range of subzero temperatures when liquid water is removed from the cell and coalesce with growing crystals outside the cell as the tissue cools. This is the result of differences in the chemical potential of supercooled water and ice at the same temperature. The lower vapor pressure of ice compared to liquid water at the same temperature forms a vapor pressure gradient. With a decline in temperature of the tissue during equilibrium freezing, the cells become increasingly dehydrated as more and more water is withdrawn to the extracellular ice (Guy, 1990).

Intracellular freezing is the term used for the formation of ice anywhere inside cells of plant tissue. In nature intracellular ice formation is thought to be universally lethal to the affected cell. Severe freezing in a plant will certainly be lethal to the plant upon thawing. In general plants adapt to regions where freezing are common where ice do not form within the cells but outside the cells in the intercellular space where the solute concentration of the water is decidedly lower (Guy, 1990). Cell dehydration is only possible as long as ice formation does

not occur in the cytosol. Steponkus (1990) stated that two conditions are required for intracellular ice formation: (a) that the cytosol must be supercooled, and (b) it must be either nucleated or seeded. In some instances intracellular ice formation is excluded and the injury plants sustain that undergo extracellular freezing can largely be attributed to deleterious effects of dehydration and physical stresses and strains of water changing to ice in the intracellular spaces of the tissue (Guy, 1990).

2.2.3 Frost

The fact that plants are poikilotherms also means that the term frost tolerance and not frost resistance should be used. A number of possible mechanisms may be involved in the process of inducing this tolerance. These include:

- Potentially toxic compound concentrations that might decrease when the solutes become concentrated.
- Toxic compounds that may become non-effective through dilution due to a higher ratio of non-toxic to toxic compounds.
- Membranes might be shielded from toxic compounds by special “protective” compounds.
- Membrane sensitivity to toxic compounds may decrease.
- Solutes such as sugars and amino acids may collectively protect and prevent injury.
- Cells may be protected from injury by the synthesis of soluble proteins (Hale & Orcutt, 1987).

Frost sensitive plants are injured under natural conditions as the consequence of ice formation between –2 and –5°C (Levitt, 1972). The acclimation ability of plants, that is those that are unable to acclimate to freezing stress, are sensitive to any form of ice formation. Plant cell walls usually do not contain strong ice nucleation sites and therefore sterile leaf discs will not nucleate ice formation until the temperature drops below –8°C. Under controlled conditions wheat leaves have no or little ice nucleating bacteria and do not freeze when exposed to temperatures as low as –8°C for up to six hours. According to Gusta and Chen (1987) freezing would however occur within minutes if ice-nucleating bacteria

were to be sprayed on the leaves at approximately –3°C. In nature plants and therefore leaf blades are not sterile and are colonised by a number of epiphytic bacteria.

Lindow, Arny and Upper (1982) found that *Pseudomonas syringae* and *Erwina herbicola* acted as active nucleation sites at temperatures as high as –2°C. Lindow (1983) identified three species of bacteria commonly found as epiphytes on leaf surfaces that are extremely effective ice nucleators at "warm" sub zero temperatures. In nature the presence of these bacteria is inevitable and causes ice formation at much higher temperatures than observed on sterile plants. Therefore, environmental factors that promote the growth of these ice nucleation active bacteria (INA) result in plants being more susceptible to frost injury the (Lindow *et al*., 1982). Lindow *et al.* (1982) also isolated strains of INA bacteria from wild populations that lack the ice nucleation gene and when these bacteria were to be sprayed onto plants it will compete with the native population. The reduction in the number of INA bacteria that exist on plants could reduce the temperature to cause frost injury to plants because ice nucleation is a function of the log of the INA bacterial population.

The incidence of frost damage is mainly attributed to environmental factors. Therefore the occurrence of frost damage will increase if the period of exposure is prolonged when sub-zero temperature decreases. Other factors such as the presence of dew also rises the freezing point of plant organs and therefore the presence of INA cannot be considered as the most important factor.

2.3 The freezing process

Ice formation in plant tissue occurs first at locations having the least negative osmotic potential, when the atmospheric or soil temperatures drop below the freezing point of water. The first nucleation event requires a nucleation site to orient the water molecules to the crystalline structure of ice and this will occur at a temperature that might be several degrees below 0°C (Burke & Lindow, 1990). Therefore, ice formation occur either as a result of heterogeneous nucleation or seeding by an ice crystal (Steponkus, 1990). The nucleation sites are very specific in shape and size and are related to a component on the cell wall. After

initiation, subsequent nucleation occurs on the surface of the ice crystal itself. Plants have been provided with a opportunity to control the location of the nucleation sites through the cell wall due to its composition and structure. When plants are cooled slowly, and continuously, the plant temperature drop below freezing point without ice formation. This is also called supercooling (Levitt, 1980).

According to Levitt (1980) ice normally forms first in the large vessels of the xylem in leaves and stems, in sub-stomatal cavities and in intercellular spaces. Once ice forms it will progress throughout the vessels and into the extracellular spaces of other tissue, but an intact plasma membrane cannot be penetrated by an ice crystal to inoculate the cytoplasm. Therefore, the ice crystal enlarges at the expense of water vapour and the surface film of liquid water on the cell wall. An accumulation of solutes and gasses that are excluded from the ice matrix occur in the liquid or unfrozen portion of the partially frozen mixture, as ice grows. Glasstone (1948) stated, that due to the dissolved cell solutes and its interaction with cellular components, the water in a cell does not freeze at once. Ice formation will continue until the chemical potential of the unfrozen water is in equilibrium with the ice, which is a direct function of the subzero temperature (Mazur, 1970). At equilibrium, the unfrozen solution will be equal to (273-T)/1.86.

$$osm = \frac{273 - T}{1.86}$$

osm = osmolality
T = temperature (°K)

Osmolality can be defined as the sum of all salutes expressed as moles of solute/kg water. Therefore the osmolality of the unfrozen portion of the solution increases linearly as a function of the subzero temperature when the solution is cooled and seeded at its freezing point (Steponkus, 1990). Thus when Mo is the original osmolality, then:

$$q = \frac{1.86\ Mo}{273 - T}$$

q = original solution that remains unfrozen
Mo = original osmolality
T = temperature (°K)

This means that when a solution is cooled and seeded at its freezing point, the osmolality of the unfrozen part of the solution increases linearly as a function of the subzero temperature (e.g., 0.53 at –1°C, 2.69 at –5°C, 5.38 at –10°C and 10.75 at –20°C) (Steponkus, 1990). Therefore if the solution is frozen, the osmolality is independent of the initial osmolality and can only be a function of the temperature (Mazur, 1970). The osmotic coefficient of the solute and the initial osmolality determine the unfrozen proportion of the original solution at any subzero temperature. From the liquidus curve of the phase diagram for the solution the unfrozen portion is most accurately calculated. The fraction (weight percent) of the unfrozen solution is calculated as the ratio of the initial solute concentration (weight percent) to the solute concentration (weight percent) in the unfrozen portion at a given subzero temperature (Rall, Mazur & McGrath, 1983 as sited by Stephonkus, 1990). According to the example of Steponkus (1990) over a range of 0 to –20°C, approximately 28% of the solution remains unfrozen at –5°C, 18% at –10°C and 14% at –20°C, during freezing of a 0.53 osm sorbitol solution. That means that if the initial osmolality of the solution were to be doubled (1.06), the osmolality of the unfrozen solution at any given subzero temperature will be the same as the more dilute solution, but less solution will have to be frozen before the unfrozen solution is sufficiently concentrated to achieve the equilibrium osmolality (± 48% of the solution will remain unfrozen at –5°C, 32% at –10°C, and 24% at –20°C).

Figure 2.2 illustrates the relationship of freezing tolerance in Johansson's wheat and rye plants to cell contraction and protoplasmic dehydration. The unhardened or non-acclimated cell was killed at –6°C. At this stage the cell volume decreased to one-sixth of the original volume and the protoplasm was dehydrated to one-half of its original volume. At this point the plasma membrane remains attached to the cell wall, causing the cell to collapse (Levitt, 1956). According to Alden and Herman (1971) the protoplasm is pushed against the outer cell wall in the form of a ring and the plasmic strains break when the cell wall collapses. The protoplasm may break away from the cell wall and shrink in size if the cells have been killed by the freeze-thaw cycle and is termed as frost or pseudo plasmolysis. The membranes of the cells are unable to regain turgor if the cells has been injured by freezing (Gusta & Chen, 1987). In contrast the hardened or acclimated cell was killed at –10°C, although the cell volume was decreased to only one-fourth of the original volume. This occurred because, at a

lower temperature, the protoplasm was dehydrated to one-third of its original volume, making it more "brittle" and therefore injured by a smaller mechanical stress due to the smaller degree of cell contraction (Levitt, 1980).

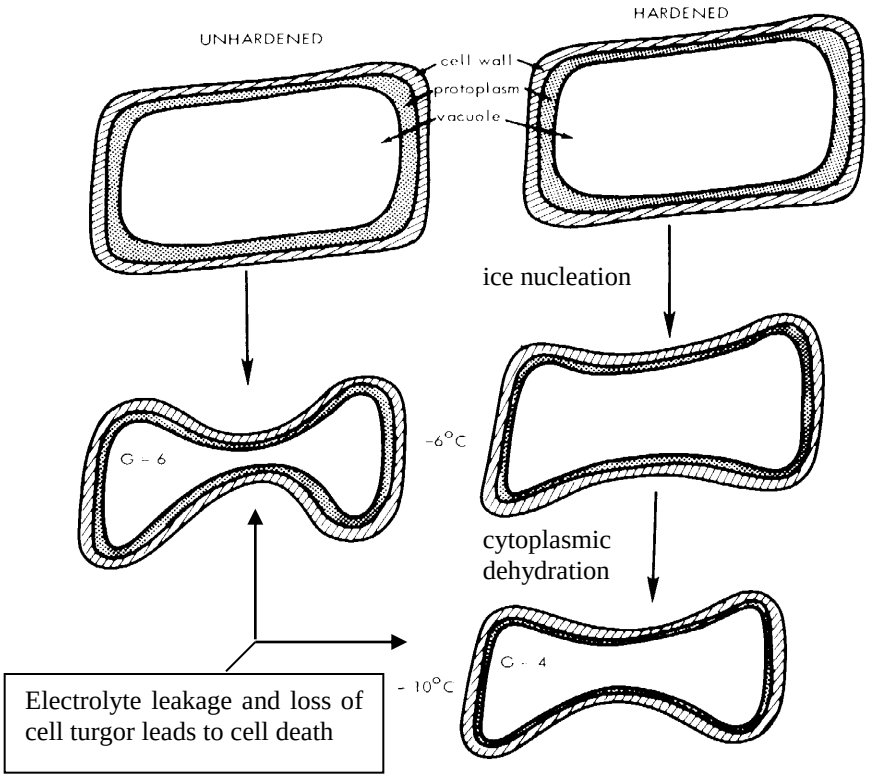


Figure 2.2 Model illustrating the freezing process in hardened and unhardened plant cells (adopted from Levitt, 1980).

During warming of the suspension and melting of the suspending medium, the gradient in chemical potential will be reversed and, if the plasma membrane remains intact, the cells will expand osmotically. This means that cells become rehydrated and expand to their original volume (Steponkus, 1990).

The survival of biological samples depends on the rate of cooling and the rate of thawing. Changes in air temperature in nature are slow and approximately 1 to 10°C h^{-1} while that of the soil is even slower at 1 to 5°C h^{-1}. Field grown plants also cool at a slow rate, in extreme situations only a few degrees per hour, and thaw at an equally slow rate. This means that

water can move to sites of lower vapour pressure created by ice. According to Mazur (1970) and Pitt and Stephonkus (1989) cooling and thawing rates are of primary importance in the cryopreservation of plant and animal cells in liquid nitrogen. To measure freezing tolerance in the laboratory the cooling and thawing rates are also important in the design and conduct of *in vitro* freeze tests (Levitt, 1980).

2.4 Freeze desiccation

To avoid ice nucleation on field grown crops is virtually impossible, because the soil freezes at temperatures just below 0°C and this serves as a nucleator for the crown and root tissues of these plants. On the other hand, although herbaceous plants are able to tolerate cytoplasmic desiccation, it does not mean that the freezing process in these plants is not controlled. To the contrary, the pattern of ice crystal growth is being influenced by the accumulation of both ice-nucleating and ice-inhibiting proteins in the apoplasm of winter rye leaves (Griffith, Ala, Yang, Hon & Moffatt, 1992; Marentes, Griffith, Mlynarz & Brush, 1993). The ice formation in rye leaves begins at specific sites and apparently grows in a controlled manner. Therefore, the presumed reason for this control is prevention of ice expansion from shearing plasmodesmata or intercellular organisation (Pearce, 1988). By making use of anti-freeze proteins, attempts have been made to genetically engineer freeze tolerance and a gene responsible for this protein has been expressed in tobacco and tomato (Hightower, Bade, Penzes, Lund & Dundmuir, 1991).

2.5 Controlling the freezing process

Secale cereale (winter rye) is a freezing tolerant cereal and it has been determined that there is a nine fold increase in the accumulation of proteins in the protoplast of winter rye leaves during cold acclimation. These apoplastic proteins may play a role in controlling the ice formation in winter rye leaves as:

- they accumulate in leaves that have been exposed to low temperatures;
- they accumulate in the apoplast where ice forms during freezing; and

- there is a quantitative correlation between the accumulation of these proteins and the increase in freezing tolerance that occurs during cold acclimation (Marentes *et al*., 1993).

The survival of freezing tolerant plants depends on its ability to control extracellular ice formation during freezing. This is accomplished by freezing tolerant plants by forming ice within their tissue. Ice does not form uniformly through the frozen plant tissue, but is rather present in discrete masses located in intercellular spaces and xylem vessels (Pearce, 1988; Pearce & Ashworth, 1992). According to Levitt (1980) no ice forms within the cell and if it would it is thought to be lethal to the organism due to damage to the cellular membranes. Heterogeneous ice nucleators in the apoplast of plant tissue are responsible for the initiation of the freezing process (Brush, Griffith & Mlynarz, 1994).

Numerous factors restrict the growth and propagation of extracellular ice through the plant. These factors include cell wall modifications, arabinoxylans and anti-freeze proteins (AFP) (Griffith *et al*., 1992). Winter rye has shown to accumulate AFP's that modifies the normal growth of ice crystals by adsorbing onto these ice crystals (Griffith *et al*., 1992; Marentes *et al*., 1993). High concentrations of AFP's cause hysteresis and at low concentrations act as potent inhibitors of the recrystallization of ice. Recrystallization occurs at temperatures just below freezing or when temperatures fluctuate in the sub zero range. During the process of recrystallization physical damage to cells could occur when large ice crystals grow at the expense of smaller ice crystals. Progress has been made in studying antifreeze proteins in winter rye.

2.6 Acclimation to freezing stress

Summer crops are sensitive to sub zero temperatures. Winter crops, such as wheat, are planted in autumn and are tolerant to prolonged exposure to freezing temperatures in late autumn and winter. This freezing tolerance is induced by environmental signals which include low temperature and/or a short photoperiod which are characteristic of autumn. During autumn the atmospheric temperatures are sub-optimal, photoperiod becomes short

and at this point wheat acclimation commences. Plants vary in the threshold of these conditions, but in controlled conditions/environments these temperatures are usually at an optimum of 2 – 5°C and an approximate photoperiod of 12 hours. The main problem with field trials and environments are that there are considerable variation year on year and that the maximum tolerance can vary.

The acclimation to freezing stress in winter cereals is induced by low temperature and is related to the genetic potential of the cereal seedling, as modulated by environmental factors. These factors are photoperiod, light intensity, soil water content and nutrition (Gusta & Fowler, 1977; Fowler, Gusta & Tyler, 1981; Limin & Fowler, 1985). In terms of mineral nutrition, hardiness was promoted with the application of phosphorous and potassium, but nitrogen increased vegetative growth and reduced the freezing tolerance of plants. Hetherington, McKersie and Keeler, (1990) also noted that through luscious growth, decreesed winter hardiness (Jung & Smith, 1959; Freyman & Kaldy, 1979) as a result of fertiliser application (nitrogen), freezing tolerance could be lowered. According to Tyler, Gusta and Fowler (1981) freezing tolerance of winter wheat was promoted by low levels of nitrogen, phosphorous and potassium.

Cold acclimation of wheat also leads to a significant rise in the protein concentration, especially in winter wheat leaves (Charest & Phan, 1990) and according to Cloutier (1983) the content and nature of proteins seem to play an important role in the cold hardening process. Charest and Phan (1990) also noted that proline accumulation was found to be very important in the crown of winter wheat varieties. Other studies have also documented proline synthesis or the presence of proline precursors in leaves and roots (Dörffling, Sculenburg, Lesselich & Dörffling, 1990) of plants under freezing stress.

Winter wheat has a broad variance in genetically fixed freezing resistance and the expression of freezing resistance is affected by environmental factors, especially low temperature (Levitt, 1980). Several physiological, biochemical and biophysical changes are involved in the process of cold hardening among which is an increase in dry matter, sugar and free amino acids (proline) (Kushad & Yelenosky, 1987), changes in the physical and chemical

composition of membranes (Uemura & Yoshida, 1984), in protein composition (Perras & Sarhan, 1989) and in the levels of abscicic acid (ABA) (Lalk & Dörffling, 1985). According to Perras and Sarhan (1989) ABA might trigger some of the processes which are responsible for freezing resistance.

Freezing tolerance is easily lost during spring when soil temperature rises above freezing and the main limitation of field survival trials in the determination of cold-hardiness of varieties are that the results are usually inconclusive as a result of complete winter kill and/or a lack thereof (Cook & Veseth, 1991; Limin & Fowler, 1993). The acclimation process is of extreme importance to the survival of wheat organs and eventually the wheat plant. Therefore, if leaves and roots of young plants (seedlings) were damaged or killed during freezing, the plant's re-growth solely depends on an undamaged crown containing the meristematic region.

2.6.1 Cold hardiness

Cold hardiness, according to Rohde and Pulham (1960), is a complex quantitative trait condition determined by the plant genotype and the environment in which the plant is grown. Wheat plants have to be exposed to low temperature for both acclimation as well as vernalization for there are a positive correlation between the cold hardiness and number of days to heading (Fowler & Carles, 1979). Winter hardiness is an important trait that influences the adaptation to winter coldness and this trait is generally estimated by artificial crown freezing tests (Andrews, Pomeroy & De la Roche, 1974). Freezing survival depends on the hardening process and this process has to be completed before the cold spell (frost) occurs and hardening must not be lost too early in spring (Cook & Veseth, 1991). Cold hardiness is not fixed and can be changed, modified or be lost with time, temperature, day length, maturity, plant water content, nutrition and physiological age (Gusta & Chen, 1987; Cook & Veseth, 1991). This process is driven by energy obtained from photosynthesis or seed energy reserves (Andrews, 1960; Olien, 1961).

Spring wheat cultivars are generally seen as less winter hardy than winter wheat cultivars (Fowler & Carles, 1979; Brule-Babel & Fowler, 1988; Roberts, 1990). Furthermore, spring wheat show an earliness in heading time and this association of earliness and frost susceptibility should be broken by wheat breeders (Fujita, Kawada & Tahir, 1992).

2.6.2 Metabolic changes

According to Levitt (1980) the number of factors involved in freezing tolerance is unknown and an unlimited number of factors has been investigated. It has also been established that the plant's metabolism changes during freezing acclimation. During these metabolic changes the plants acquire freeze tolerance through the accumulation of specific metabolites. Various attempts has been made to correlate acclimation with metabolic changes and the following have been observed (Levitt, 1980):

a) The accumulation of different substances:

The accumulation of sugars; amino acids; proteins; nucleic acids; lipids and certain growth regulators proved to be closely correlated to freezing tolerance.

- An increase in the sugar content changes the osmotic potential and the accumulated sugars may depress the freezing point of plant tissue. From late fall to late winter the relationship between sugar content and freezing tolerance may become more pronounced.
- Amino acid accumulation did no show a constant correlation to freeze acclimation. Though this correlation to be inconsistent the specific amino acid, proline, has been reported to accumulate at hardening temperatures.
- Striking parallels exist between the soluble protein content of the plant and freezing tolerance. The synthesis of these proteins is also associated with an increase in the amount of mRNA, tRNA and polysomes.
- Lipids also accumulate during acclimation and low temperatures increase the degree of unsaturated fatty acids.
- Hardening is also accompanied by a change in growth regulators. Different plant species indicated an increase in ABA (an inhibitor) and a decrease in the content of auxins and gibberillin (GA).

b) Changes in metabolic rates:

Levitt (1980) concluded that the accumulation of substances was supported by a visible increase in the amount of protoplasm per cell. This lead to the following questions: Which metabolic processes were responsible for the accumulation? What is the purposed of these accumulated substances? It was established that the accumulation of substances during the fall was partly due to a decrease in the breakdown reactions associated with growth in the winter annuals. Therefore the accumulation of substances during hardening was effected by both the photosynthetic and respiratory rates.

- The relationship between freezing tolerance and photosynthetic rate is very complex. It has been established that during periods of low temperature hardening plants need CO_2 and light to produce and support the accumulation of proteins, sugars, lipids and other substances.
- An inversely relation exist between freezing tolerance and respiration rate. This relationship is also complex and based on species and cultivar differences as well as the different stages of hardening.

2.6.2.1 Sugars

Numerous studies have demonstrated quantitative and qualitative changes in the free saccharide content of plants exposed to low temperatures (Levitt, 1980; Guy, Huber & Huber, 1992; Sasaki, Ichimura & Oda, 1996). Sucrose, glucose and fructose increase gradually during cold acclimation and these levels of accumulation correlate positively with the degree of freezing tolerance. Sucrose is the most commonly accumulated sugar in response to low temperatures in plants. The sucrose content in some plants can be as high as tenfold. Lesser amounts of glucose and fructose are also accumulated (Guy *et al*., 1992). Low temperatures also lead to the synthesis of fructan that is dependent upon sucrose accumulation (Pollock & Lloyd, 1987).

Winter cereals showed that fructans are the principal storage carbohydrate in the crown (Olien & Clark, 1995). When *Agropyron desertorum, A. cristatum* and *Agrostis alba* L. were transferred from 20°C to 5°C, fructan concentrations increased three to tenfold although

starch became the most prevalent carbohydrate (Chatterton, Harrison, Bennett & Thornley, 1987). Cold stress also induced the accumulation of fructan in the leaf blades of barley (*Hodeum vulgare*) and wheat seedlings. It seems that fructans act as a short-term storage form of carbohydrates, it regulates sucrose levels and provides osmoregulation. Plants may be protected through the hydrolisation of fructan to soluble sugars because this response is more pronounced in hardy winter rye than in relatively susceptible barley (Olien & Clark, 1995).

2.6.2.2 Lipids

Miller, De la Roche and Pomeroy (1974) observed during the hardening of four wheat cultivars that there was marked growth alterations in the fatty acid composition and unsaturation of the mitochondrial phospholipids. Winter hardy wheat cultivars showed that their structural transitions occurred at lower temperatures in cold grown material and were quantitatively greater. Farkas, Deri-Hadlackzy and Belea (1975) established a correlation between the degree of lipid unsaturation and cultivar hardiness.

According to Lynch and Steponkus (1987) and Uemura and Steponkus (1994) the lipid components of plant cells change dramatically as the plant acclimates to freezing stress and earlier work showed that the lipids became more unsaturated with acclimation. Recent studies have analyzed changes in the lipid composition of the plasmalemma and this was made possible due to the importance of the plasma membrane in freezing tolerance (Steponkus, 1984) and the recent improvements in the isolation of purified plasma membrane fractions. Uemura, Joseph and Steponkus (1995) observed significant changes in the lipid composition of the plasma membrane as plants acclimated. It is known that winter oats is less hardy than winter rye. Uemura and Steponkus (1994) established that there was a vast difference between spring oat leaves and that of winter rye in their lipid composition of plasma membranes, when isolated. The fatty acid unsaturation and proportion of phospholipid classes changed slightly during hardening when the plasma enriched fraction from cold hardened winter rye seedlings were analyzed (Uemura & Yoshida, 1984). This

suggested that the fatty acid changes may not have been as dramatic as once thought for cold hardened winter cereals.

2.6.2.3 Abscisic acid (ABA)

Abscicic acid is defined as a plant hormone that mainly acts to inhibit growth, promotes dormancy and to help the plant to tolerate stress conditions. The application of the growth regulator, ABA, may induce the development of freezing tolerance (Chen & Gusta, 1983). Lalk and Dörffling (1985) found an increase in ABA levels in two winter wheat varieties during a hardening program lasting five weeks. This was also supported by Machakova, Hanisova and Krekule, (1989) who reported an increase in ABA levels in wheat in response to cold hardening under growth chamber conditions. This observation demonstrates the positive effects of exogenously applied ABA, freezing resistance in the whole plant and at cell level (Lalk & Dörffling, 1985). Chen and Gusta (1983) support the hypothesis that ABA is intimately involved in the processes that are responsible for freezing tolerance.

2.6.2.4 Proline

The function of proline in freezing tolerance might be more direct than that of ABA as several researchers have shown that applied proline acts as a cryoprotectant (Withers & King, 1979). According to Duncan and Widholm (1987) treatments that increase the level of endogenous proline also increase the resistance to chilling and freezing stress. It has also been established that varieties with higher freezing tolerance accumulate proline faster and reach higher levels than less freezing tolerance genotypes. Proline is also being used as a chemical marker for freezing resistance in breeding programs for winter hardiness in this crop.

Different researchers have investigated the difference in freezing tolerance of wheat and rye (Dörffling *et al*., 1990). They also determined that the grade of frost tolerance and the level of proline accumulation were directly proportional. Furthermore, these researchers also established that there were significant differences between frozen young shoots of different

varieties. The proline level in the leaves and roots of wheat varieties increased with frost tolerance and ceased when leaves started to fall. Therefore the higher the frost tolerance the higher the accumulation of proline.

Proline accumulation is a common metabolic response of higher plants to different stress factors such as water deficits, salinity stress, high and low temperature (Lalk & Dörffling, 1985). Proline is of utmost importance to plants in their metabolic processes and it can serve as a nitrogen and carbon source during the recovery of plants after exposure to stress (Jager & Meyer, 1977). Proline also protect proteins during dehydration, it could be involved in osmoregulation and acts as an enzyme regulator.

2.6.2.5 Proteins

During stress related conditions, newly synthesized proteins appear and they are more or less specific to a given environmental stress condition. According to Griffith, Antikainen, Hon, Hakaskimaunsbauch, Yu, Chun, and Yong (1997) the development of freezing tolerance are based upon cold acclimation results in altered gene expressions leading to the synthesis of specific proteins and certain enzymes. When the plants are exposed to low temperatures there appears to be a decline in the abundant pre-existing proteins that formed during normal temperature exposure. Though there appears to be a decline, new transcripts and polyploids are synthesized, and it appears that they play a role in the acclimation of plants to freezing stress.

The amount of soluble proteins increases in cold and desiccation-hardened plants and soluble proteins during cold hardening also undergo changes in electrophoretic mobility (Cloutier & Siminovitch, 1982; Siminovitch & Cloutier, 1982). Some overwintering plants survive freezing temperatures by forming ice in intercellular spaces and in xylem tracheids and vessels within their tissues (Pearce, 1988). Brush *et al*. (1994) established that winter rye leaves produce intrinsic ice nucleators that have been shown to initiate the formation of extracellular ice during freezing under controlled conditions, during cold acclimation. Griffith *et al*. (1992) and Marentes *et al*. (1993) have also shown that winter rye accumulated

antifreeze proteins (AFP's), that had the ability to modify the growth of ice and inhibit the recrystallization of ice. This was also supported by Hon, Griffith, Chong and Yang (1994) who established that five antifreeze proteins were present as the most abundant proteins in apoplastic extracts of cold-acclimatised winter rye leaves.

AFP's lower the freezing temperature more than the melting temperature and therefore depress the freezing temperature of a solution noncolligatively. Hysteresis (that is the difference between freezing and melting temperatures) is measured by observing the growth of a seed crystal microscopically (De Vries, 1986) and AFP's are often referred to as thermal hysteresis proteins in frost-tolerant organisms.

Chun, Yu & Griffith (1998) also established that AFP's accumulate in the leaves of winter cereals during cold acclimation, where they may inhibit recrystallization during freezing and thawing cycles and provide nonspecific disease resistance. These researchers also established that the antifreeze activity and apoplastic protein content were not correlated with freezing tolerance (defined as the % survival at –11°C), but they were positively and significantly correlated with winter field survival rates. Furthermore it was determined that the total leaf fresh weight (negatively correlated) and antifreeze activity (positively correlated) together accounted for approximately 55% of the variation in winter survival. This indicated that high antifreeze activity and slow vegetative growth at a low temperature are both important quantitative traits for winter survival.

2.7 Anatomical and morphological changes

Wheat plants are exposed to adverse environmental conditions throughout the growing season. During the growing season the wheat plant progresses through a vegetative and reproductive stage, where both these stages include growth and development during which the plant go through different sets of complex reactions. These growth periods and processes are subjected to different environmental conditions that include water logging, drought, high and low temperatures, etc. The interaction between these growth stages and different environmental conditions leads to different reactions within the plant. Different plant parts

posses different levels of cold hardiness. The ability of roots and crowns of Norstar (a winter wheat) and Puma (a rye) to hardening has been investigated. Chen, Gusta and Fowler (1983) established that the roots were only able to harden between –6 and –7°C and that of the crowns between –20 and –30°C. It has also been established that when plant parts were stored at temperatures of –3 to –5°C for a period of four months, the crowns were still alive while the adventitious roots died.

As early as 1929 it has been reported that younger tillers survived the winter better than older tillers on the same plant and therefore tillers on the same plant does not possess the same degree of winter survival (Gusta & Chen, 1987). In contrast to this Legge, Fowler and Gusta (1983) reported that not the younger but rather the intermediate to younger tillers, were the more cold hardy tillers on the same plant. According to Legge (1979); sited by Gusta and Chen (1987), tillers regenerated from adventitious buds rather than from the intercalary meristem following freezing may be due to injury to the xylem vessels and the cells of the central as well as the lower part of the crown (Olien, 1961). Large ice crystals could form and mechanically damage the tissue of the central and lower crown due to the high water content in xylem vessels as well as the presence of large vacuolated cells. According to Pauli (1961) the damage to the tissue would reduce the connection between the shoots and roots. Beard and Olien (1963) also found that it would damage tissue that normally gives rise to adventitious roots. Gusta and Chen (1987) suggested that small undifferentiated cells and their less rigid tissues compared to that of the stem region of the parent tiller may lead to injury escape by axillary buds.

According to Gusta and Fowler (1977) an inverse relationship exists between cold hardiness and the number of tillers, number of leaves, crown root number and crown root length. The high correlation between plant erectness, the water content of crowns and leaves, the crown phosphorous content, and the total crown sugar content were found to be good indicators of winter survival in a controlled test. The importance of wheat crowns in winter survival cannot be emphasized enough. Ferguson and Boatwright (1968) suggested that deep crowned cultivars survive the winter better than shallow crowned cultivars. The depth of seeding, soil temperature, light, genotype and other environmental factors influence the depth

at which crowns are found. However, in contrast Fowler and Gusta (1977) found no correlation between crown depth and winter survival for four different wheat cultivars.

Single and Marcellos (1974) determined that wheat leaves possess a considerable degree of frost tolerance and the reproductive tissue of the developing ear was considerably less resistant to freezing and might even be injured at –1.8°C. Through the process of supercooling the floral parts in the leaf sheath may avoid freezing, even though the rest of the plant is frozen. Exposed floral and reproductive tissue may also supercool when exposed to frost depending on how glaucous the lemma, pale and awns are. The waxy surface on these floral parts prevents contacts between atmospheric freezing nuclei and internal moisture. When frost occur during the development of young ears, that is any time from the onset of stem elongation, damage might usually not be recognised until heading is complete.

When the growth point (developing ear) is killed before heading the main stem will remain intact but will eventually die and this (if early enough) will lead to the initiation of new tillers in the crown of the plant, if not damaged. According to Cook and Veseth (1991) frost damage might also be confused with drought stress where drought could also lead to empty, bleached tips on the ears. Before ear emergence the floral parts of the ear within the flag leaf sheath may avoid freezing through supercooling, even though the rest of the plant is frozen. The reason for this is the inability of the ice front to travel across the node of the stem or rachis to the developing ear. After ear emergence, atmospheric ice or ice nucleating bacteria might initiate nucleation.

2.8 Wheat growth and development

To understand the effect of freezing and/or frost stress it is necessary to understand wheat growth and development. The effect of these and other environmental factors on crop growth and yield depend upon the developmental stages when these factors act (Fisher, 1985; Thorne & Wood, 1987; Miralles & Slafer, 1999; Saqib, Akhtar & Qureshi, 2004). According to Landes and Porter (1989) grain yield is more sensitive to environmental factors during more sensitive growth stages than less sensitive growth stages. Therefore, crop development was

defined as the sequence of phenological events that is controlled by external factors, which determine the morphological changes and/or functions of some organs (Miralles & Slafer, 1999).

Miralles and Slafer (1999) stated that the wheat development is a continuity of vegetative, reproductive and grain filling phases through which the crop initiates and grows its organs and completes its life cycle. In turn the interactions between genetic and environmental factors determine the duration of each growth stage and the number of primordia initiated. A nondestructive identification method (external morphological stages) by which the developmental progress is determined provides no information on the sequence and timing of events in the shoot apex, where the actual development occurs (Miralles & Slafer, 1999). It is also difficult to extrapolate between external and internal developmental events due to the different responses of leaf appearance and apex development to major environmental factors. As a result, some scales have been developed to describe major developmental stages. Some use morphological changes (Gardner, Hess & Trione, 1985) and some are more precise and make use of apex dissection (Haun, 1973; Zadoks, Chang & Konzak, 1974, ARC - Small Grain Institute, 2004). During the discussion of the results in this thesis the scale that is in use by the Small Grain Institute in South Africa will be referred to. Figure 2.3 shows a schematic classification of the apex or growth point (ARC - Small Grain Institute, 2004). These different stages mark changes in phasic development. There are three major phases: the vegetative phase, initiation of leaves, the reproductive phase, from floret development until the number of fertile florets is determined and the grain filling phase, when the grain first develops the endosperm cells and grows to determine the final grain weight and yield (Miralles & Slafer, 1999).

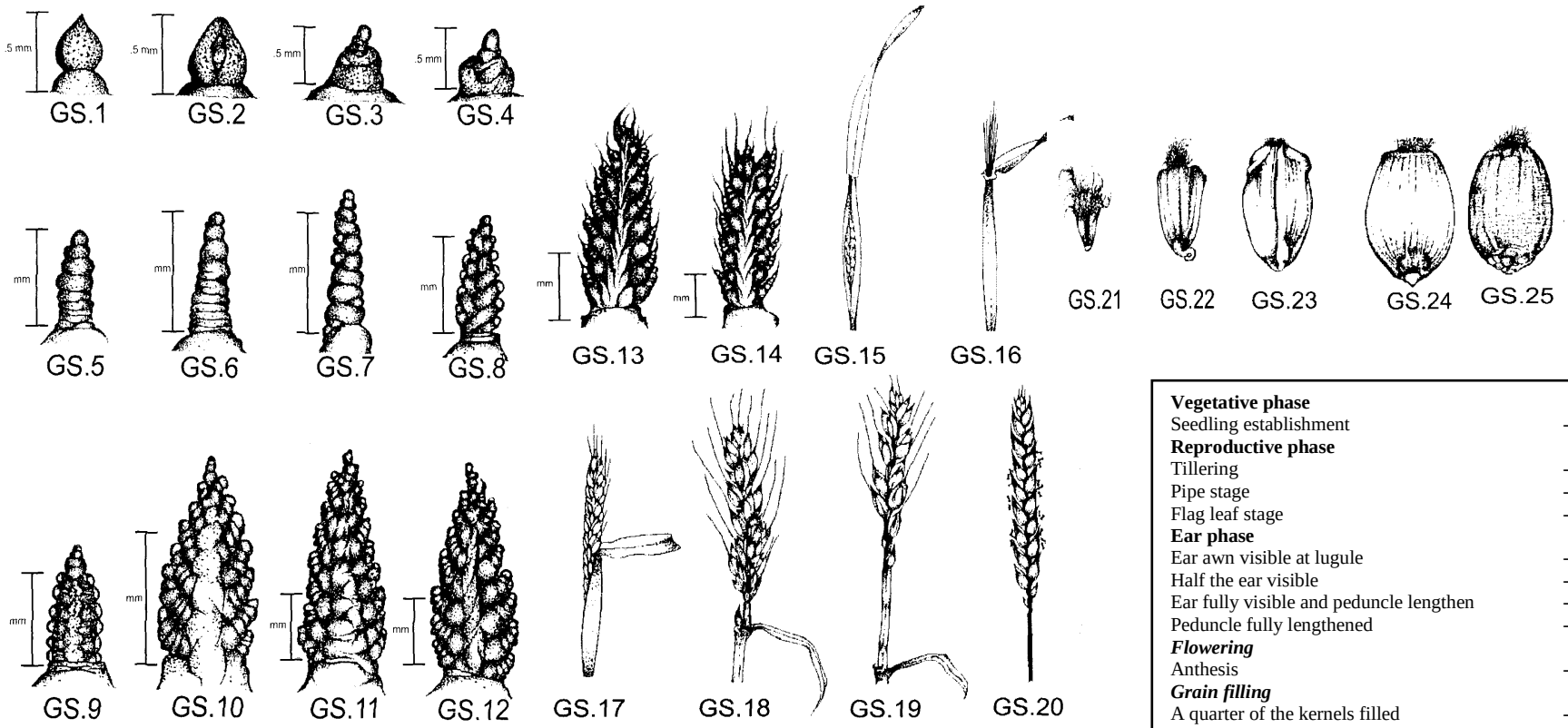
Wheat yield is determined by the interaction of the plant (its yield components) and the environment. The different yield components are initiated and formed through a process of growth and development during the life cycle of the crop. Some of the developing stages are more sensitive to environmental changes than other stages. Kirby (1988) and Siddique, Kirby and Perry (1989) emphasized that the period between terminal spikelet initiation and anthesis is of paramount importance. The reason for this being that the relationship between

the number of kernels per unit area is better correlated to yield than the weight of individual kernels (Fisher, 1985; Thorne & Wood, 1987). Rawson (1971) concluded that there was an association between the development stages and the yield components during the pre-anthesis period. This association is of paramount importance in determining which factors and how these factors modify or inhibit the duration of the mentioned phases.

2.8.1 Vegetative phase

Germination and seedling establishment

Evans, Wardlaw and Fisher (1975) stated that the minimum moisture needed for the germination of wheat is 35 to 45% of the kernel dry weight and at temperatures of 4 to 37°C, with the optimum being 12 to 25°C. This process is started through imbibition, which reinitiates the metabolic activity in the nondormant seed, followed by leaf initiation in the shoot apex (Miralles & Slafer, 1999). During germination the seminal roots extend first, followed by the coleoptile. Seminal roots at the level of the seed are associated with the scutellar and epiblast nodes. Adventitios roots are also produced in association with the coleoptilar node in addition to the seminal roots. The elongation of the internode between the coleoptilar node and the first foliar node causes a distance between the adventitious roots originating in the crown and the more complex later formed roots. The extent of the elongation of this internode varies with planting depth (Simmons, 1987).



Vegetative phase	
Seedling establishment	- 0
Reproductive phase	
Tillering	- 5
Pipe stage	- 10
Flag leaf stage	- 15
Ear phase	
Ear awn visible at lugule	- 16
Half the ear visible	- 17
Ear fully visible and peduncle lengthen	- 18
Peduncle fully lengthened	- 19
Flowering	
Anthesis	- 20
Grain filling	
A quarter of the kernels filled	- 21
Half of the kernels filled	- 22
Three quarters of the kernels filled	- 23
Mature kernels and looses chlorophyll	- 24
Physiologically mature	- 25

Figure 2.3 A schematic classification of the apex or growth point (adopted from ARC - Small Grain Institute, 2004)

Depending on the planting depth and the rate of leaf initiation of a specific cultivar one to three more leaf primordia are initiated before seedling emergence and these primordia add to those initiated in the main/mother plant (Hay & Kirby, 1991). The shoot apex retains the shape of a dome during a period of which the length depends strongly on the genotype and environmental conditions, after seedling emergence (Miralles & Slafer, 1999). According to Fisher (1973) it elongates and the initiation of the leaf primordia may continue as single ridges around the elongated apex until the onset of floral initiation. Leaf primordia are initiated at a single rate on a thermal time basis despite the morphological changes in the apical meristem during the vegetative phase. When the apex changes from a vegetative to a reproductive phase at floral initiation, leaf initiation ceases and the maximum number of leaves in the main shoot is determined (Miralles & Slafer, 1999). Gardner *et al*. (1985) showed that after floral initiation the first double ridge is initiated, when leaf and spikelet primordia appear as double ridges around the shoot apex. The upper ridge is the spikelet primordia, while the lower ridge is the leaf primordia. This double ridge stage has been used to indicate the end of the vegetative phase, but Kirby (1990) have shown that the first spikelet primordium may be initiated before the first double ridge appears.

Seedling appearance is frequently taken as the appearance of the tip of the first leaf through the coleoptile and occurs as soon as the coleoptile emerges through the soil. Several leaves have already been initiated by the time the first leaf appears. This ensures a limited duration for reproductive development given by the number of primordia that have to appear until flag leaf appearance and the length of the phyllochron and the period between flag leaf appearance and anthesis (Miralles & Slafer, 1999). According to Hay and Kirby (1991) the longer the period from seedling emergence to floral initiation the higher the number of leaf primordia that have to appear after floral initiation. This also means a prolonged duration of the reproductive phase from floral initiation to anthesis. Leaf appearance and the rate of appearance is influenced by both the genetic background and variation of cultivars as well as environmental conditions, including the date and location of planting, temperature and photoperiod (Rawson, 1971; Bauer, Frank & Black, 1984; Hay & Kirby, 1991; Kirby, 1992).

Cultivars have also shown differences in their rate of appearance of leaves between the main shoot and tillers on the same plant and mature leaves vary with their position on the plant. According to Simmons (1987) the maximum leaf area per shoot is achieved at the time of flag leaf expansion, which occurs just before heading.

Tillering

The number of tillers produced can influence grain yield. Miralles and Slafer (1999) stated that besides the bud corresponding to the main shoot apex, axillary tiller buds are developed in each phytomer and each of these buds has the potential to further develop into leafy tillers. The ordered sequence of primary tiller formation in relation to degree unit accumulation has been described by Rickman, Klepper and Peterson, (1983). The authors reported that stress factors (dry or crusting soil) might hinder the formation of some tillers, but as soon as the stress was removed the tillers formed normally. According to Miralles and Slafer (1999) the emergence of tillers is closely related to leaf emergence. The appearance of the first primary tiller coincides with that of the fourth leaf and this occurs at approximately three phyllochrons after seed emergence. Miralles & Slafer (1999) also stated that the subsequent primary tillers appear at regular intervals of one phyllochron. This meant that the relationship between the number of primary tillers and the number of visible leaves on the main shoot is linear with a slope close to one and an abscissa intercept corresponding to the number of leaves appeared before the onset of tillering. Tillers from different higher orders (that is secondary, tertiary, etc.) may eventually appear from the axillary buds developed in each tiller phytomer, with a similar relationship to tiller leaf number than that described for the main shoot (Miralles & Slafer, 1999).

A few tillers may form during autumn or winter if conditions are mild in winter wheat and during spring with increasing temperatures there is a rapid increase in tiller number (Simmons, 1987). The main shoot and earlier formed tillers are most likely to complete development and form grain, for winter or spring wheat, and later formed tillers usually senesce prematurely in a crop community environment. The number of tillers that senesce varies with cultivars and other factors such as temperature, plant population and nitrogen nutrition. According to Simons (1982) and Miralles and Slafer (1999) cessation of tillering

and the onset of tiller senescence are commonly associated with the completion of spikelet initiation on the main shoot and the beginning of stem elongation. The beginning of stem elongation is related to the initiation of the terminal spikelet on the main shoot apex and the length of the tillering period is therefore directly related to plant development for a particular plant density (Hay & Kirby, 1991). Frost injury during this stage is usually restricted to the leaves of the plants because the apex is near the soil surface and protected by the leaves. At this stage the plant growth rate might be retarded and might also reduce the number of tillers. When temperature increases the growth of new leaves and tillers usually resume and the crop may recover fully (Shroyer *et al*., 1995).

2.8.2 Reproductive phase

Stem development elongation (jointing, boot and heading stages)

Stem elongation coincides with the growth of leaves, tillers, roots and that of the inflorescence, which undoubtedly raises the question of assimilate supply and possible assimilate competition (Patrick, 1972). The lower internodes of stems remain short, whereas the fourth internode elongates first in spring wheat with a total of nine leaves. According to Simmons (1987) a higher numbered internode elongates and forms more leaves in winter wheat. The elongation of an internode starts as soon as the preceding internode has reached half of its final length and this sequence continuous until stem elongation is complete just before anthesis. Damaged tillers remain green when the growth point was injured by frost and the growth of the stem stops immediately (Shroyer *et al*., 1995).

Floret initiation begins in the spikelets first initiated shortly after spikelet initiation. Initiation of the florets starts at a third from the bottom of the spike and progresses up and downward until completion. The development of different floret pieces begins in the basal positions of each spikelet and progresses from there toward the distal position (Sibony & Pinthus, 1988). Kirby (1988) stated that three to five florets are initiated in each spikelet at the time of terminal spikelet initiation and no florets are initiated after the appearance of the flag leaf ligule. Many of the initiated florets abort during the short period of booting to heading and anthesis. Freezing or frost to the flag leaf may trap the spike inside the flag leaf sheath.

”Frost rings” may also occur and inhibit the translocation of photosynthate, which might lead to the death of the ear. Freezing temperatures that are severe enough to injure leaves and lower stems are nearly always fatal to male flower parts, but less severe freezing may cause male sterility without any symptoms on the plants vegetative parts (Warrick & Miller, 1999).

2.8.3 Ear phase

Anthesis

On the same plant there are different stems and ears (primary, secondary, tertiary, etc.) and they all differ in age. These differences in duration of development among shoots (that is of the same plant), spikelets of the same spike and florets of the same spikelet all serve to synchronize development (Simmons, 1987). Kirby (1974) showed that the time differential between two florets may be six hours at meiosis, although the difference in time of initiation between the first two florets in a spikelet might have been more than two days. Each wheat floret has two lodicules that are situated at the base of the ovary (Craig & O’Brien, 1975). These lodicles swell during anthesis and its function is to open the flower for anther extrusion. The process of anthesis is complete within 20 minutes from the swelling of the lodicules and the closure of the palea and the lemma.

Frost or freezing injury during the flowering stage causes either partial or complete sterility and therefore void or partially filled spikes. Because some florets were at a sensitive stage when they were frozen, one or both ends or the center of the spikes might be void of grain and grain might develop in other parts of the spikes (Shroyer *et al*., 1995).

Grain filling (Milk, soft dough and hard dough stages)

During anthesis the fertile florets are fertilized and they become potential grains. After anthesis the dry weight accumulation is slow, during which time the endosperm cell division occurs and cell numbers increase rapidly. According to Warrick and Miller (1999) developing kernels grow to full size within 12 to 14 days after anthesis, but the maximum grain weight is not reached for another two weeks. Frost injury during this stage (milk stage) could cause kernel development to cease, or slightly injured kernels could grow to their normal size but the grain might be lean, light and/or shriveled at maturity (Shroyer *et al*.,

1995; Warrick & Miller, 1999). Furthermore, wheat that has been injured by frost or freezing stress during this stage often shatters easily at maturity. The shriveled kernels also cause the grain to have a low hectoliter mass and the germination percentage may be reduced (Warrick & Miller, 1999). During the dough stage starch deposition starts one to two weeks after anthesis and initiates a period of nearly linear increase in kernel dry weight. Depending on temperature, water stress and genotype this growth period lasts two to four weeks (Simmons & Crookston, 1979). The kernel growth rate may be enhanced when the availability of assimilates increases during this brief period of dry weight accumulation and adversely a reduction in assimilates may inhibit the growth rate in particular when this occurs during the early growth rate of the kernel (Simmons, Crookston & Kurle, 1982). Though the availability of assimilates are important the importance of temperature on kernel growth can not be neglected. According to Bhullar and Jenner (1983) the direct effect of temperature on kernel growth and development is more important than the indirect effects such as the availability of photosynthate.

The spikelet and floret position on the wheat's ear play an important role in the growth and final weight of individual kernels (Kirby, 1974). The largest kernels are usually those kernels that were formed in the centrally positioned spikelets and in the proximal florets of each individual spikelet. Furthermore, a significant correlation was found by Kirby (1974) between the final kernel weight and the time of floret initiation. This indicated that the duration and sequence of ovary formation within the spike might be of importance in establishing grain weight.

According to Shroyer *et al*. (1995) and Warrick and Miller (1999) kernels reach their full size halfway through the dough stage and at this point of growth the kernel development is nearly complete. At this stage wheat is more tolerant to freezing stress than at earlier growth stages during late winter and the beginning of spring. This could be attributed to the reduction in the water content of the kernel. A slightly wrinkled kernel with a low hectoliter mass might be the only visible sign of frost or freezing damage at this stage.

Frost damage during the dough stage could also result in the reduction of germinating kernels when wheat seed are produced. This could occur due to the higher water content of the embryo or germ in relation to that of other kernel parts. The more complex cellular contents and structures of the embryo also make it more vulnerable to frost (Shroyer *et al*., 1995).

2.9 Wheat quality

The effects of soil, climate, genotype, kernel components and the interaction between the mentioned determine the actual quality of wheat. Finny, Yamazaki, Youngs and Rubenthaler (1987) stated that the basic definition of wheat quality usually varies from one class of wheat to another. Therefore the suitability of wheat might be the simplest definition of wheat quality where wheat that is desired has good quality and wheat that is not desired has poor quality. Wheat quality can thus not be expressed in terms of a single property. Quality depends on several milling, baking, processing and physical dough characteristics of which each is important in the production of bread, pastry or pasta products.

Finny *et al*. (1987) also stated that important bread (hard) wheat milling properties are relative hardness or softness, hectoliter mass, siftability of flour, break flour yield, middlings flour yield, total flour yield, flour ash content and wheat to flour protein conversion. Some of these properties are associated with the basic ingredients of the wheat grain. Most importantly the protein content of wheat only contributes to approximately 12% of the wheat grain. Nevertheless, the contribution of protein to bread quality cannot be underestimated. The remainder of the wheat grain consists of a variety of ingredients and is illustrated in Figure 2.4 (Stone & Savin, 1999).

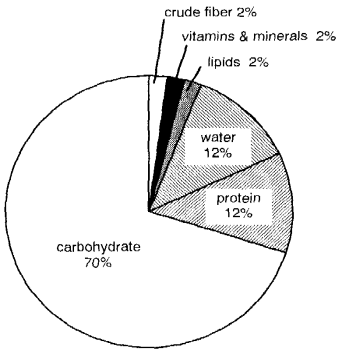
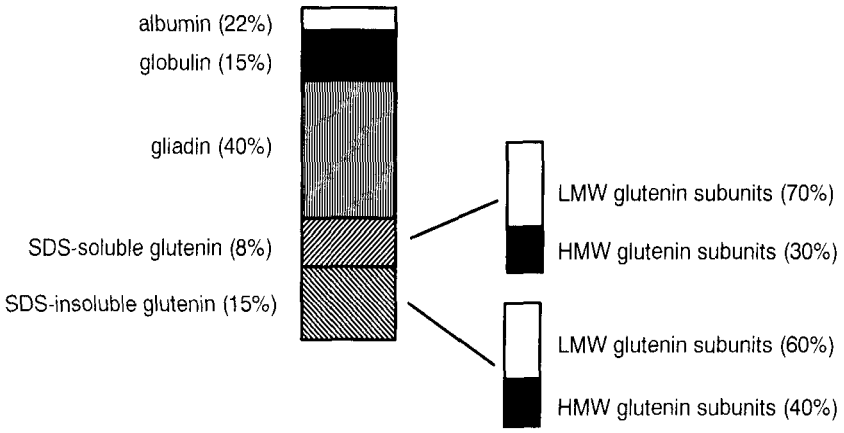


FIGURE 2.4 The basic ingredients of wheat grain (Stone & Savin, 1999)

2.9.1 Wheat proteins

According to Weegels, Hamer and Scholfield (1996) proteins are recognised as the most important components governing bread-making quality. Wheat grain proteins have been classified as albumins, globulins, gliadins and glutenins on the basis of their solubility (Osborne, 1907 as sited in Stone & Savin, 1999). The metabolic proteins (albumin and gliadin) make up 20 to 30% of the total grain protein (Figure 2.5).

Stone and Savin (1999) stated that albumin and globulin play a minor role in the protein interactions that are required for the formation of cohesive gluten and therefore have a minor impact on dough strength and bread making quality. Their importance is discarded because they reside primarily in the embryo part of the grain, a fraction that is deliberately excluded from the white flours used in bread and pastry making, and because they are not chemically disposed toward the protein-protein interactions.



Note: Bars show the percentage of each protein fraction. SDS is sodium dodecyl sulfate and LMW and HMW are low and high molecular weight, respectively.

FIGURE 2.5 Protein composition of a typical wheat grain (Stone & Savin, 1999)

The remainder of the grain consists of storage proteins that compose 70 to 80% of wheat grain. The storage proteins are responsible for determining the physical properties of dough and consequently many aspects of grain quality. The primary role of these proteins is to store energy and nutrients for germinating wheat seedlings; therefore it is a mere fortuity that

these proteins have secondary properties that made them useful for humans and human consumption (Stone & Savin, 1999).

In mature grain, gliadins and glutenins, (the storage proteins) are aggregated in polymers with different sizes and solubility. The gliadins and glutenins each make up approximately 30 to 40% of the total protein in the wheat grain (Stone & Savin, 1999). The distribution of monomeric and polymeric proteins as well as their solubility play a critical role in governing wheat flower properties, including baking quality (Gupta, Masci, Lafiandra, Bariana & MacRithcie, 1996). The smaller of the two proteins is the gliadins and their size ranges approximately from 30 to 80 kDa. The gliadins are nonaggregating and therefore they are unlikely to contribute strength to gluten. According to Gupta, Khan and MacRitchie (1993) gliadins is largely responsible for extensibility in doughs, or the ability to strech without breaking.

The glutenins differ from the gliadins in numerous aspects. Glutenins is a family of individual proteins that vary in size from 12 to 130 kDA, but it is not usually as individuals that these glutenin molecules exert their particular influence on dough strength (Stone & Savin, 1999). Glutenins form strong bonds and are therefore aggregating proteins. This property enables formed gliadin polypeptides to form macromolecules with molecular weights of up to 10 million, which make them among the largest proteins in the natural world (Wrigley, 1996). These gluten macromolecules are inevitable to the formation of the cohesive gluten network required for dough strong enough to withstand the stresses of bread making (Stone & Savin, 1999).

According to Gupta *et al*. (1993) glutenins can be divided into subclasses on the basis of their solubility in dilute detergent, particularly SDS (sodium dodecyl sulfate). The SDS soluble glutenin proteins tend to be smaller and contribute less to dough strength than the insoluble glutenins. The greater the proportion of high molecular weight (HMW) glutenin subunits in a given glutenin molecule, the greater its ability to form a macromolecule and in turn the greater the contribution is to dough strength. The greater contribution to dough strength could be explained as the SDS soluble glutenin contains a smaller proportion of HMW

glutenin subunits than that of the SDS insoluble glutenin (Gupta *et al*. 1993). The ration between soluble and insoluble proteins that affects the bread making quality, is a function of the protein composition that is genetically controlled. Environmental factors such as temperature, water and nitrogen nutrition also affects this ratio (Jia, Masbou, Aussenac, Fabre & Debaeke, 1996; Daniel & Triboï, 2002).

2.9.2 Carbohydrates

The wheat grain consists of approximately 70% (Figure 2.5) carbohydrate that includes starch, sucrose, glucose, fructose and other less abundant sugars. The sugars contribute to less than three percent of the carbohydrates (Abou-Guendia & D'Appolonia, 1973). These sugars play an important role in the baking process for they contribute to the sugars required by yeast for the production of leavening gas (Stone & Savin, 1999).

Wheat starch (thousands of glucose molecules linked together) occurs in small granules, densely packed in the endosperm of wheat and serves as stored energy to a germinating seed. Starch granules occur in three distinct groups or sizes (A, B, and C granules), although the starch granule properties and the physiology of their synthesis are also different. The largest starch granules (A-type) are 10 to 50 µm in diameter and they are lenticular in shape, which distinguish them from the other starch types (B and C granules). This type of starch contributes to approximately 80% of the total starch content, but contributes to only 10% of the total granule number. Granules with a diameter of 5 to 10 µm and spherical in shape are the B-type granules. These granules contribute 15% ot the total mass of granules and as well as the number of granules. C-type granules contribute 75% to the total number of granules and 5% to the total starch mass. This is due to the size of the granules for they are 2 to 5 µm in diameter and they also have a spherical shape (Stone & Savin, 1999). Starch is insoluble in water, but during the milling process 10 to 35% of the granules are damaged and these are prone to swelling and are partially soluble. Varying from crystalline to amorphous, the starch granules are not uniform due to their dependence on the chemical form of starch present in the granule.

Amylose and amylopectin, the two main fractions of wheat starch, respectively make up approximately 25 and 75% of the total starch mass (Leloup, Colonna & Buleon, 1991). According to Stone and Savin (1999) the relationship between amylose and amylopectin is analogous to that between gliadin and glutenin. The size and structure of the starch fractions also differ as discussed previously, therefore they have different physical and chemical properties, and the balance of these properties affects the functionality of starch.

One of the properties of starch is its thickening effect which, as an additive, is of importance in the food industry. Amylose is a more effective thickening additive than amylopectin due to the following: amylopectin is more highly branched and much larger, with individual molecules making up tens of thousands of glucose units. Amylose on the other hand is an essential linear polymer that consists of 1-4 linked α-D-glucose and in wheat is about five chains long and in contrast to amylopectin is slightly branched. For a given mass amylose, the linear structure of amylose is longer than that of amylopectin, therefore making it more prone to entanglement with starch and other molecules (Stone & Savin, 1999).

McGee (1991) as sited in Stone and Savin (1999) stated that:

- starch does not only contribute to making up the rest of the bulk of the loaf, but it also reinforces the gluten network by providing a semisolid structure to which gluten can adhere;
- starch can move around the dough and therefore fill up spaces that are created during the baking process with change in the shape of the loaf;
- starch play an important role in the regulation of water distribution through a loaf of bread. With a temperature increase during the baking process water is forced out of the coagulating protein structures. During this stage the starch absorbs more water as it has reached its gelatinisation temperature (> 60°C). Protein does not provide enough water during this process to be absorbed by starch as starch can absorb over ten times its weight and therefore the bread is composed of partially gelatinised starch.

According to Stone and Savin (1999) the partially gelatinised amylose becomes less viscous after the removal from the oven and cooling and this firming is responsible for the ease in

slicing bread. Amylopectin is slower in resuming to a crystalline form than amylose and during this crystallisation process of amylopectin water is “squeezed” out of the lattice, from where it migrates down a concentration gradient and finally out of the loaf and therefore this is associated with the dryness of bread. Based on this amylose and particularly amylopectin and therefore starch as a whole, plays an important role in the staling of a loaf of bread.

Starch is just as important to quality than protein due to the fact that starch, relative to protein, is very stable between genotypes and environments. Starch properties are important in determining the actual quality of grain, and therefore the fact that they are not highly variable means that there is not a high priority placed on measuring the starch content to determine the value of wheat and wheat quality. With the increased importance of noodle wheat this view has changed somewhat (Stone & Savin, 1999).

2.9.3 Lipids

Figure 2.5 clearly show that lipids compose only two percent of the mass of wheat flour. If lipids were to be removed from dough it would not be able to rise during baking, therefore making lipids absolutely essential for bread making. Wheat flour lipids are closely associated with starch granules as well as gluten proteins. It was also suggested that lipids are involved in the binding of gliadin to glutenin within the gluten structure, and of gluten to starch within the dough (Stone & Savin, 1999). According to Stone & Savin (1999) lipid content and composition are not often used as a guide to grain quality, despite its importance, and this might largely be because the role of lipids in determining quality is poorly understood.

Frost damage in South Africa usually occurs during the booting, flag leaf and flowering stages and therefore losses are mainly limited to yield for no seed growth or development had started prior to these stages. Quality losses are restricted to the grain filling stages with regard to the hectoliter mass of the grain and evidently a slight yield loss. Questions from farmers are not restricted to yield losses only, but also possible quality losses with regard to protein content. These questions prompted this study and the attempt to find answers.

CHAPTER 3

MATERIAL AND METHODS

3.1 Wheat cultivars and treatments

3.1.1 Experiment 1

A pot experiment was conducted to determine the effect of frost damage to three types of wheat grown in a naturally lit glasshouse at the University of the Free State, Bloemfontein. A randomised block design with five replicates (pots, each pot containing two plants) was used.

Three (3) wheat cultivars (different with respect to genotype) were selected from a range of South African cultivars. These cultivars represented the different growing habits namely a winter (SST 399 – C2), intermediate (PAN 3377 – C3) and spring type (Kariega – C4). The selection was based on research entitled "Genetic variability of tolerance to freezing in South African wheat cultivars" (Jacobs, 1999). A Canadian winter wheat cultivar (Norstar – C1) was selected as the control and included as the fourth cultivar. All cultivars were vernalised at a temperature of 4°C for a period of four (4), two (2) and one (1) week for the winter, intermediate and spring types, respectively, before it was planted.

All cultivars were planted on the same day in a sandy loam soil and grown under controlled conditions at a temperature regime of 20/10 °C (day/night, 12 h each). Four (4) seeds per pot were planted at a depth of 20 mm and thinned to two (2) seedlings per pot two (2) weeks after emergence. Pots used were 200 mm in diameter and 220 mm deep. The plants were watered by means of a computerised dripping system and nutrients were provided on a weekly basis through the dripping system. The water content was maintained at field capacity throughout the growth period.

Plants were hardened at temperatures below 10°C for two (2) weeks before they were subjected to five different freezing temperatures [0 – (control), –3, –6, –9 and –12°C] at four different growth stages (tillering, flag leaf, flowering and hard dough stage).

3.1.2 Experiment 2

Based on the results obtained with Experiment 1, the effect of frost damage to two types of wheat was conducted the following growing season in the glasshouse. A randomised block design with five replicates (pots, each pot containing two plants) was used.

The temperature selection for the latter two experiments (Experiment 2 and 3) was based on results in Experiment 1, since no damage was obtained during experiment 1 at temperatures of –6°C or higher. Therefore the range started at –5°C where no damage would be encountered, –7°C where damage would be encountered and –9°C where severe frost damage would be encountered, as was observed in Experiment 1. Throughout the trial period visual symptoms of frost damage was collected by means of photographs and these were also verified with symptoms that was observed in the field, especially that of the 2003 growing season, where severe frost damage was encountered during spring (3 and 4 October 2003). These visual symptoms were used to compile a guide on frost damage for South African conditions (see Chapter 8). The temperature range changed from 0, –3, –6, –9 and –12°C in Experiment 1 to –5, –7 and –9°C in Experiment 2 while only two cultivars [a South African winter (SST 399 – C1) and intermediate type (PAN 3377 – C2)] were used in the latter.

Both cultivars were vernalised at a temperature of 4°C for a period of four (4) and two (2) weeks for the winter and intermediate type, respectively. These cultivars were planted on the same day, in a sandy loam soil, and grown under controlled conditions at a temperature regime of 20/10 °C (day/night). Four (4) seeds per pot were planted at a depth of 20 mm and thinned to two (2) seedlings per pot two (2) weeks after emergence. The pots used, were 200 mm in diameter and 220 mm deep. The plants were watered by means of a computerised dripping system and the water content was maintained at field capacity throughout the growth period.

The plants were hardened at temperatures below 10°C for two (2) weeks before it was subjected to three different freezing temperatures (–5, –7, –9°C) at three different growth stages. These stages were defined as; early flag leaf (growth point stage 13), flag leaf (growth point stage 15) and late flag leaf stage (growth point stage 17) (Joubert system – ARC Small Grain Institute, 2004).

3.1.3 Experiment 3

This experiment was conducted to evaluate the effect of frost temperatures on wheat during different flowering stages. The same methodology was used for this experiment as set out in section "3.1.2 Experiment 2".

The plants were also exposed to three different freezing temperatures (–5, –7 and –9°C) at three different flowering stages. These stages were defined as no flowering (0%), 50% flowering and 100% flowering.

3.2 Freezing test

Freezing tests were conducted using chambers cooled through conduction-convection. Three chambers were used, measuring 3(w) x 4(d) x 2(h) m. The different chambers were able to operate at 10 to 0°C, 10 to –7°C and 10 to –13°C, respectively.

Plants were removed from the glasshouse (early in the morning) when it reached the appropriate growth stage. The base temperature of the glasshouse (± 7°C) was used in the cold and freezing rooms to start the freezing process. With the exception of the control (0°C), plants were supercooled to –2°C. The temperature was decreased in equal increments to the desired temperature over a period of 1 – 3 hours. After reaching the desired temperature the plants was kept at this temperature for one (1) hour. Refrigeration and air circulation were then stopped, the chamber slightly opened to allow slow thawing and the plants were transferred to the glasshouse the next morning.

3.3 Observations

3.3.1 Quantitative evaluation

On completion of the tests the plants were transferred to the glasshouse for recovery. The plants were still watered and supplied with the necessary nutrients until they reached physiological maturity. Each pot contained two plants that was cut just above the soil level and the total dry matter of each plant was determined. During the trial the primary spike was used to determine the growth stage of the plants when the treatment was applied. This was done to determine the effect of frost injury on the primary and secondary spikes separately, which were not at the same growth stage. All other parameters besides the dry matter were quantified and this included number of spikes, number of spikelets, spikelets per spike, number of kernels, kernels per spike, kernel weight, kernel weight per spike and mass per 100 kernels. The above parameters were used to evaluate the effect of frost injury in Experiments 1, 2 and 3.

3.3.2 Qualitative evaluation

Though three trials were conducted, the qualitative evaluation was restricted to Experiment 1. The reason for this being that the effect of frost injury at the flag leaf (Experiment 2) and flowering stages (Experiment 3) were detrimental to seed set resulting in low yields and therefore not enough material was generated to execute qualitative tests.

The treatments restricted the amount of qualitative parameters as a result of the limited yields obtained. Therefore, with the available material, only the water-soluble protein, total protein content and stirring number was determined.

3.3.2.1 Determination of total water-soluble protein levels in wheat kernels (µg/g)

3.3.2.1.1 Preparation of material and extraction of total water-soluble proteins

a) The total seed yield was ground with a Kenwood coffee mill and the flour was sieved through a 0.5 mm sieve.
b) The flour was ground further to a fine powder using a mortar and pestle. Subsequently, proteins were extracted in 6 cm^3 g^{-1} fresh weight with a 12.5 mM Tris-HCl buffer (pH = 6.8) containig 10 mM mercapto-ethanol, 2 mM EDTA and 2 mM PMSF. The crude

extract was centrifuged at 12000 g for 10 minutes and the water-soluble protein content determined in the supernatant.

3.3.2.1.2 Protein assay

The water-soluble proteins were assayed in quadruplicate according to the method of Bradford (Boyer, 1993) using the Biorad colour reagent. Bovine gamma globulin was used as standard. The mean of the four replicates was calculated. Spectrophotometer readings were obtained by means a Model EL_X 808 micro plate reader.

3.3.2.2 Total protein content (%)

The total protein content in the kernel flour was determined after preparation as described in section 3.3.2.1.1 a), by means of the DICKEY-John protein analyser.

3.3.2.3 Stirring number

The stirring number (SN) was determined by using a Rapid Visco Analyser – Mini 3 (RVA, Newport Scientific, Australia) according to the American Association of Cereal Chemists (2000). The moisture content of the flour had to be determined by means of a Marconi moisture meter, model TF 933C. The samples were weighed according to their moisture content by making use of a moisture adaptation table. The method included a sample size of ±4.00 g (dry matter), a water volume of 25.0 ml (dH_2O) and a block temperature of 91°C. Three replications were conducted and the results will be represented as Rapid Visco Units (RVU's). The RVU's can be used to determine the falling number (FN) by means of the following formula:

$$Y\ (FN) = [1.2868\ X\ SN\ (RVU's)] + 35.792$$

3.4 Statistical analysis

Differences between treatments were determined by Analysis of Variance (ANOVA) using Number Cruncher Statistical System (NCSS 2000) General Linear Model procedure (Hintze, 1998). Treatment means were compared using least significant differences (LSD) at the 5% level of significance.

CHAPTER 4

ASSESSMENT OF FROST STRESS TOLERANCE IN SOUTH AFRICAN WINTER, INTERMEDIATE AND SPRING WHEAT

4.1 INTRODUCTION

Yields from wheat cultivars depend largely on how well they have adapted to the local climatic environment (Halse & Weir, 1974). Planting dates in South Africa differ considerably. Generally the dry land winter wheat production (including different cultivars) commences for early planting, from 15 April until 31 May and for late sowing from 15 May to 30 June. In the warmer regions planting under irrigation commences 1 May to 30 June and for the cooler irrigated regions from 20 May to 25 July (ARC – Small Grain Institute, 2004).

The development of wheat is a continuity of three growth stages namely the vegetative, reproductive and grain filling phases through which the crop initiates and grows its organs and completes its life cycle. In turn the duration of each growth stage as well as the number of primordia that is initiated is being determined by the genetic and environmental interaction (Miralles & Slafer, 1999). Some of the developing growth stages are more sensitive to stress or environmental changes than other growth stages. Kirby (1988) and Siddique *et al*. (1989) showed that the period between terminal spikelet initiation and anthesis was of importance in this regard. The reason is that the relationship between the number of kernels produced per unit area is better correlated to the yield than the weight obtained by individual kernels (Thorne & Wood, 1987). There is also a strong association between the development stages and the yield components during the pre-anthesis period (Rawson, 1971). This association is of paramount importance in determining which factors and how these factors modify or inhibit the duration of the mentioned phases. It is therefore important to investigate the differences of different types (spring, intermediate and winter wheat) of wheat and their response to frost stress.

Yield of especially irrigated wheat can significantly be influenced by the time of planting. Under dry land conditions similar responses have been reported. Furthermore, the reduction in yield associated with late planting have been attributed to moisture stress at and after anthesis which may reduce the number of kernels set or result in a lower photosynthetic area duration (Fisher & Kohn, 1966a, b, c). Other factors such as temperature and dry matter production may also influence the yield of field grown wheat. Marcellos and Single (1972) and Solfield, Evans, Cook and Wardlaw (1977) established that poor yields may be caused by higher post-anthesis temperatures which can reduce the period of grain filling and therefore kernel weight. The lower yields could also be ascribed to lower dry matter production due to late planting and ultimately a shorter growing season (Doyle & Fisher, 1979). Until now the emphasis was on late planting, but early planting can also result in a reduction of the yield. According to Single (1961) and Marcellos (1977) a major constraint to yield is the likelihood of frost damage during early spring. Both these constraints (frost damage during early spring and high temperature during late spring with associated water stress during grain filling) are features of the South African summer rainfall, wheat production areas. The length of the growing season for each cultivar is also used to determine the optimum planting date to avoid these stresses. Therefore obtaining data on the effect of frost damage to South African cultivars and under South African conditions has become vital.

The phenomenon of frost during early spring has become more important due to frequent questions being asked on the effect of frost damage on wheat. These questions were not confined to yield only but also to:

- ***?*** the reaction of different cultivars to frost damage?
- ***?*** the effect of frost damage to the quality of the grain?
- ***?*** the effect of frost damage at different growth stages on the quality of the grain?

4.2 MATERIALS and METHODS

See Chapter 3, section 3.1.1; 3.2; 3.3.1 and 3.4.

4.3 RESULTS

Plant dry matter production and yield components were used to evaluate the influence of frost stress on wheat production. The influence of different sub zero temperatures on different growth stages are presented in Figures 4.1 – 4.25. For all parameters the different cultivars were grouped together and the different cultivars were annotated as follows: Canadian winter type – C1; South African winter type – C2; South African intermediate type – C3 and South African spring type – C4. Analysis of variance for the different parameters is presented in Appendix 4.1-4.72.

Some of the parameters were fractionated in three different components (primary, secondary and total) where applicable. This was done where spike components were used. The reason for this was that the primary spike was used as indicator of the specific growth stage and therefore not all spikes were at the same stage of growth and development.

4.3.1 Dry matter

Dry matter production in above soil vegetative parts of the different cultivars was influenced significantly as shown by the interaction of temperature and growth stage (Appendix 4.1 – 4.4). Tendencies for dry matter production in vegetative plant parts differed slightly between cultivars due to different growth patterns. Cultivar 1 and cultivar 2, both winter type cultivars, reacted similarly to decreasing temperatures at the different growth stages. Figure 4.1 clearly show that there were no significant differences in dry matter production between temperatures 0 and –6°C, for all growth stages. In contrast a slight increase or stimulation of dry matter production was observed at the tillering and flag leaf stages. This phenomenon was even more evident in cultivar 1 than cultivar 2 between –3 and –6°C.

Early growth stages were more sensitive to sub zero temperatures –9 and –12°C than the later flowering and hard dough stages, with regard to total biomass (Figure 4.1 – C1 and C2). These temperatures killed growth points within the tiller and/or the flag leaf sheath and therefore growth and development in these tillers were terminated. Cultivar 3 showed a similar response at –9 and –12°C, but at –9°C the reaction was less pronounced.

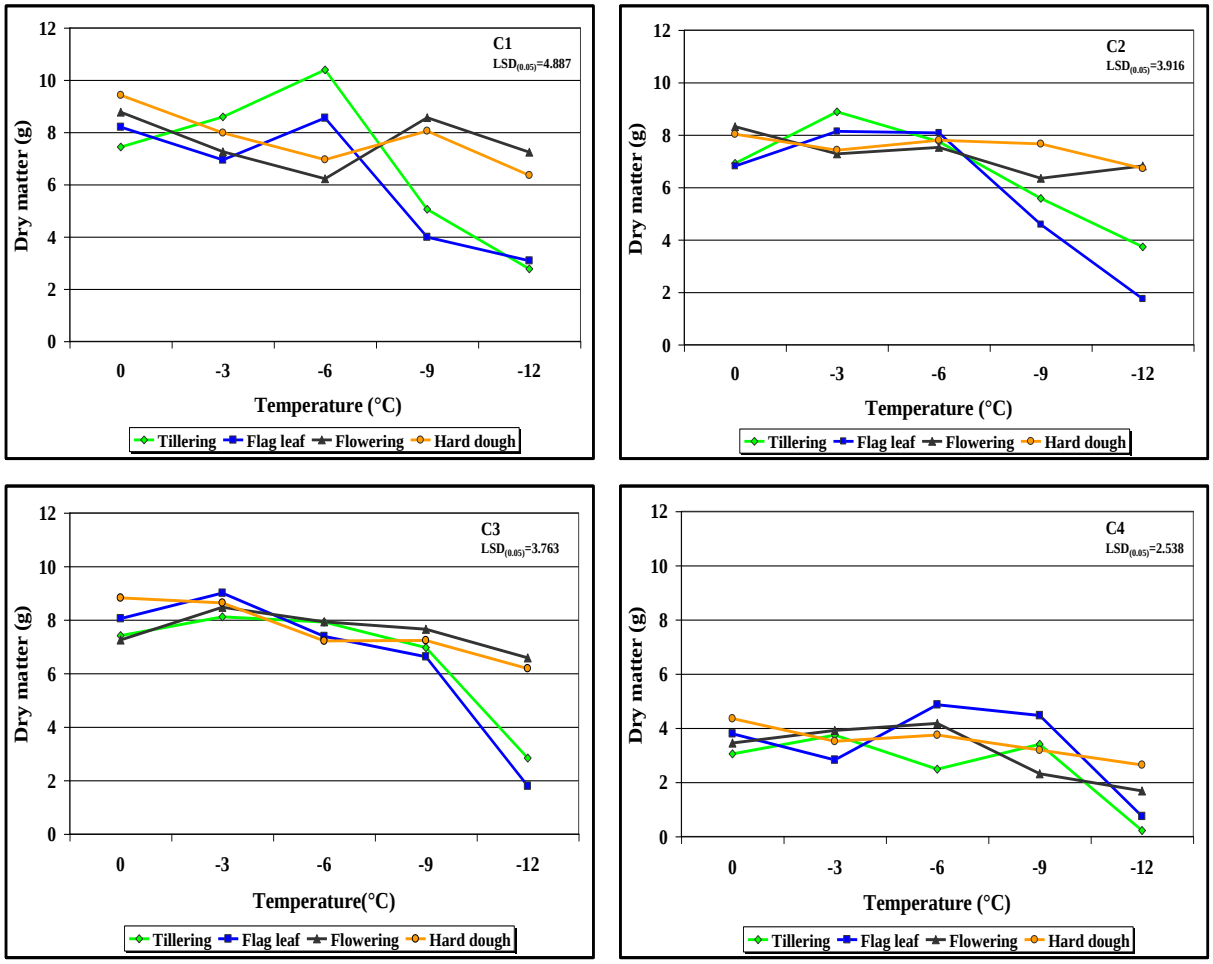


Figure 4.1 Dry matter production as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

No significant differences in dry matter production were found for cultivar 4 from 0 to –6°C for the different growth stages. However, at –12°C this cultivar showed the same tendency to decrease dry matter production as was the case in cultivar 1, 2 and 3 where the tillering

and flag leaf stages seemed to be more sensitive to frost than the flowering and hard dough stages.

In summary all cultivars showed a reduction in dry matter production at –9 and –12°C during the tillering and flag leaf stages and to a lesser extent at the flowering and hard dough stages (Figure 4.1). At the latter growth stages the plants are fully-grown and the ratio of wheat's grain to the total above ground biomass is low. Therefore, the effect of frost damage at these stages is not significant on total above ground dry matter.

4.3.2 Spikes per plant

The number of spikes per plant ultimately determines the yield. This is only true if any form of environmental stress, insects and/or diseases does not damage the spikes. The number of spikes per plant is, therefore, a handy parameter and the influence of temperature at different growth stages necessary to consider.

Appendix 4.5 – 4.8 clearly indicate a significant difference in the number of spikes per plant as a result of the interaction between different temperatures and growth stages. Figure 4.2 shows that cultivars 1 and 2 reacted similarly at the different growth stages to the decrease in temperature. The number of spikes per plant increased from –3 to –6°C with a significant drop in spike number at –9 and –12°C during the tillering and flag leaf stages. Although not significant, both cultivars showed a higher number of spikes per plant at the tillering stage than the flag leaf stage. This phenomena is ascribed to the fact that the plants are younger at the tillering stage and that the low temperatures could stimulate the initiation of more tillers as part of the compensation ability that wheat has. Cultivars 3 and 4 did not show the same tendency to increase or stimulate spike formation at –3 and –6°C (Figure 4.2 – C3 and C4), but the decrease in spike number was the same as was observed for cultivar 1 and 2, especially at –12°C.

Significant differences in the number of spikes per plant were not observed for each of the individual cultivars during the flowering and hard dough stages at the different temperatures.

This clearly indicates that the number of spikes per plant can only be influenced at temperatures below –6°C and mainly before the spikes have emerged from the flag leaf sheath.

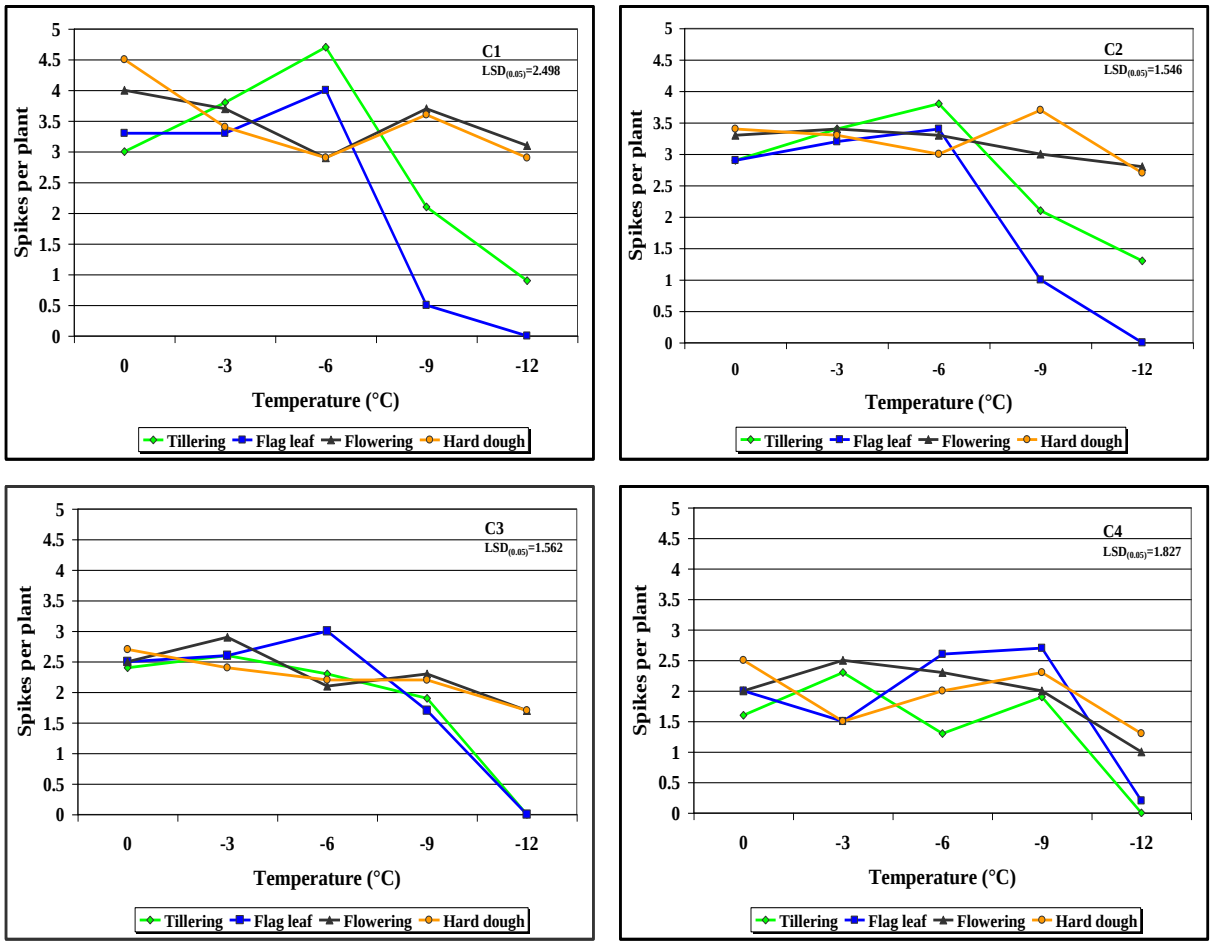


Figure 4.2 **Number of spikes per plant as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).**

The number of spikes per plant for cultivars 1 and 2 did not differ significantly between the tillering and flag leaf growth stages at temperatures of –9 and –12°C. Both these cultivars showed that they were more sensitive to frost stress during the flag leaf than the tillering growth stage. This was evidently not the case for cultivars 3 and 4 where both cultivars showed no or very small differences between the two growth stages.

4.3.3 Spikelets per spike

4.3.3.1 Spikelets per primary spike

The number of spikelets per spike is an important plant component that has an indirect influence on yield determination. Data for the number of spikelets per primary spike showed significant differences for the different cultivars as a result of the interaction between temperature and growth stage (Appendix 4.9 – 4.12).

Figure 4.3 (C1 to C4) clearly shows that the number of spikelets per primary spike was not influenced during the flowering and hard dough stages for the different cultivars. This was also expected, because all spikelets have already been formed at these stages. Bearing this in mind the discussion on the number of spikelets *per se* will be based on the tillering and flag leaf stages. During these stages the formation of the spikelets have been initiated but the spikelets could either be damaged by sub zero temperatures or primary spikes could be killed that would lead to a decline in the average number of spikelets per primary spike. The latter is usually the result of what happens in practice and therefore the explanation of the decline in the number of spikelets.

In cultivar 1 (Figure 4.3 – C1) the number of spikelets were negatively affected by sub zero temperatures below –6°C. The number of spikelets were respectively reduced by 10.61% and 47.91% at –9 and –12°C during the tillering stage compared to the average number of spikelets at 0°C for the different growth stages (control). During the flag leaf stage the number of spikelets were reduced by 81.99% at –9°C and by 100% at –12°C indicating that the flag leaf stage was more sensitive to frost stress than the tillering stage.

A similar tendency to decrease the number of spikelets was observed for cultivar 2 but the decline case was more severe during the tillering stage than was the case in cultivar 1 at –9°C (Figure 4.3 – C2). Cultivar 2 showed a decline of 30.60 and 43.22% during the tillering stage at –9 and –12°C, respectively. During the flag leaf stage the decline was 44.48 and 100% at –9 and –12°C respectively emphasizing that these two cultivars were more sensitive

to frost stress during the flag leaf stage with regard to the number of spikelets per primary spike.

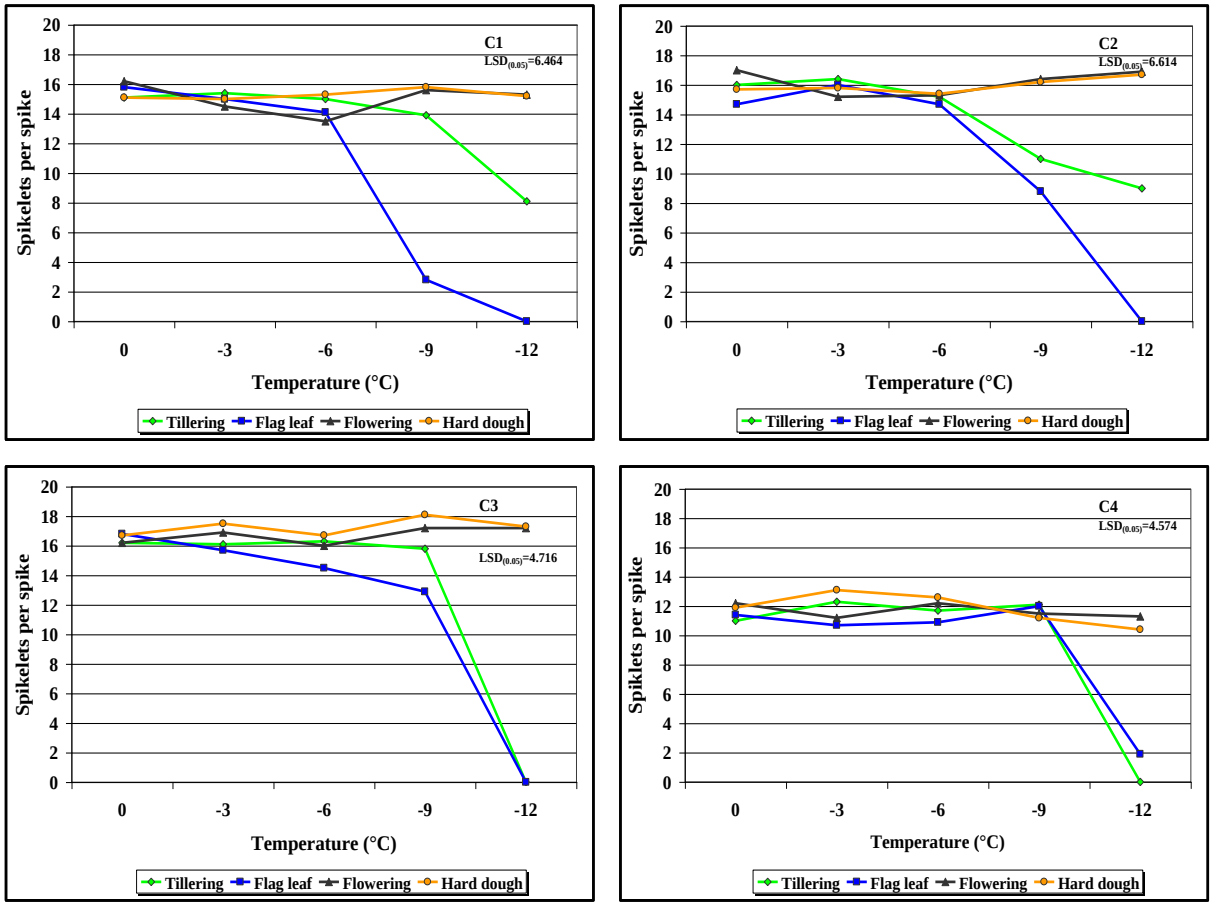


Figure 4.3 Number of spikelets per primary spike as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

Cultivar 3 (Figure 4.3 – C3), the intermediate cultivar, showed that it was more tolerant to progressive cooling in terms of the reduction in the number of spikelets per primary spike than the previously mentioned cultivars. This cultivar only experienced a reduction of 4.10% at –9°C during the tillering stage. Although the reduction in the number of spikelets was once again more severe at –9°C during the flag leaf stage (21.70% reduction), this was only significantly lower than that of the hard dough stage at the same temperature. At –12°C, during both the tillering and flag leaf growing stages, the reduction was 100%.

Contradictory to cultivars 1 – 3 cultivar 4 did not show a reduction in the number of spikelets during the tillering and flag leaf stages at –9°C, but at –12°C both the tillering and flag leaf stages showed a severe reduction in the number of spikelets. Both these stages at –12°C resulted in a significantly lower number of spikelets per primary spike than all other treatment combinations.

The number of spikelets per primary spike for the different cultivars were not significantly influenced by sub zero temperatures until –6°C, but below this temperature and specifically at –9°C, the number of spikelets per spike decreased, with even a 100% reduction at –12°C. This was seen during the tillering and the flag leaf stage, where the flag leaf stage showed the highest sensitivity to sub zero temperatures.

4.3.3.2 Spikelets per secondary spike

The number of spikelets per secondary spike differed significantly for all cultivars as shown by the interaction between temperature and growth stage (Appendix 4.13 – 16).

The number of spikelets per secondary spike, for cultivar 1 (Figure 4.4 - C1), did not differ significantly between the different treatment combinations with the exception of the treatment where the number of spikelets at –9°C during the flag leaf stage was significantly lower than at –6°C during the tillering stage. At –12°C the number of spikelets was reduced by 100% in the flag leaf stage and this was not significantly lower that that of the tillering stage at –12°C, the flag leaf stages at –6 and –9°C, the flowering stage at 0, –3 and –6°C and the hard dough stage at –6°C. The number of spikelets was reduced by 84.88% at –9°C during the flag leaf stage and this was only significantly lower than that at –6°C during the tillering stage. The increase in the number of spikelets per secondary spike for this cultivar could be ascribed to the fact that –6°C stimulated the development of new/more tillers during this early growth stage.

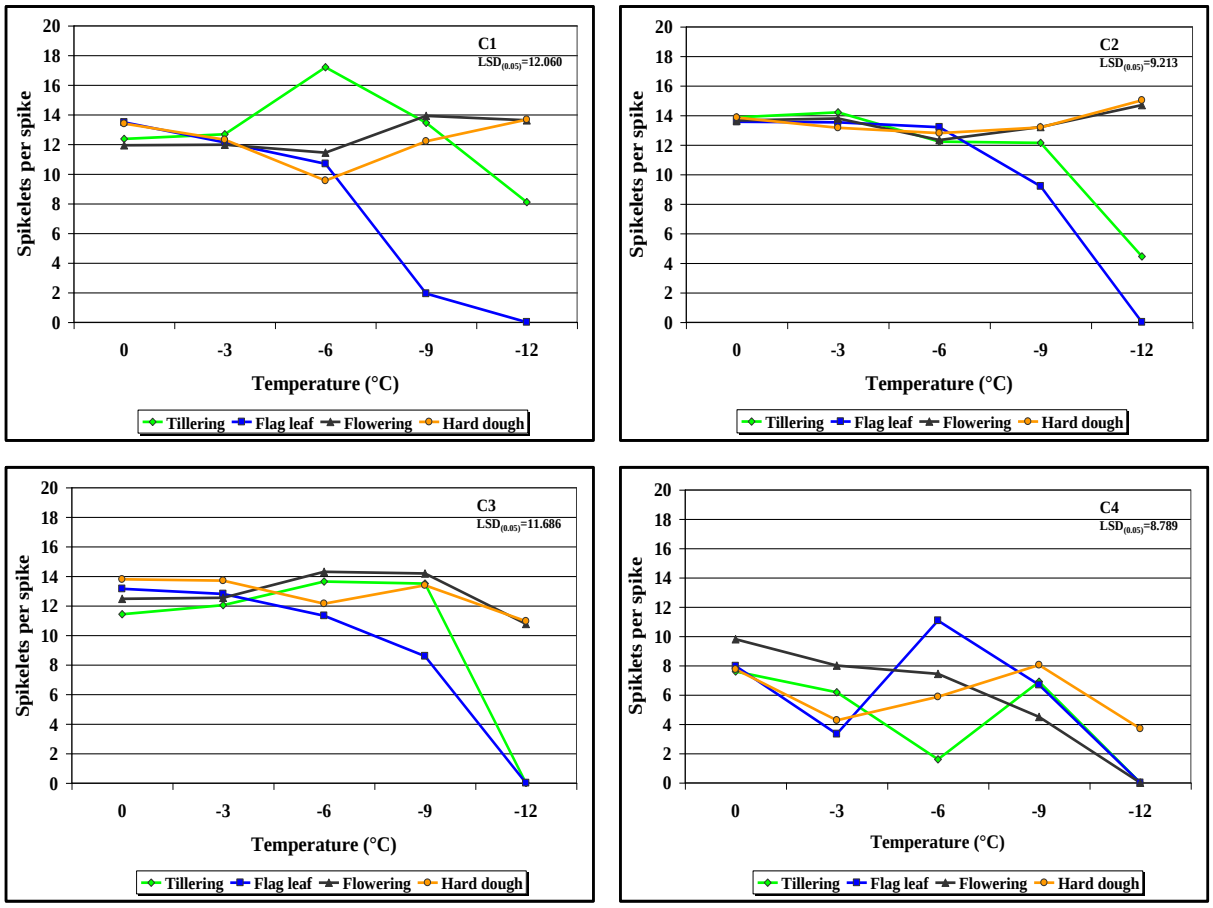


Figure 4.4 **Number of spikelets per secondary spike as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).**

The number of spikelets per secondary spike for the different growth stages of cultivar 2 at –6°C showed a slight reduction of 7.98% compared to the average of the control (0°C; (Figure 4.4 - C2). During the tillering stage, at –12°C, and the flag leaf stage at –9°C, a reduction of 67.6 and 33.01% were encountered respectively. Although a severe reduction of 33.01% was calculated it still did not differ significantly from all other combination treatments. A 100% reduction in the number of spikelets per secondary spike occurred during the flag leaf stage at –12°C. This was significantly lower for all combination treatments with the exception of the tillering stage at –12°C and the flag leaf stage at –9°C (Appendix 4.14).

Cultivar 3 showed a significant reduction (100%) in the number of spikelets per secondary spike during the tillering and flag leaf stages at –12°C (Figure 4.4 - C3). Results obtained from cultivar 4 (Figure 4.4 – C4) did not show the same tendencies than that obtained from the previously discussed cultivars. Therefore, the results for the different cultivars during different growth stages at different temperatures showed that the number of spikelets per secondary spike was mainly reduced during the tillering and more importantly the flag leaf stage. This was found at –12°C and to a lesser degree at –9°C.

4.3.3.3 Average number of spikelets per spike

Significant differences were obtained for the average number of spikelets per spike dually affected by the interaction of different temperature and different growth stages for the different cultivars (Appendix 4.17 –4.20).

Cultivar 1 (Figure 4.5 - C1) showed that the flag leaf growth stage was the most sensitive and that –9 and –12°C resulted in a reduction of 78.15 and 100% respectively for the mentioned growth stage. Cultivar 2, also a winter type, showed that only –12°C during the flag leaf stage had a significant reducing effect on the average number of spikelets per spike (Figure 4.5 – C2).

Cultivars 3 and 4 (Figure 4.5 – C3 and C4) showed similar reactions to the applied stress temperatures during the different growth stages. The only difference was between the flag leaf stage of these cultivars where cultivar 4 showed a reduction of 82.38% in the average number of spikelets per spike and cultivar 3 a reduction of 100%. A 100% reduction was observed for both cultivars during the tillering stage at –12°C and this was significantly lower than for all other treatment combinations accept that of the flag leaf stage at –12°C.

The number of spikelets per spike (primary, secondary and average) clearly showed that the tillering and flag leaf stages were more sensitive than the flowering and hard dough growth stages. The flag leaf stage seemed to be the most sensitive to sub zero temperatures.

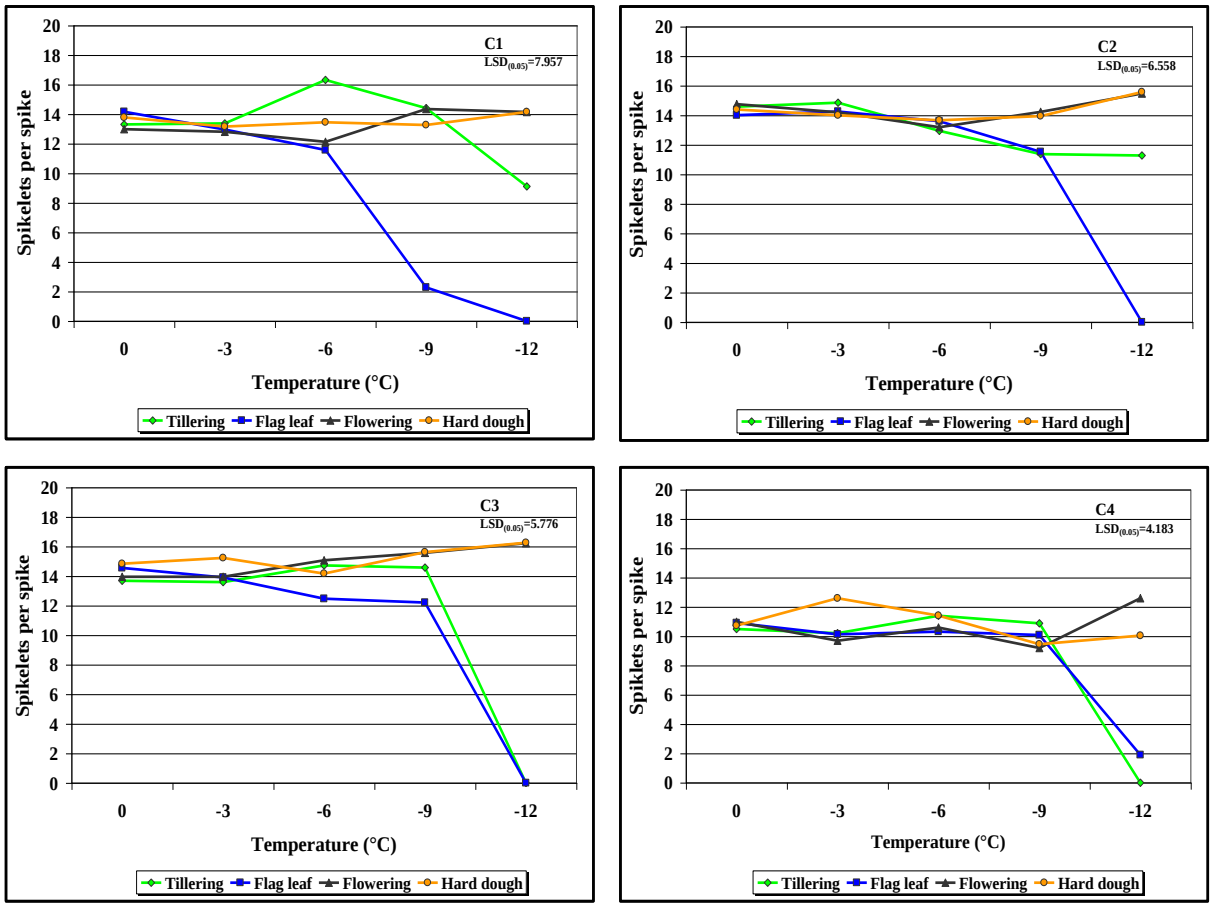


Figure 4.5 **Average number of spikelets per spike as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).**

Growth stage played a major role, but equally important was the influence of temperature and that is the reason for significant differences obtained through the interaction of these two main effects. The number of spikelets per primary spike clearly showed that temperatures below –6°C had an inhibiting effect. This was less evident at –9°C, during the tillering stage of the different cultivars for the number of spikelets per secondary spike.

4.3.4 Kernel count

It was envisaged that the number of kernels produced by the primary and secondary spikes could provide an indication of the effect of sub zero temperatures at different growth stages as well as the reaction of the different cultivars to the main treatments and/or treatment combinations with regard to seed set.

4.3.4.1 Number of kernels produced by primary spikes

The number of kernels produced by the primary spikes showed significant differences for the treatment combinations for the different cultivars (Appendix 4.21-4.24). In cultivar 1 (Figure 4.6 – C1) there were no significant differences between the number of kernels for all treatment combinations with the exception of the flag leaf stage at –9 and –12°C. These treatments resulted in a significantly lower number of kernels with a reduction of 88.15 and 100% respectively. Although not significant, a similar tendency to reduce the kernel number during the tillering stage as was the case for the flag leaf stage at the mentioned temperatures, was observed.

Cultivar 2 showed no reduction in the number of kernels at the different growth stages until the temperature decreased below –6°C. A reduction of 21.11% was the result at –9°C during the tillering stage, but still this was not significantly lower than the kernel count during the different growth stages at 0, –3 and –6°C. With a decrease in temperature to –12°C during this period, the kernel count reduction was 44.41%. This was significantly lower than that at 0°C during the flowering stage, –3°C during the flag leaf stage and –6°C during the flag leaf and hard dough stages, but significantly higher than the kernel count at –12°C during the flag leaf growth stage. The reduction in the kernel count was more severe during the flag leaf stage with a reduction of 54.47 and 100% at –9 and –12°C respectively. The latter two treatments did not contribute to significant differences in the kernel count (Figure 4.6 – C2).

Cultivar 3 showed no significant differences in the number of kernels produced between the different growth stages with a decline in temperature until –6°C, including –9 and –12°C, for the flowering and hard dough stages (Appendix 4.23). A slight increase in the number of

kernels produced was encountered from 0 to –6°C during the tillering and flowering stages (Figure 4.6 – C3). From this point onwards the number of kernels decreased with a decline in the temperature where the tillering stage seemed to be more sensitive than the flowering stage. The flag leaf stage was the most sensitive in this cultivar and a decrease in the number of kernels was encountered from –6°C. Significant decreases in the number of kernels produced occurred at –9°C during the flag leaf stage (36.89%) and –12°C during the tillering and flag leaf stages (100%). The number of kernels at –9°C during the flag leaf stage was significantly lower than that at –3°C during the flowering and hard dough stages, –6°C during the tillering and flowering stages, –9°C during the flowering and hard dough stages and –12°C during the hard dough stage. The number of kernels produced during the tillering and flag leaf stages at –12°C were significantly lower than all other treatment combinations.

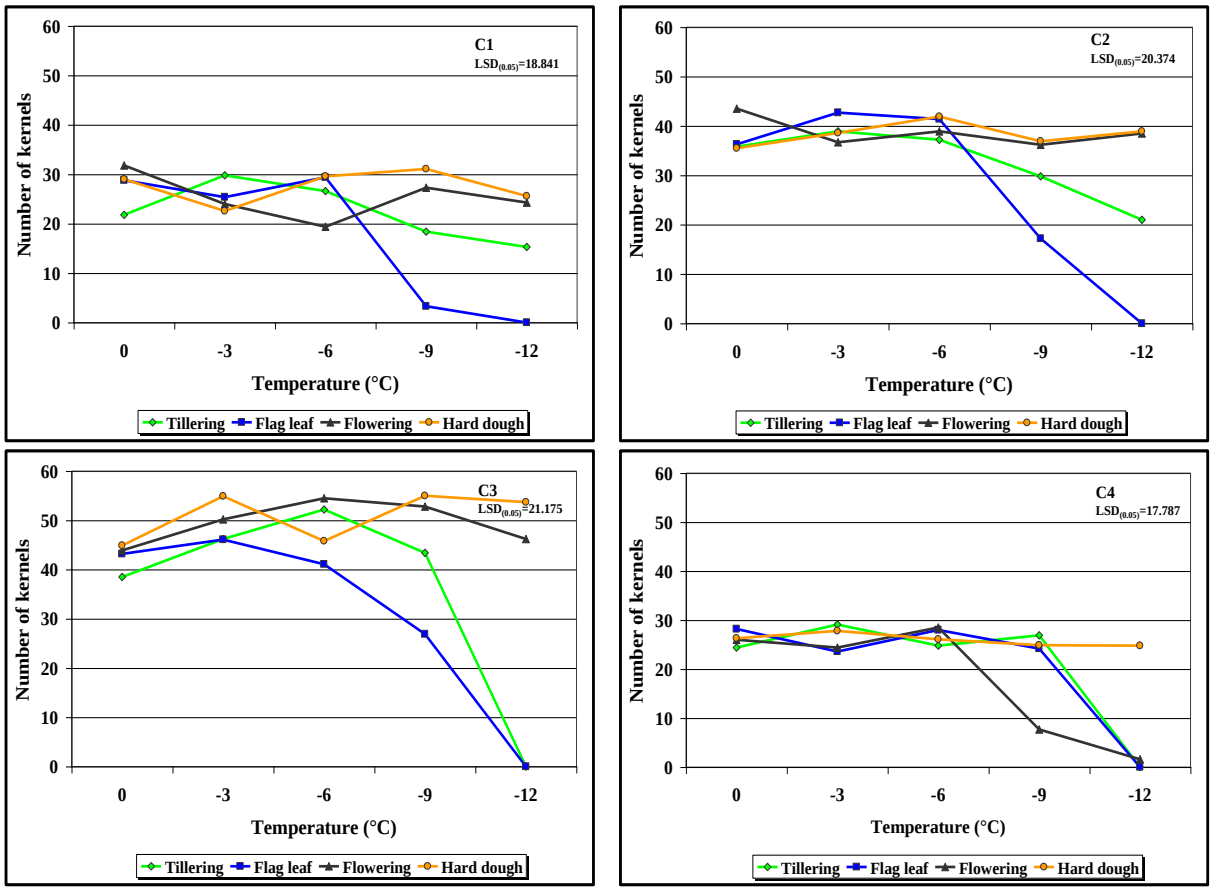


Figure 4.6 **Number of primary kernels as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).**

Although cultivar 4 showed significant differences in the number of kernels produced (Appendix 4.24) it differed from the other cultivars in reaction to the treatment combinations. No differences were observed up to –9°C for the different growth stages with the exception of the flowering stage at –9°C (81.94% reduction). The number of kernels produced was significantly lower at –12°C during the tillering (100% reduction), flag leaf (100% reduction) and flowering (96.25%) stages compared to other treatment combinations with the exception of the flowering stage at –9°C.

From the above it is clear that the tillering and flowering stage were more sensitive to sub zero temperatures for cultivars 1 – 3 and that a reduction in the number of kernels produced occurred at temperatures below –6°C, with the exception of the flag leaf stage of cultivar 3. Cultivar 4 reacted differently from the other cultivars at –9°C where the flowering stage was the most sensitive.

4.3.4.2 Number of kernels produced by secondary spikes

Significant differences in the number of kernels produced by the secondary spikes were found for the different treatment combinations of cultivars 1 and 2 (Appendix 4.25 and 4.26). Cultivars 3 and 4 showed significant differences in the number of kernels produced for the main effect of temperature (Appendix 4.27 and 4.28).

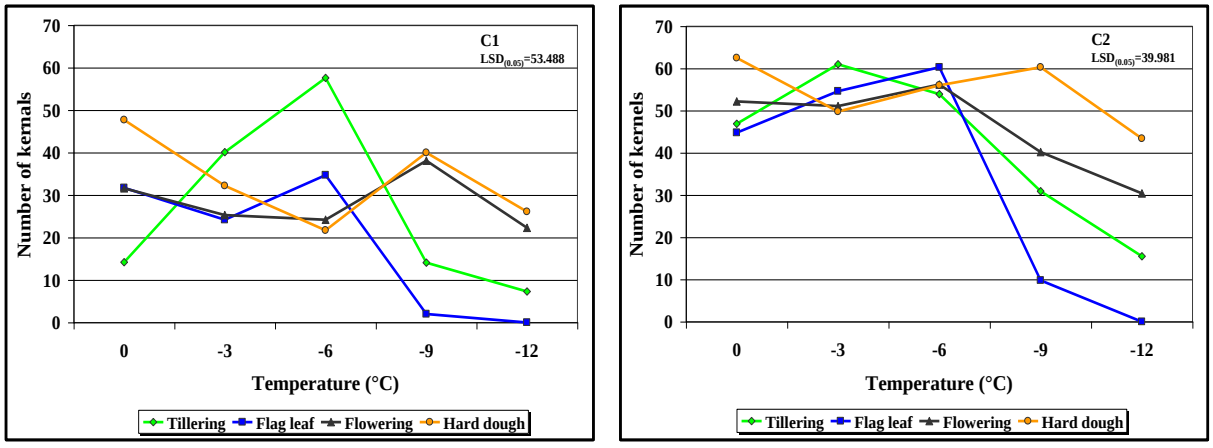


Figure 4.7 Number of secondary kernels as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type).

The only differences in the number of kernels produced by the secondary spikes of cultivar 1 we observed during the flag leaf stage at –9 and –12°C which were significantly lower than that of the tillering stage at –6°C (Figure 4.7 – C1).

Cultivar 2 showed significant differences in the reduction of the number of kernels per secondary spikes. No negative effects and/or differences occurred until –6°C. Temperatures below –6°C had a negative effect on the number of kernels produced during all growth stages with the exception of the hard dough stage at –9°C. Although not significant, the number of kernels decreased during the tillering stage at –9°C (40.12%) and the flowering stage at –9°C (22.09%) and –12°C (41.09%). No significant differences occurred between the tillering and flag leaf stages at –9 and –12°C or the flowering stage at –12°C. Once more the tillering and flag leaf stages seemed to be more sensitive, followed by the flowering stage, with the flag leaf stage being the most sensitive growth stage. The reduction in the number of kernels during the tillering stage at –12°C and the flag leaf stage at –9 and –12°C were 69.96, 81.01 and a 100% respectively (Figure 4.7 – C2).

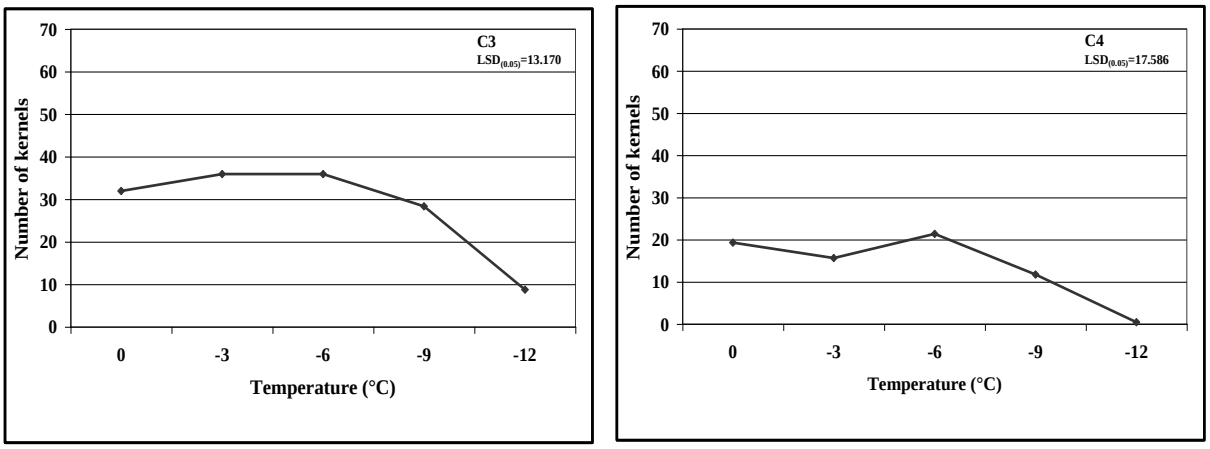


Figure 4.8 Number of secondary kernels as affected by different temperatures for different cultivars (C3 - intermediate type and C4 – spring type).

Both cultivars 3 and 4 showed a reduction in the number of kernels produced at temperatures below –6°C. In cultivar 3 the reduction was only significantly lower at –12°C (72.61%) and the reduction for cultivar 4 was also significantly lower at –12°C (97.80%) but this was not significantly lower than at –9°C (Figure 4.8 – C3 and C4).

Although cultivars 3 and 4 did not reacted the same as cultivars 1 and 2, it is evident that temperatures below –6°C had a negative effect on the number of kernels produced by the secondary spike. In cultivars 1 and 2 the tillering and flag leaf stages were more sensitive than the flowering and hard dough stages, while in cultivar 2 the flowering stage was more sensitive to treatments at sub zero temperatures in terms of the number of kernels produced by secondary spikes.

4.3.4.3 Total number of kernels produced by primary and secondary spikes

Calculation of the total number of kernels produced by primary and secondary spikes revealed significant differences between the different treatment combinations for the different cultivars (Appendix 4.29-4.32).

In cultivar 1 (Figure 4.9 – C1) the total number of kernels produced by the primary and secondary spikes for the different growth stages at 0°C were determined at 59.15. Using this as a reference, the number of kernels decreased slightly for the different cultivars at –6°C, with the exception of the tillering stage at –3°C and the tillering and flag leaf stages at –6°C, but there were no significant differences. Below –6°C a marked reduction was encountered during the tillering, and to a large extent, the flag leaf stage. The tillering stage showed a reduction of 45.05 and 61.79% at the –9 and –12°C, respectively. The only significant reduction was that of the flag leaf stage at –9 and –12°C, which were 91.04 and a 100% respectively. The results from both these treatments differed significantly from that of the hard dough at 0°C, the tillering stage at –3 and –6°C and the hard dough stage at –9°C.

No significant differences and/or reductions were found between the different growth stages at 0, –3 and –6°C. Exposure to temperatures lower than –6°C had a slight to severe negative effect of the number of kernels produced by cultivar 2 (Figure 4.9 – C2). The tillering and flag leaf stages showed the largest reduction of 32.08 and 59.16% at –9 and –12°C respectively for the tillering stage and 69.79 and 100% at –9 and –12°C, respectively, for the flag leaf stage.

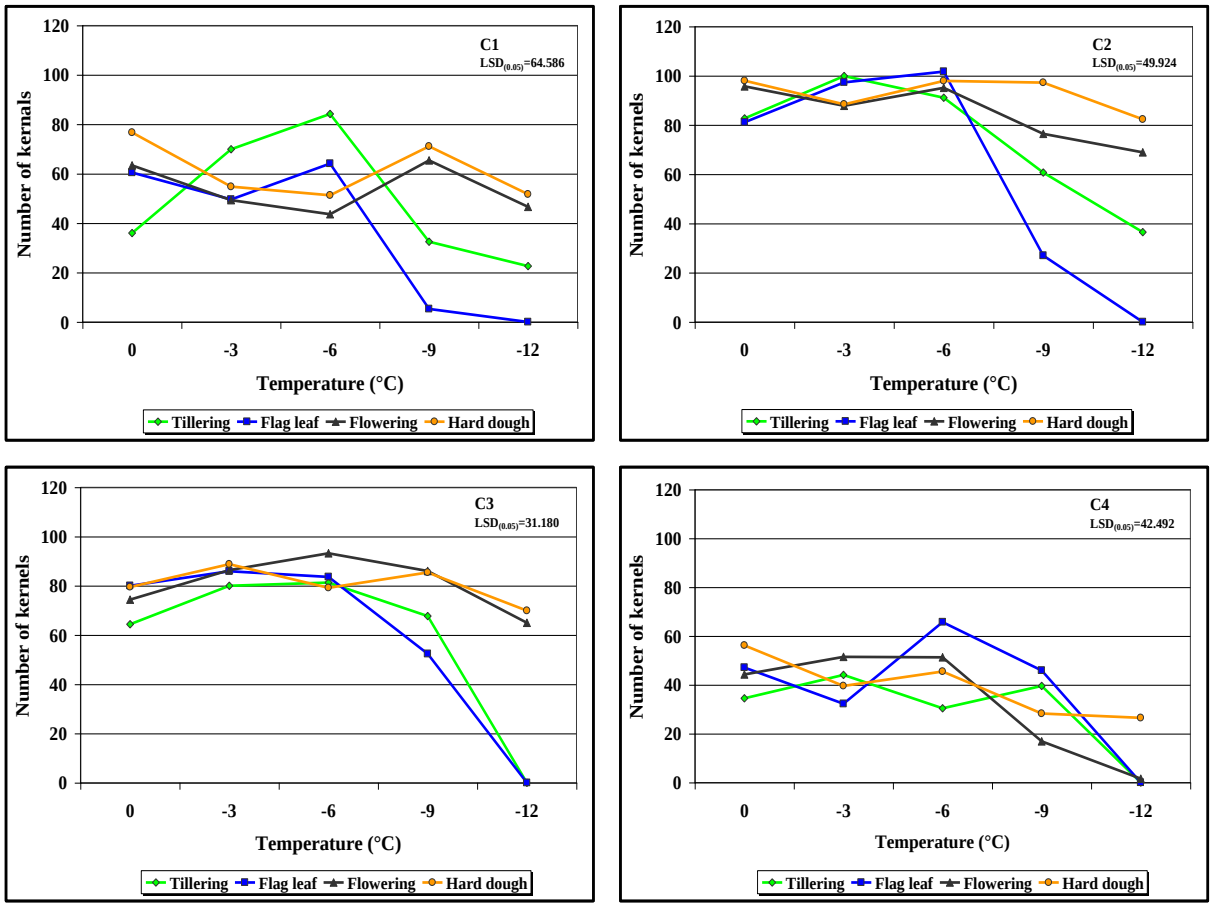


Figure 4.9 **Total number of kernels as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).**

In cultivar 3 the reduction in the total number of kernels was significant at the tillering and the flag leaf stages when exposed to –12°C. The only other significant difference was observed at the tillering stage at –3°C (significantly higher) and the flag leaf stage at –9°C (Figure 4.9 – C3). Cultivar 3 showed a slight increase in the total number of kernels from 0 to –6°C after which a slight to severe reduction occurred.

Cultivar 4 also did not show a reduction in the total number of kernels produced by the primary and secondary spikes when the temperature was decreased to –6°C. A slight reduction was encountered at –9°C but only at –12°C the reduction was significant for the tillering, flag leaf and flowering stages.

The decrease in the number of kernels (primary, secondary and total) clearly showed that the tillering and flag leaf stages were more sensitive than the flowering and hard dough growth stages while the flag leaf stage seemed to be most sensitive to sub zero temperatures. However, in cultivar 3 the flowering stage was also effected by temperatures lower than –6°C.

For this it is clear that growth stage and the degree of low temperature exposure as well as the interaction between these two main factors played a major role in determining the degree of frost damage in terms of kernel number.

4.3.4.4 Contribution of the primary kernel number to the total number of kernels produced

Wheat plants were exposed to different stress temperatures at different growth stages. These growth stages were determined primarily on the basis of development of the primary spike in order to distinguish between different stages of development of the primary spike and later maturing (secondary) spikes. Subsequently, the influence of temperature and growth stage on the contribution of the primary spike's kernel production to that of the total kernel production was investigated for the different cultivars. The data are presented in Tables 4.1 – 4.4 and expressed as a percentage of the total production.

The results in Tables 4.1 – 4.4 did not reveal clear tendencies for the treatment combinations but some tendencies came to the fore with regard to the main effects namely growth stage and temperature.

Table 4.1: Contribution (%) of the primary kernels to the total number of kernels produced for cultivar 1 (winter type)

Growth stage	Temperature (°C)					
	0	**-3**	**-6**	**-9**	**-12**	***Average***
Tillering	60.6	42.6	31.6	58.4	67.7	***52.2***
Flag leaf	47.6	51.2	45.9	62.3	0.0	***41.4***
Flowering	50.2	48.7	44.5	41.7	52.2	***47.5***
Hard dough	37.8	41.2	57.7	43.7	49.5	***46.0***
Average	***49.0***	***45.9***	***44.9***	***51.5***	***42.4***	

Table 4.1 showed variation in the contribution of the primary spike to the total kernel production at differing temperatures and growth stages and this phenomenon was similar to that of the main effect, temperature. The flag leaf stage seemed to be extremely sensitive at –12°C as the growth point was totally destroyed. Growth stage as a main effect showed that the contribution of the primary spikes to total kernel production lowered with progressive development of the growth point to the flag leaf stage. The contribution at the flowering and hard dough stages slightly less than the tillering stage but still higher than that of the flag leaf stage, showing that the flag leaf stage was the most sensitive growth stage.

Table 4.2: Contribution (%) of the primary kernels to the total number of kernels produced for cultivar 2 (winter type)

Growth stage	Temperature (°C)					
	0	**-3**	**-6**	**-9**	**-12**	***Average***
Tillering	43.3	38.9	40.8	49.1	57.5	***45.9***
Flag leaf	44.8	43.9	40.7	63.7	0.0	***38.6***
Flowering	45.5	41.8	40.9	47.4	55.9	***46.3***
Hard dough	36.2	43.7	42.8	38.0	47.3	***41.6***
Average	***42.4***	***42.1***	***41.3***	***49.5***	***40.2***	

The tendencies observed in cultivar 2 (Table 4.2) correspond to that of cultivar 1. Below –6°C a higher contribution was observed at the different growth stages with the exception of the flag leaf stage at –12°C and the hard dough stage at –9°C. With regard to temperature and growth stages as main effects the same results were obtained as for cultivar 1.

Table 4.3: Contribution (%) of the primary kernels to the total number of kernels produced for cultivar 3 (intermediate type)

Growth stage	Temperature (°C)					
	0	**-3**	**-6**	**-9**	**-12**	***Average***
Tillering	59.8	57.8	64.2	64.1	0.0	***49.2***
Flag leaf	54.0	53.7	49.2	51.3	0.0	***41.6***
Flowering	59.0	58.1	58.5	61.4	71.1	***61.6***
Hard dough	56.7	61.8	57.8	64.4	76.8	***63.5***
Average	***57.3***	***57.8***	***57.4***	***60.3***	***37.0***	

Table 4.3 showed that there were no differences in the contribution of the primary spike kernel production to that of the total kernel production from 0 to –9°C. At –12°C the primary growth point was totally destroyed at the tillering and flowering stages. A higher contribution was obtained at the flowering and hard dough stages at –9 and –12°C than at 0 to –6°C. The reason for this is that the secondary spikes are at this stage more sensitive to frost damage than the primary spike and therefore has a low contribution to the total number of kernels produced. This phenomenon was also observed for growth stage as a main factor.

Table 4.4: Contribution (%) of the primary kernels to the total number of kernels produced for cultivar 4 (spring type)

Growth stage	Temperature (°C)					
	0	**-3**	**-6**	**-9**	**-12**	***Average***
Tillering	70.7	66.0	81.6	67.9	0.0	***57.2***
Flag leaf	59.9	73.1	42.6	52.7	0.0	***45.7***
Flowering	58.7	47.4	55.6	45.6	100	***61.4***
Hard dough	46.7	70.2	57.4	88.0	98.6	***71.2***
Average	***59.0***	***64.2***	***59.3***	***63.6***	***49.7***	

Cultivar 4 (Table 4.4) a spring type cultivar with a low tillering ability showed no consistency in its reaction to the treatment combinations and/or its reaction to the main effects (temperature and growth stage). As a result of the low tillering ability the contribution by the primary spikes was higher compared to previously discussed cultivars.

The winter type cultivars reacted similarly to the applied treatments. No consistency was to be found between the different types of cultivars in their reaction to the subjected treatments. This inconsistency could be ascribed to the different cultivar types growth abilities, especially their compensation ability with regard to tillering.

4.3.5 Number of kernels per spike

It was envisaged that the number of kernels produced by the primary and secondary spikes could provide an indication of the effect of sub zero temperatures at different growth stages and the reaction of the different cultivars to the main treatments and/or treatment

combinations with regard to seed set. This could potentially further provide data to be used for determining the effect of stress temperatures on the different spikes, as all spikes were not at the same stage of development when exposed to low temperature stress.

4.3.5.1 Number of kernels produced per primary spike

Indeed the number of kernels produced per primary spike revealed significant differences for the treatment combinations and for the different cultivars (Appendix 4.21 – 4.24). The data has already been presented in Figure 4.6 and discussed (see section 4.3.3.1). However, only one primary spike is formed while this is not true for secondary spikes and therefore the number of kernels per secondary spike will be discussed comprehensively.

4.3.5.2 Number of kernels per secondary spike

Changes in the number of kernels per secondary spike did not show similar tendencies under the influence of different treatment combinations (Appendix 4.33 – 4.36). In cultivar 1 a decrease in the number of kernels per secondary spike was observed during the flag leaf stage at –9 and –12°C, but this did not differ significantly from the other treatment combinations (Figure 4.10 – C1). Both the main effects (growth stage and temperature) did not have any significant effect on the number of kernels per secondary spike for cultivar 1.

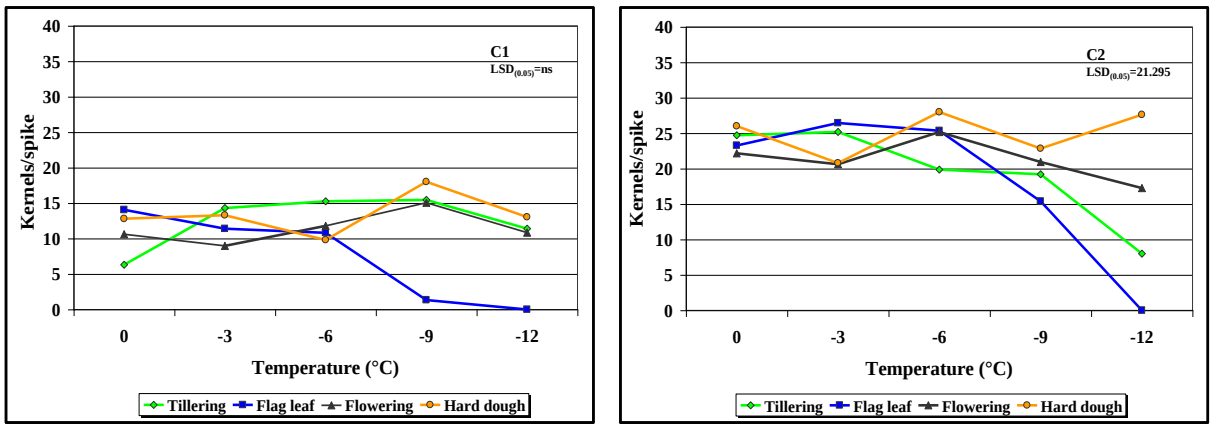


Figure 4.10 Number of kernels per secondary spike as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type).

The number of kernels produced per secondary spike in cultivar 2 differed significantly under the influence of different treatment combinations (Appendix 4.34). Figure 4.10 – C2 clearly shows that the treatment combination had no negative effect until –6°C. Below –6°C there was a slight decrease in the number of kernels per spike for all the growth stages although not significant. This decrease was more prominent for the flag leaf stage at –9°C (35.95%). At –12°C the effect was more severe with the exception of the hard dough stage that showed no negative effect. The only significant reduction was that of the flag leaf stage at –12°C (100%) and it differed from the tillering stage at 0 and –3°C, the flag leaf stage at 0, –3 and –6°C, the flowering stage at 0 and –6°C and the hard dough stage at 0, –6, –9 and –12°C.

In cultivar 3 the treatment combinations did not lead to significant differences in the number of kernels produced per secondary spike, but when analysed separately, both the main effects showed significant differences (Appendix 4.35). The F-test showed that the number of kernels per secondary spike was significantly ($P < 0.05$) reduced during the tillering stage. The differences due to growth stage was, however, not significant when compared by means of Tukey's test at the 5% significant level. Further, the number of kernels was least affected during the hard dough and flowering stages followed by the flag leaf stage and then the tillering stage (Figure 4.11).

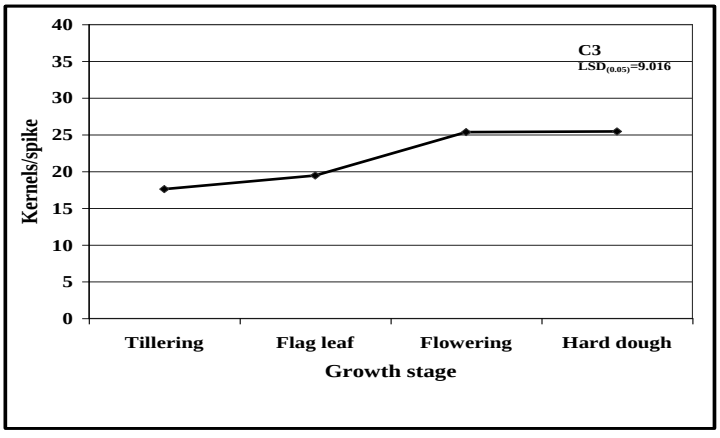


Figure 4.11 Number of kernels per secondary spike as affected by growth stage.

Temperature had a significant influence on the number of kernels per secondary spike for cultivar 3 (Figure 4.12). The number of kernels increased slightly as temperature was decreased to –6°C after which a significant reduction in the number of kernels per secondary spike occurred with a further decrease in temperature to –12°C (Appendix 4. 35).

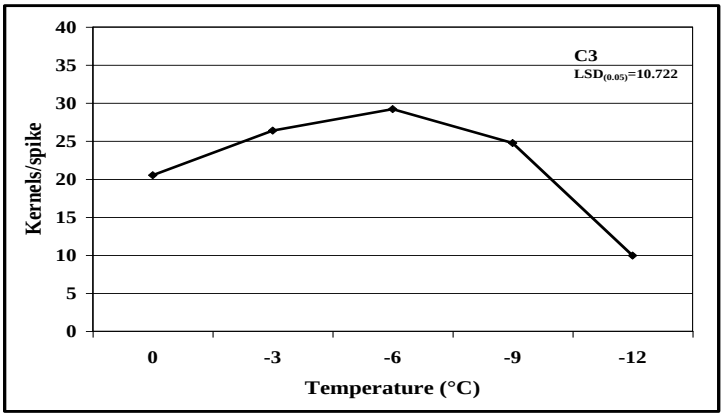


Figure 4.12 Number of kernels per secondary spike as affected by temperature.

In cultivar 4 a decrease in temperature led to a reduction in the number of kernels, but this was only significant at –9°C (53.73%) and at –12°C (100%; Figure 4.13; Appendix 4.36). The number of kernels per secondary spike at –12°C differed significantly from all other temperatures while that at –9°C was significantly lower than at 0°C but simultaneously significantly higher than at –12°C.

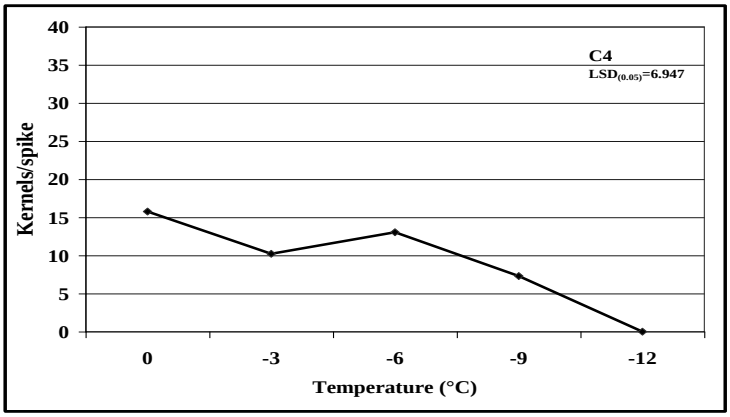


Figure 4.13 Number of kernels per secondary spike as affected by temperature.

4.3.5.3 Average number of kernels produced per spike

Results of the average number of kernels per spike, including primary and secondary spikes, are presented in Figure 4.14. Growth stages and temperature interactions, were responsible for significant differences in the number of kernels per spike for different cultivars (Appendix 4.37 – 4.40).

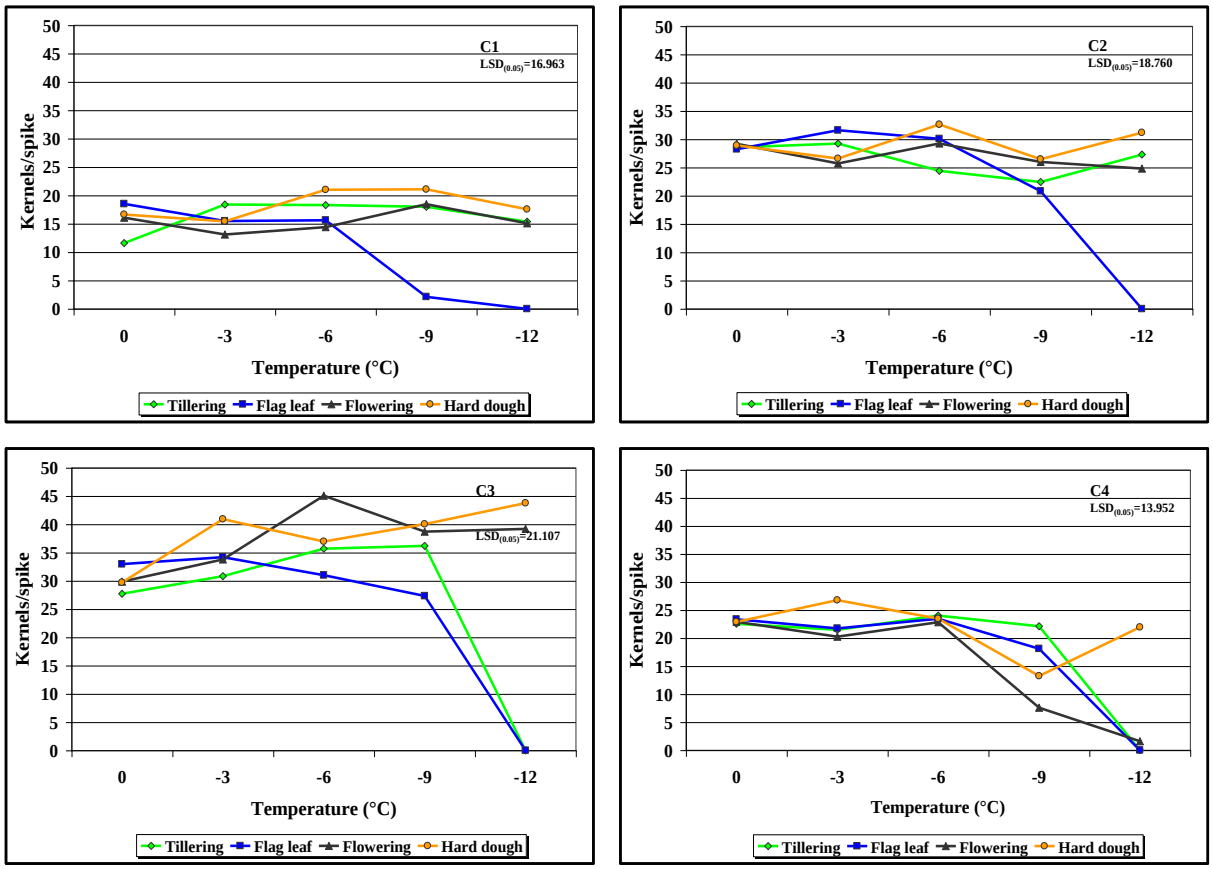


Figure 4.14 Average number of kernels per spike as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

In cultivar 1 no differences in the average number of kernels per spike were observed between the different treatment combinations, with the exception of significant reductions in the flag leaf stage at –9 (86.51%) and –12°C (100%). Statistically, these results did not differ from each other, but the number of kernels per spike was significantly lower at –9°C during the flag leaf stage compared to the hard dough stage at –6 and –9°C. The average number of

kernels per spike at –12°C during the flag leaf stage was also significantly lower than that of the tillering stage at –3, –6 and –9°C, the flag leaf stage at 0°C and the hard dough stage at –6, –9 and –12°C (Figure 4.14 – C1). In cultivar 2 (Figure 4.14 – C2) exactly the same tendency was observed except that the reduction in kernel number was not as marked at –9°C as was the case in cultivar 1.

Cultivar 3 reacted similarly to cold treatment than cultivar 2 with the exception that there was a reduction in the average number of kernels per spike at –12°C during both the tillering and flag leaf stages. This indicates that the tillering stage of cultivar 3 was more sensitive than that of the previously mentioned cultivars (Figure 4.14 –C).

Cultivar 4 showed no differences and or reductions in the average number of kernels per spike during the different growth stages at a temperature range of 0 to –6°C. A slight reduction was encountered at –9°C during the different growth stages (tillering – 3.52%, flag leaf – 21.07%, flowering – 66.88% and the hard dough stage – 42.24%). Only a reduction of 66.88%, that of the flowering stage, was significantly lower than that of all growth stages at 0 and –6°C, the flag leaf and hard dough stages at –3°C, the tillering stage at –9°C and the hard dough stage at –12°C. The average number of kernels per spike produced during the tillering, flag leaf and flowering stage were significantly lower at –12°C than that of all growth stages at 0, –3 and –6°C, the tillering and flag leaf stages at –9°C and the hard dough stage at –12°C.

In summary, whether expressed per primary, secondary or average number of spikes, the reduction in kernel number under the influence of temperatures below –6°C showed that the flag leaf stage was the most sensitive growth stage in all cultivars followed by the tillering and hard dough stages in cultivar 4.

4.3.6 Kernel weight

Preceding parameters contributed to the final yield of each plant. The role it played and the effect it had are portrayed by the yield contribution of the primary and secondary spikes.

Therefore the discussion from hereon will focus mainly on the kernel weight of the primary and secondary spikes and its contribution to the average kernel weight produced by every individual plant as effected by the treatments. The reason for also including kernel weight additional to kernel number as a parameter was to ascertain whether the effect of freezing treatment could be attributed to either damage to flowers or translocation of photosynthate assimilates during the grain filling stage or both.

4.3.6.1 Primary kernel weight

The kernel weight recorded in the primary spikes showed significant differences as a result of the treatment combinations (Appendix 4.41 – 4.44) for all cultivars (Figure 4.15).

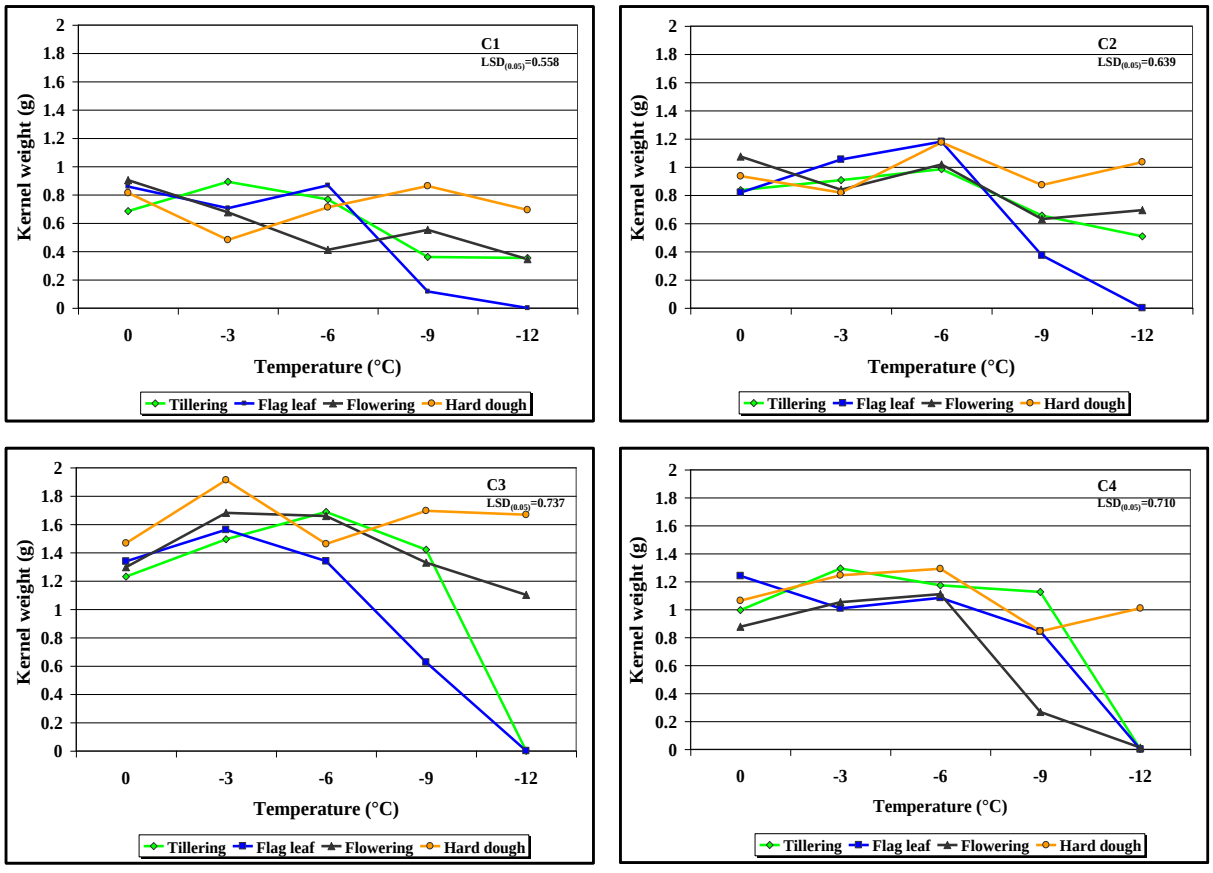


Figure 4.15 Primary kernel weight as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

In cultivar 1, with the exception of the hard dough growth stage, all growth stages were negatively affected at temperatures below –6°C (Figure 4.15 – C1). Not withstanding the fact that a decline in sub zero temperature during the tillering, flag leaf and flowering stages led to a decrease in kernel weight, only the flag leaf stage at –9 and –12°C and the flowering stage at –12°C were significantly negatively influenced. The flag leaf stage at –9 and –12°C yielded significant lower kernel weights than all growth stages at 0°C; the tillering, flag leaf and flowering stages at –3°C; the tillering, flag leaf and hard dough stages at –6°C and the hard dough stage at –9 and –12°C. In the flowering stage the kernel weight was only significantly lower at –12°C than compared to at 0°C.

In cultivar 2 (Figure 4.15 – C2) temperatures below –6°C resulted in a loss of kernel weight during the different growth stages. The growth stage least affected by this was the hard dough growth stage. The tillering and flowering stages showed losses at –9°C of 28.49 and 31.11% and at –12°C of 44.50 and 24.28%, respectively. At –12°C the reduction (100%) in kernel weight was significantly pronounced in all the different growth stages than at 0, –3 and –6°C; the tillering and hard dough stages at –9°C and the flowering and hard dough stages at –12°C. The flag leaf stage at –9°C showed a reduction of 59.28% and this was significantly lower than the flowering stage at 0°C; the flag leaf stage at –3°C; the flag leaf, flowering and hard dough stages at –6°C and the hard dough stage at –12°C. A reduction of 44.50% was calculated during the tillering stage at –12°C and this was only significantly lower than the kernel weight of the flag leaf and hard dough stages at –3°C.

In cultivar 3 a slight increase in kernel weight was observed during the different growth stages at –3°C (Figure 4.15 –C). This was followed by a marginal reduction at –6°C, with the exception of the tillering stage, and a definite reduction during the tillering, flag leaf and flowering stages at –9 and –12°C. The flowering stage at –12°C showed a reduction of 17.40% which was significantly lower than the kernel weight of the hard dough stage at –3°C. The flag leaf stage at –9°C showed a reduction of 53.11% while the tillering and flag leaf stages, at –12°C, showed a reduction of 100%. These results at –12°C were significantly lower than the kernel weight obtained during the different growth stage at 0, –3 and –6°C;

the tillering flowering and hard dough stages at –9°C and the flowering and hard dough stages at –12°C.

In cultivar 4 no yield losses during the different growth stages at temperatures of 0, –3 and –6°C were observed. At –9°C the loss in kernel weight was marked, especially during the flowering stage (74.41% reduction) (Figure 4.15 – C4). At –12°C the tillering, flag leaf and flowering stages showed a kernel weight loss of 100%. This was significantly lower in the latter growth stage at 0, –3 and –6°C, the tillering, flag leaf and hard dough stages at –9°C and the hard dough stage at –12°C. The flowering stage at –9°C did not differ significantly from the flowering stage at 0°C, the flag leaf and hard dough stage at –9°C and the tillering, flag leaf and flowering stage at –12°C, but was significantly lower in kernel weight than the remaining treatment combinations.

Primary kernel weight measurements revealed that the different growth stages were negatively and severely affected when the temperature was decreased, especially below –6°C. Previous parameters showed that the flag leaf stage was more sensitive to sub zero temperatures than other growth stages followed by the tillering stage. Kernel weight measurements showed that the flowering stage was slightly more sensitive than the tillering stage and even more sensitive than the flag leaf stage of cultivar 4.

4.3.6.2 Secondary kernel weight

Only the main effect, temperature, was responsible for significant differences in the secondary kernel weight for different cultivars while growth stages was responsible for significant differences in cultivar 2 (Appendix 4.45 – 4.48).

In cultivar 1 a significant reduction in kernel weight was observed at –12°C and this was only significantly lower than that at 0 to –6°C. The kernel weight obtained at –9 and –12°C for cultivar 2 was significantly lower than the kernel weight at 0 to –6°C, but it did not differ from each other. In cultivar 3 the kernel weight at –9°C was significantly lower than that at 0

to –6°C, but significantly higher than at –12°C. The kernel weight of cultivar 4 at –12°C was significantly lower than at the other temperatures (Figure 4.16 – C1, C2, C3 and C4).

Different cultivars, representing different growth types, showed similar tendencies in the reduction of the secondary kernel weight at temperatures below –6°C. The reduction at –9°C was high for cultivars 1 to 3 (47.33, 49.13 and 44.31% respectively) while that of cultivar 4 was less (32.16%). Kernel weight reduction at –12°C for cultivars 1 to 3 was 76.00, 68.34 and 84.55% respectively while cultivar 4 showed a reduction of 99.03%.

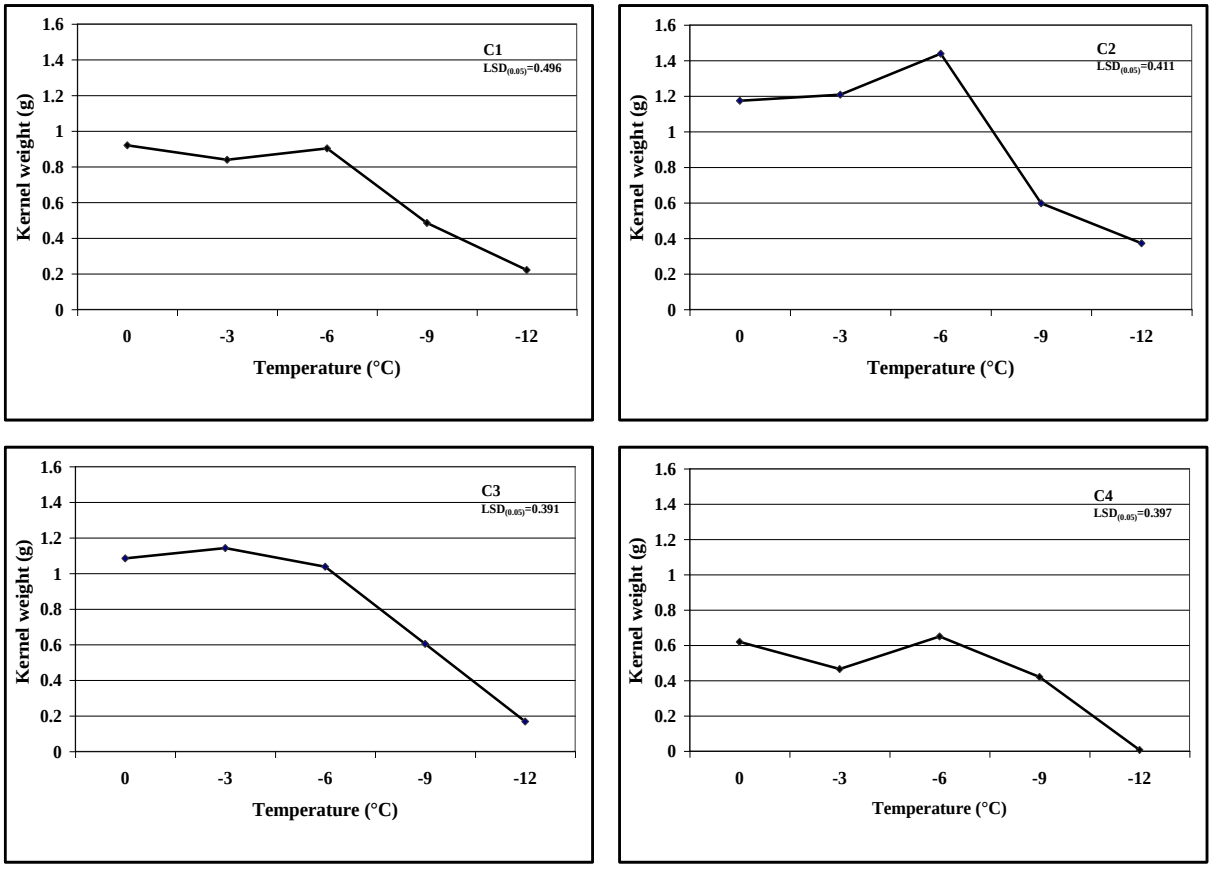


Figure 4.16 Secondary kernel weight as affected by different temperatures for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

4.3.6.3 Total kernel weight

The total kernel weight measurements, including primary and secondary spike kernels, showed significant differences as a result of the treatment combination's in all cultivars (Appendix 4.49 - 4.52 and Figure 4.17).

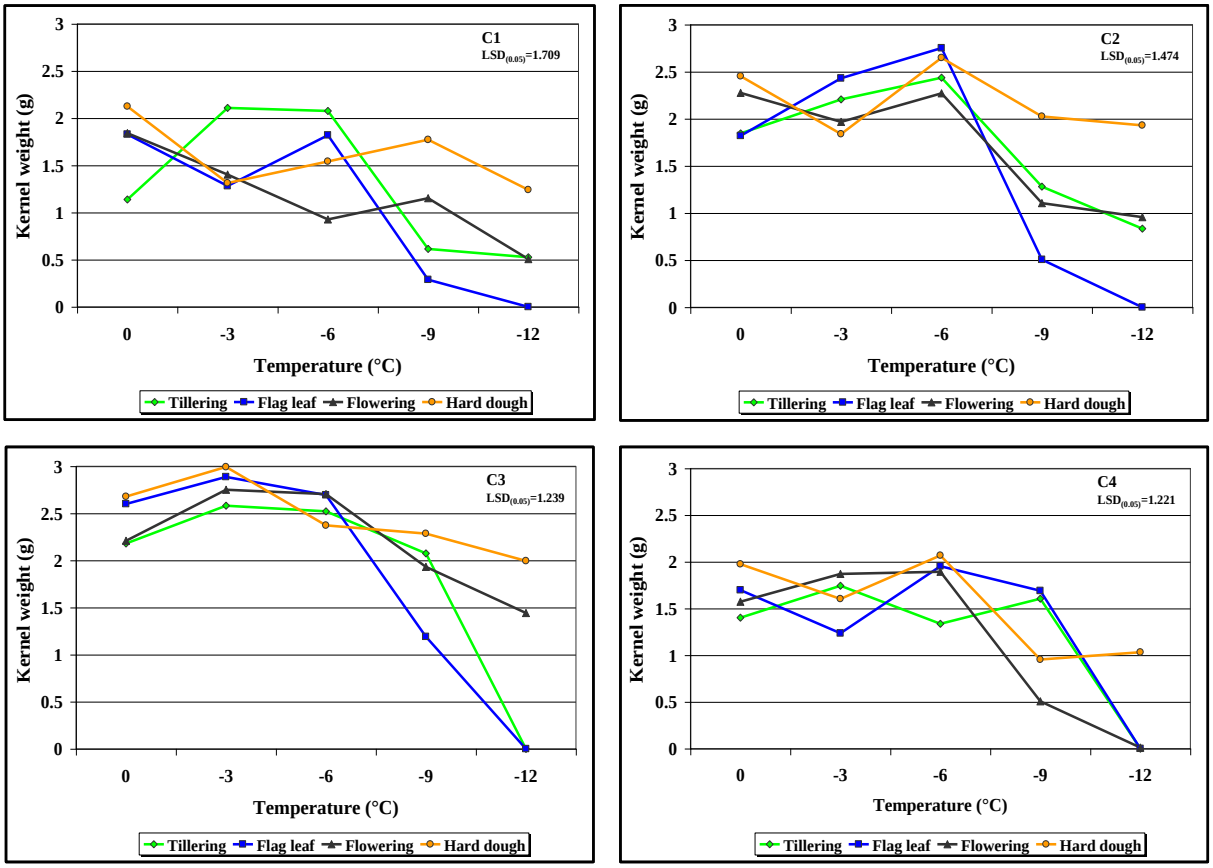


Figure 4.17 Total kernel weight as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

In cultivar 1 a respective reduction in kernel weight during the flowering stage at –3, –6 and –9°C of 46.55, 34.32 and 70.76%, was observed (Figure 4.17 – C1). The reduction in kernel weight at the tillering stage exposed to –9 and –12°C were 64.56 and 69.67% and that of the flag leaf stage 83.39 and 100.00% at –9 and –12°C, respectively. Although the reduction in kernel weight was severe at the tillering and flag leaf stages, only the flag leaf stage at –9 and

–12°C showed a more pronounced reduction compared to hard dough stage at 0°C and the tillering stage at –3 and –6°C. A 100% reduction at –12°C during the flag leaf stage was significantly lower than the flag leaf stage at 0 and –3°C, the flowering stage at 0°C and the hard dough stage at –9 °C.

In cultivar 2 no significant differences and/or reductions were observed during the different growth stages at temperatures of 0 to –6°C compared to the average of the control (i.e. 0°C). The hard dough stage showed no reduction in kernel weight, but at the tillering stage reductions of 38.69 and 60.06%, the flowering stage a reduction of 47.09 and 54.17% and the flag leaf stage a reduction of 75.78 and 100.00% at –9 and –12°C, respectively were calculated (Figure 4.17 – C2). The flowering stage at –9°C showed a significantly lower kernel weight than that of the flag leaf and hard dough stages at –6°C while this was not the case at the tillering and flowering stages. A severe reduction was observed during the flag leaf stage at –9°C and this was significantly lower than that of the flowering and hard dough stages at 0°C, the tillering and flag leaf stages at –3°C, all the stages at –6°C and the hard dough stage at –9°C. No yield was obtained when plants in the flag leaf stage was exposed to –12°C and this was significant compared to all other growth stages at 0, –3 and –6°C as well as that of the hard dough stage at –9 and –12°C.

Similarly to cultivar 2, cultivar 3 also showed a 100% reduction in kernel weight during the tillering and flag leaf stages at –12°C. This was significantly lower than the kernel weight measured for all the other treatment combinations with the exception of the flag leaf stage at –9°C. The flag leaf stage of cultivar 3 at –9°C showed a reduction of 50.71% and this was significantly lower than the kernel weight obtained during the flag leaf and hard dough stages at 0°C, the different growth stages at –3°C and the tillering, flag leaf and flowering stages at –6°C. A reduction of 40.34% in kernel weight was observed at –12°C during the flowering stage and this was significantly lower than that of the flag leaf and flowering stages at –3 and –6°C as well as the tillering and flag leaf stages at –12°C (Figure 4.17 – C3).

The tillering and flag leaf stages showed a 100% reduction at –12°C while this was 99.28% at the flowering stage (Figure 4.17 – C4). This was statistically significant compared to the

kernel weight values for all growth stages at 0, –3 and –6°C as well as that of the tillering and flag leaf stages at –9 °C. At the flowering stage and at –9°C the kernel weight reduction was 69.50% and this was significantly lower than the tillering stage at 0°C; the flag leaf stage at –6°C; the flowering stage at –3 and –6°C; and the hard dough stage at 0 and –6°C.

In cultivars 1 to 4 (Figure 4.17 – C1 – C4) the kernel weight was not affected for the different growth stages at temperatures of 0 to –6°C. The flag leaf stage once more seemed to be the most sensitive growth stage followed by the tillering and flowering growth stages.

From the results it was clear that growth stage and temperature, both separately and in combination, were determining factors for the significant reduction in kernel weight. In both the primary and secondary spikes temperatures below –6°C had the most marked inhibiting or stress related effect on kernel weight and this was also seen in the calculated total.

4.3.6.4 Contribution of the primary kernel weight to the total kernel weight produced

With reference to section 4.3.4.4 (*Contribution of the primary kernel number to the total number of kernels produced)* it was evident that there was a difference in the sensitivity of the different growth stages to cold treatment in terms of the number of kernels produced in both the primary and later maturing (secondary) spikes. In order to ascertain whether the influence of temperature and growth stage on kernel weight was due to a reduced number of kernels or due to a reduction in phytomass or both, the contribution of primary kernel weight to the total weight was investigated. The data is presented in Tables 4.5 – 4.8 and are expressed as a percentage of the total production.

The results in Table 4.5 indicate that the contribution of individual kernel weight in primary spikes to the total weight was less of an important factor than the number of primary kernels. During the tillering stage, however the percentage contribution to the total kernel weight increased when the plants were exposed to temperatures below –6°C. This was also the case with the flowering and hard dough stages. This means that the secondary spikes must have been damaged and therefore the contribution of the primary spikes was higher. The flag leaf

stage showed the opposite indicating that the contribution of the primary spikes to total kernel weight was lower. Considering the number of kernels produced (section 4.3.4.4 – Table 4.1), the contribution of kernel weight to the total weight was less and therefore these kernels were smaller. This indicates that the primary spike was more sensitive during the flag leaf stage than during the other growth stages, especially in terms of the number of spikes produced. Secondary spikes were less sensitive during this stage and therefore the damage was limited to the primary spikes.

Table 4.5 Contribution (%) of the primary kernel weight to the total kernel weight produced for cultivar 1 (winter type)

Growth stage	Temperature (°C)					
	0	**-3**	**-6**	**-9**	**-12**	***Average***
Tillering	60.1	42.3	37.0	58.6	67.1	***53.0***
Flag leaf	75.3	33.4	41.7	19.0	0.0	***33.9***
Flowering	49.0	48.2	44.3	47.9	68.0	***51.5***
Hard dough	38.2	36.6	46.1	48.6	55.8	***45.1***
Average	***55.7***	***40.1***	***42.3***	***43.5***	***47.7***	

In cultivar 2 (Table 4.6) decreasing temperatures was associated with a slight decrease in the contribution of the primary kernel weight to that of the total kernel weight at temperatures between 0°C to –6°C. Below –6°C the percentage contribution was higher with the exception of the flag leaf stage at –9 and –12°C and the hard dough stage at –9°C.

Table 4.6 Contribution (%) of the primary kernel weight to the total kernel weight produced for cultivar 2 (winter type)

Growth stage	Temperature (°C)					
	0	**-3**	**-6**	**-9**	**-12**	***Average***
Tillering	46.8	41.1	40.4	51.1	60.9	***48.1***
Flag leaf	45.0	43.3	42.9	29.1	0.0	***32.1***
Flowering	47.2	42.6	44.8	57.1	72.4	***52.8***
Hard dough	38.1	44.5	44.3	43.0	53.6	***44.7***
Average	***44.3***	***42.9***	***43.1***	***45.1***	***46.7***	

In cultivar 3 (Table 4.7) the contribution of the primary kernel weight to the total weight was slightly higher with a decrease in temperature with the exception of the tillering and flag leaf stages at –12°C, where kernel weight was restricted.

Table 4.7 Contribution (%) of the primary kernel weight to the total kernel weight produced for cultivar 3 (intermediate type)

Growth stage	Temperature (°C)					
	0	**-3**	**-6**	**-9**	**-12**	***Average***
Tillering	56.4	57.9	66.9	68.4	0.0	***49.9***
Flag leaf	51.5	54.1	49.7	52.5	0.0	***41.6***
Flowering	58.7	61.1	61.3	68.8	76.4	***65.3***
Hard dough	66.4	63.9	61.6	74.2	83.5	***69.9***
Average	***58.3***	***59.3***	***59.9***	***66.0***	***40.0***	

Cultivar 4 (Table 4.8), a spring type cultivar with a low tillering ability, showed no consistency in its reaction to the treatment combinations. On average the percentage contribution of the primary kernel weight to the total weight was markedly higher than in the other cultivars. The percentage contribution during the tillering stage was consistent with the exception of the –12°C treatment, while the contribution during the flag leaf stage was lower at temperatures below –3°C. The flowering stage was also consistent in its contribution but it was lower than the tillering, flag leaf and hard dough stages with the exception of treatment –12°C where all secondary spikes were killed.

Table 4.8 Contribution (%) of the primary kernel weight to the total kernel weight produced for cultivar 4 (spring type)

Growth stage	Temperature (°C)					
	0	**-3**	**-6**	**-9**	**-12**	***Average***
Tillering	71.0	74.1	87.7	70.0	0.0	***60.6***
Flag leaf	73.0	81.6	55.5	49.9	0.0	***52.0***
Flowering	55.7	56.2	58.6	52.7	100.0	***64.6***
Hard dough	53.8	77.6	62.4	88.4	97.7	***76.0***
Average	***63.4***	***72.4***	***66.0***	***65.3***	***49.4***	

The winter type cultivars reacted similarly to the applied treatments. All cultivars seemed to be extremely sensitive at the flag leaf stage at –9 and –12°C. Regarding the reaction of

different cultivars to cold treatment, no consistency was observed and this could be ascribed to different growth habits, especially their compensation ability with regard to tillering.

4.3.7 Kernel weight per spike

4.3.7.1 Kernel weight per primary spike

The kernel weight per primary spike showed significant differences between treatment combinations for the different cultivars (Appendix 4.41 – 4.44). The data has already been presented in Figure 4.15 and discussed (see section 4.3.6.1).

4.3.7.2 Kernel weight per secondary spike

Kernel weight per secondary spike did not reveal similar tendencies in reaction to the different treatment combinations for the different cultivars (Appendix 4.53 – 4.56). In cultivar 1 no significant differences in kernel weight per secondary spike for either of the different treatment combinations and/or the main effects (growth stage and temperature) (Appendix 4.53; Figure 4.18 – C1) observed.

Significant differences in kernel weight produced by the secondary spikes were, however, observed between the treatment combinations (Appendix 4.54) in cultivar 2. Kernel weight per secondary spike did not differ significantly at the different growth stages between 0 and –6°C. Temperatures lower than –6°C led to a reduction in the kernel weight per secondary spike for all growth stages at –9 and –12°C with the exception of the hard dough stage at –12°C. At –9°C the flag leaf and flowering stages showed reductions of 63.2 and 56.0%, respectively. This was only significantly lower than the kernel weight per spike at the hard dough stage at –6°C. This was topped with reductions of 70.3 and 71.6% at the tillering and flowering stage, respectively. A 100% reduction was obtained at the flag leaf stage at –12°C and this was significantly lower than the kernel weight per secondary spike for the different growth stages at 0 and –6°C; the tillering and flag leaf stages at –3°C and the hard dough stage at –12°C. The kernel weight per secondary spike obtained at the flowering stage at –12°C was significantly lower than that of the flag leaf stage at –3 and –6°C as well as that of

the hard dough stage at –6°C. The kernel weight per spike at the tillering stage and at –12°C showed the same tendency as the flowering stage at –12° with the exception of the flag leaf at –3°C.

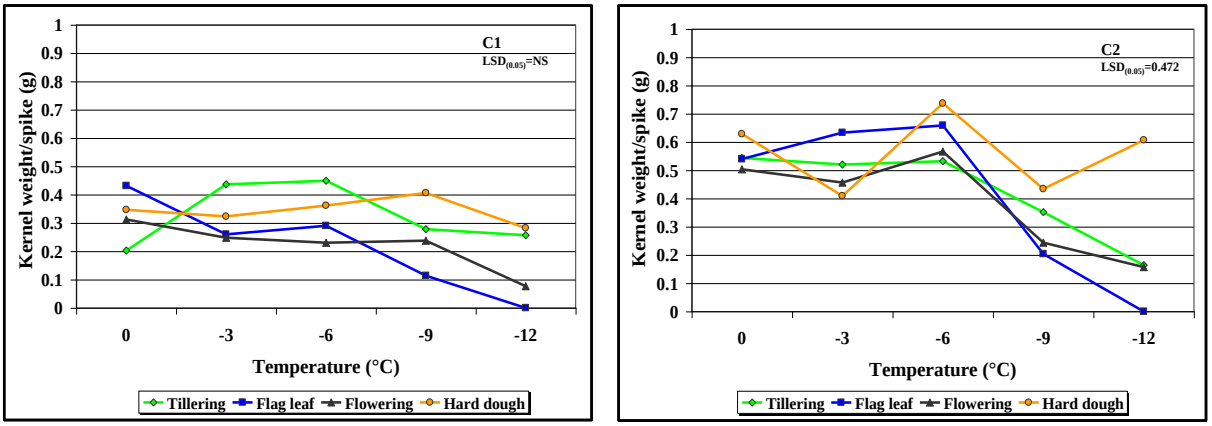


Figure 4.18 Kernel weight per secondary spike as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type).

In cultivar 3 (Figure 4.19 – C3) the kernel weight per secondary spike increased between 0 and –6°C. A decrease in temperature led to a sharp reduction of 22.2% at –9°C and 74.7% at –12°C compared to the control at 0°C. Although a reduction in kernel weight of 22.2% was observed at the –9°C this was not significantly lower than the kernel weight per spike obtained at temperatures of 0 to –6°C while the reduction (74.7%) at –12°C was significantly lower than that at 0 to –9°C.

A reduction in kernel weight per spike was observed at all temperatures below 0°C in cultivar 4. This indicated that the spring type cultivars are more sensitive to sub zero temperatures than the intermediate or winter type cultivars. The reduction at –3, –6, –9 and –12°C were 42.8, 25.1, 52.3 and 97.7%, respectively. Kernel weight per spike obtained at –12°C was significantly lower than that of the other temperatures. A reduction of 52.3% at –9°C was also significantly lower than the kernel weight per secondary spike at 0°C.

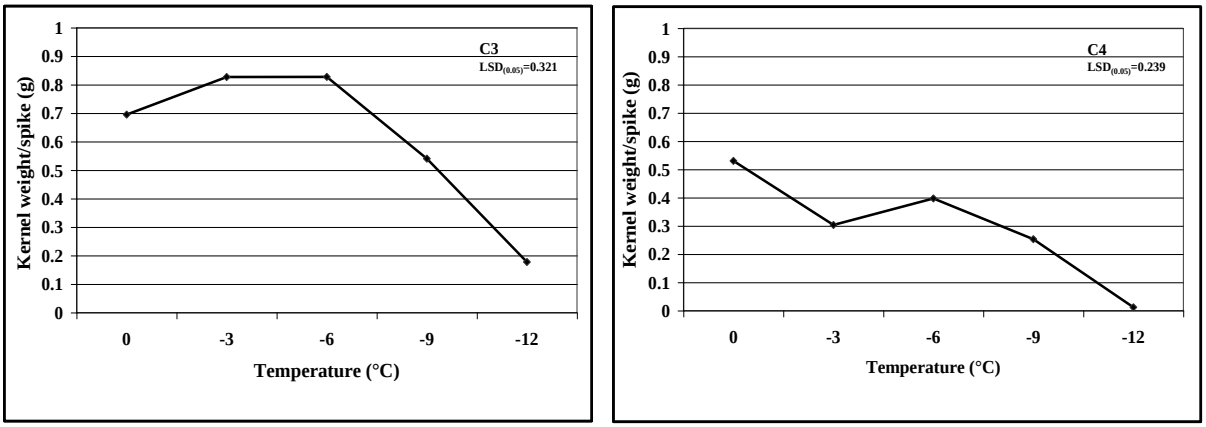


Figure 4.19 Kernel weight per secondary spike as affected by different temperatures in different cultivars (C3 - intermediate and C4 – spring type).

Figure 4.19 clearly show that the average kernel weight per spike of cultivar 4 (0.299g/spike) was lower than that of cultivar 3 (0.613g/spike). This phenomenon could be an indication that cultivar 4 is more sensitive to sub zero temperatures than cultivar 3.

4.3.7.3 Average kernel weight per spike

The average kernel weight per spike (average of the primary and secondary spikes) was significantly different in the different cultivars as a result of different treatment combinations, with the exception of cultivar 1 (Appendix 4.57 – 4.60). Both the main effects had a significant influence on the kernel weight per spike produced in cultivar 1. In the latter no reduction in kernel weight per spike was observed at temperatures between 0 and –6°C, but a significant reduction of 29.2 and 49.1% at –9°C and –12°C, respectively, was calculated. The only significant difference in the kernel weight per spike was between –12°C and 0 to –6°C (Figure 4.20), where the kernel weight was much lower at the former treatment.

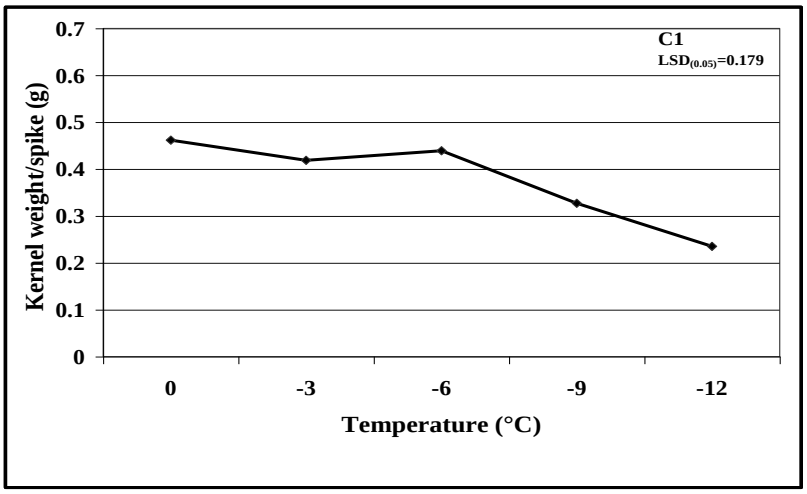


Figure 4.20 Average kernel weight per spike as affected by different temperatures for cultivar 1 (C1 – winter type).

The results in Figure 4.21 indicated that the tillering and hard dough stages of cultivar 1 were not negatively influenced in terms of the average kernel weight per spike at the tillering stage (0.429g) and the hard dough stage (0.454g) compared to the control (0°C; 0.4617g/spike). The flag leaf and flowering stages were, however, highly sensitive to cold treatment and showed reductions of 37.7 and 30.6% respectively.

In cultivar 2 no differences in kernel weight for the different growth stage at 0 to –6°C was observed. Temperatures below –6°C reduced the kernel weight per spike with the exception of the tillering and hard dough stages at –12°C. The kernel weight at the hard dough stage and –3°C was significantly higher than in the flag leaf and flowering stages at –9 and –12°C. A reduction of 100% was observed in the flag leaf stage at –12°C and this was significantly lower than in all growth stages at 0 to –6°C, the tillering stage at –12°C and the hard dough stage at –9 and -12°C (Figure 4.22 –C2).

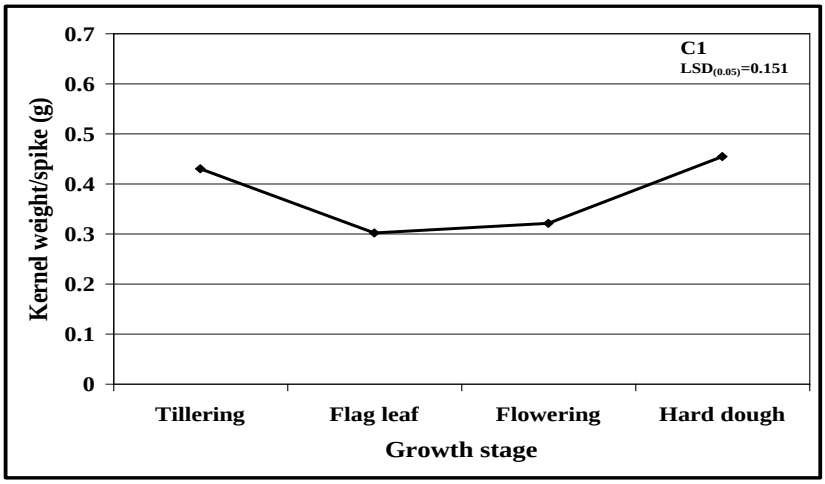


Figure 4.21 **Average kernel weight per spike as affected by different growth stages for cultivar 1 (C1 – winter type).**

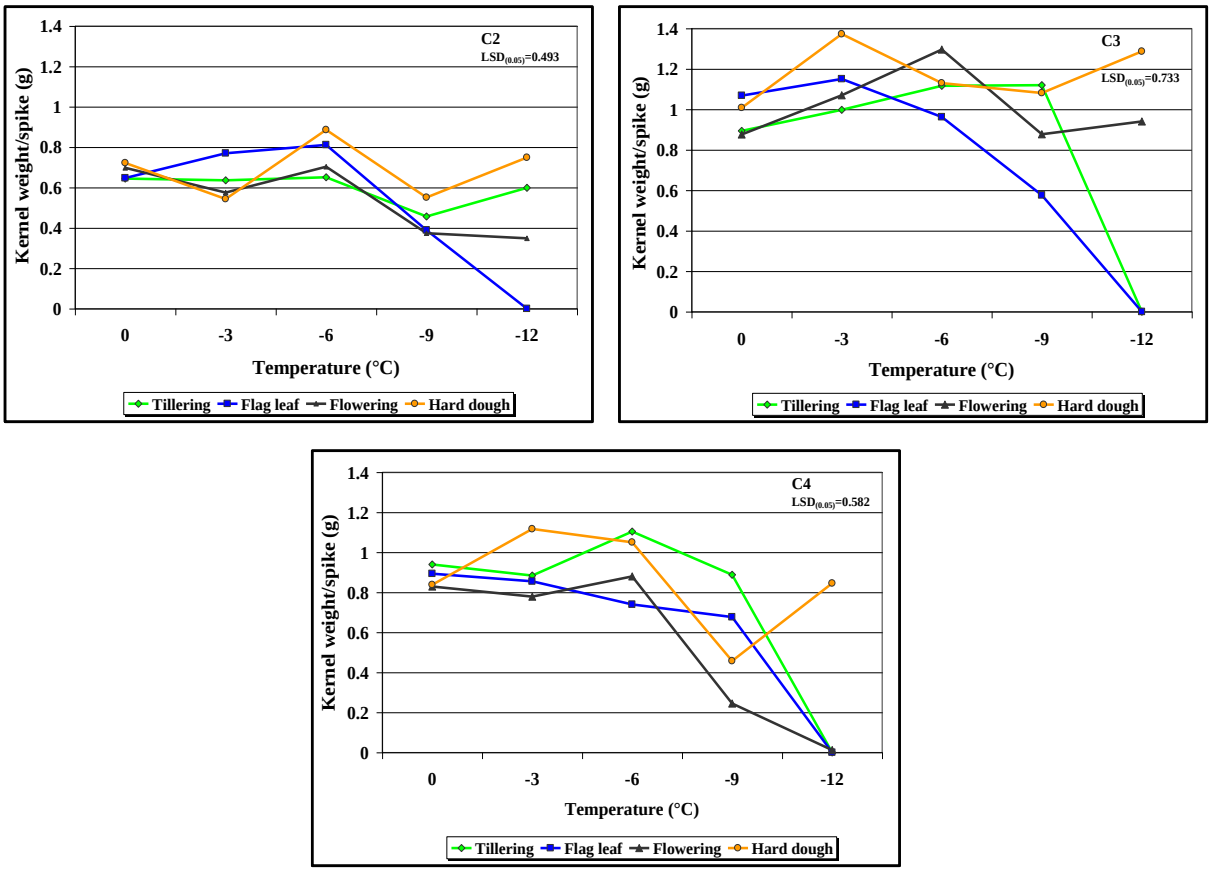


Figure 4.22 **Average kernel weight per spike as affected by different temperatures at different growth stages for different cultivars (C2 – winter type, C3 – intermediate type and C4 – spring type).**

In cultivar 3 the kernel weight per spike was reduced by a 100% during the flag leaf and tillering stages at –12°C (Figure 4.22 – C3). This was significantly lower than the kernel weight per spike for all treatment combinations with the exception of the flag leaf stage at –9°C that showed a reduction of 40.30%. The latter was only significantly lower than that of the hard dough stage at –3°C.

Cultivar 4 did not show any significant differences in kernel weight per spike between the different growth stages at 0 to –6°C, the tillering and flag leaf stages at –9°C and the hard dough stage at –12°C (Figure 4.22 – C4). However, a slight reduction occurred at temperatures below –6°C with the flowering stage being the most sensitive growth stage at –9°C (72.01% reduction). At –12°C a reduction of 98.63% was observed in the flowering stage while this was a 100% in the tillering and flag leaf stages.

Collectively, the reduction in kernel weight per primary spike, per secondary spike and the average kernel weight per spike emphasised the sensitivity of wheat to temperatures below –6°C with the flowering stage being the most sensitive growth stage.

4.3.8 Mass per 100 kernels

Hectolitre mass could not be determined as the yields were too low. Instead the mass per 100 kernels were calculated to show the effect of the main and/or combination treatments on the grain produced.

4.3.8.1 Mass per 100 kernels produced by primary spikes

Significant differences in the mass per 100 kernels between different treatment combinations and cultivars were observed for the primary spikes (Appendix 4.61 – 4.64). In cultivar 1 the mass per 100 kernels was reduced by 100% during the flag leaf stage at –12°C and this differed significantly from that of other growth stages at 0 to –6°C; the tillering, flowering and hard dough stages at –9°C and the hard dough stage at –12°C (Figure 4.23 – C1). The flag leaf stage at –9°C showed a reduction of 76.03% and this was significantly lower than that of all other growth stages at 0°C; the tillering, flag leaf and flowering stages at –3°C; the

tillering, flag leaf and hard dough stages at –6°C and the hard dough stage at –9 and –12°C. During both the tillering and flowering stages at –12°C the mass per 100 kernels was significantly lower than that of the tillering stage at 0°C compared to the average of the control. The flowering stage was the most sensitive growth stage at –6°C where a reduction of 40.27% was observed. At temperatures below –6°C all growth stages were negatively affected with the exception of the hard dough stage.

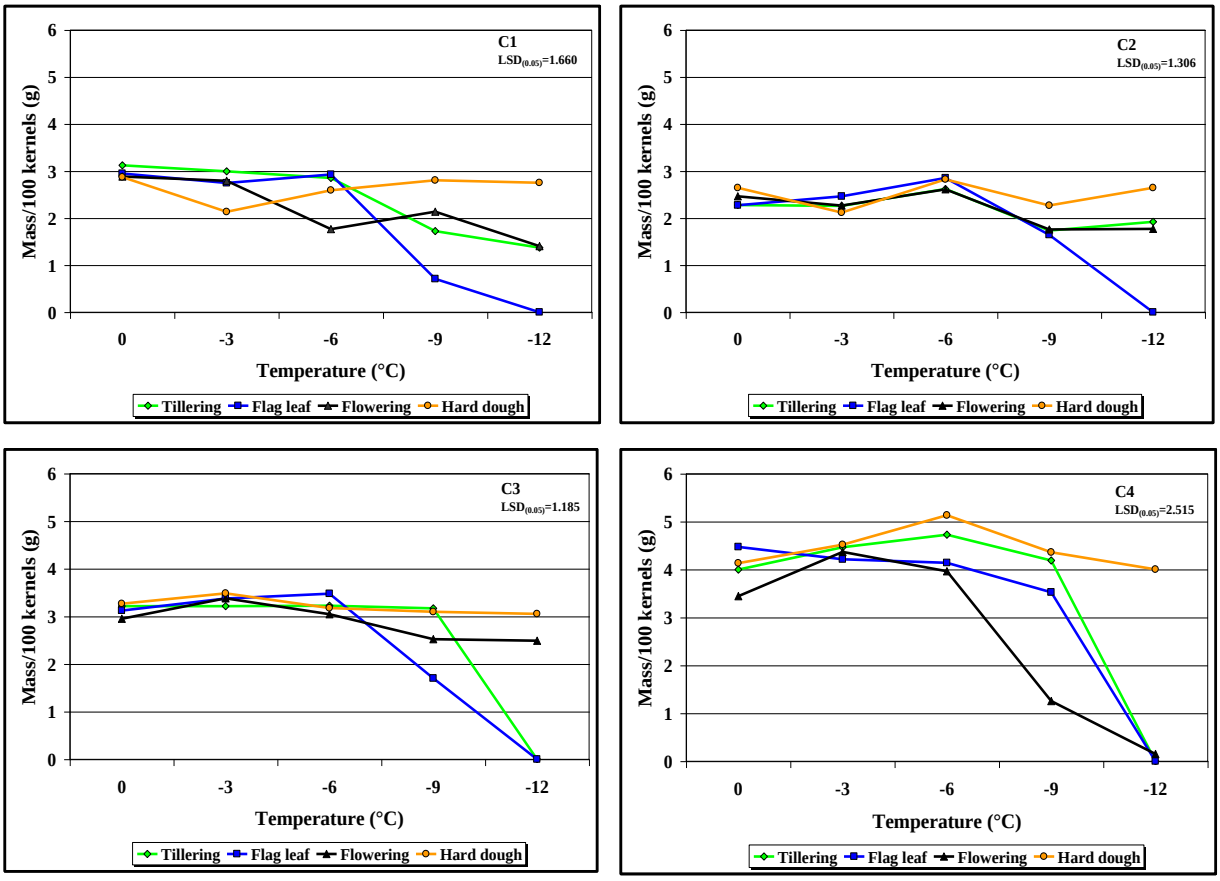


Figure 4.23 Mass per 100 kernels (primary spike) as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

In cultivar 2 a reduction in the mass per 100 kernels was observed during the tillering, flag leaf and flowering stages only at –9 and –12°C. However, a significant reduction (100%) was only calculated in the flag leaf stage at –12°C and this was significantly lower with respect to all other treatment combinations.

The flag leaf stage was the most sensitive growth stage in cultivar 3 as reduction in the mass per 100 kernels of 45.81 and 100% were observed at both –9 and –12°C, respectively (Figure 4.23 – C3). Although a significant reduction of 100% was observed at the tillering and flag leaf stages, this was only at –12°C. At –9°C the mass per 100 kernels was significantly higher in the flag leaf stage than the tillering and flag leaf stages at –12°C, but significantly lower than the remainder of the treatment combinations.

In cultivar 4 no significant differences in the mass per 100 kernels between different growth stages at 0, –3 and –6°C; the tillering, flag leaf and hard dough stages at –9°C and the hard dough stage at –12°C were observed (Figure 4.23 – C4). At –9°C the mass per 100 kernels was significantly lower in the flowering stage than the tillering, flag leaf and hard dough stages at 0°C; the different growth stages at –3 and –6°C; the tillering and hard dough stages at –9°C and the hard dough stage at –12°C.

In summary, treatment at temperatures below –6°C resulted in a reduction in the mass per 100 kernels produced by the primary spikes.

4.3.8.2 Mass per 100 kernels produced by secondary spikes

With the exception of cultivar 2, all other cultivars showed significant differences in the mass per 100 kernels obtained as a result of temperature (Appendix 4.65 and 4.67 – 4.68). The mass per 100 kernels of cultivar 2 showed significant differences as a result of the interaction between temperature and growth stage (Appendix 4.66).

In cultivar 1 and 3 a respective reduction of 10.87 and 16.73% was observed in the mass per 100 kernels at temperatures ranging from 0 to –6°C, but this was not statistically significant (Figure 4.24 – C1 and C3). At temperatures below –6°C a sharp reduction in the mass per 100 kernels was observed with a reduction of 39.40 and 63.91% at temperatures of –9 and –12°C, respectively, for cultivar 1. Cultivar 3 showed a similar tendency, but in this case the reduction for the respective temperatures were 41.84 and 81.86%. Cultivar 4 differed from

cultivar 1 and 3 with respect to the main effect of temperature in that the mass per 100 kernels was reduced at –3, -6, -9 and –12°C by 32.23, 26.51, 19.65 and 100%, respectively.

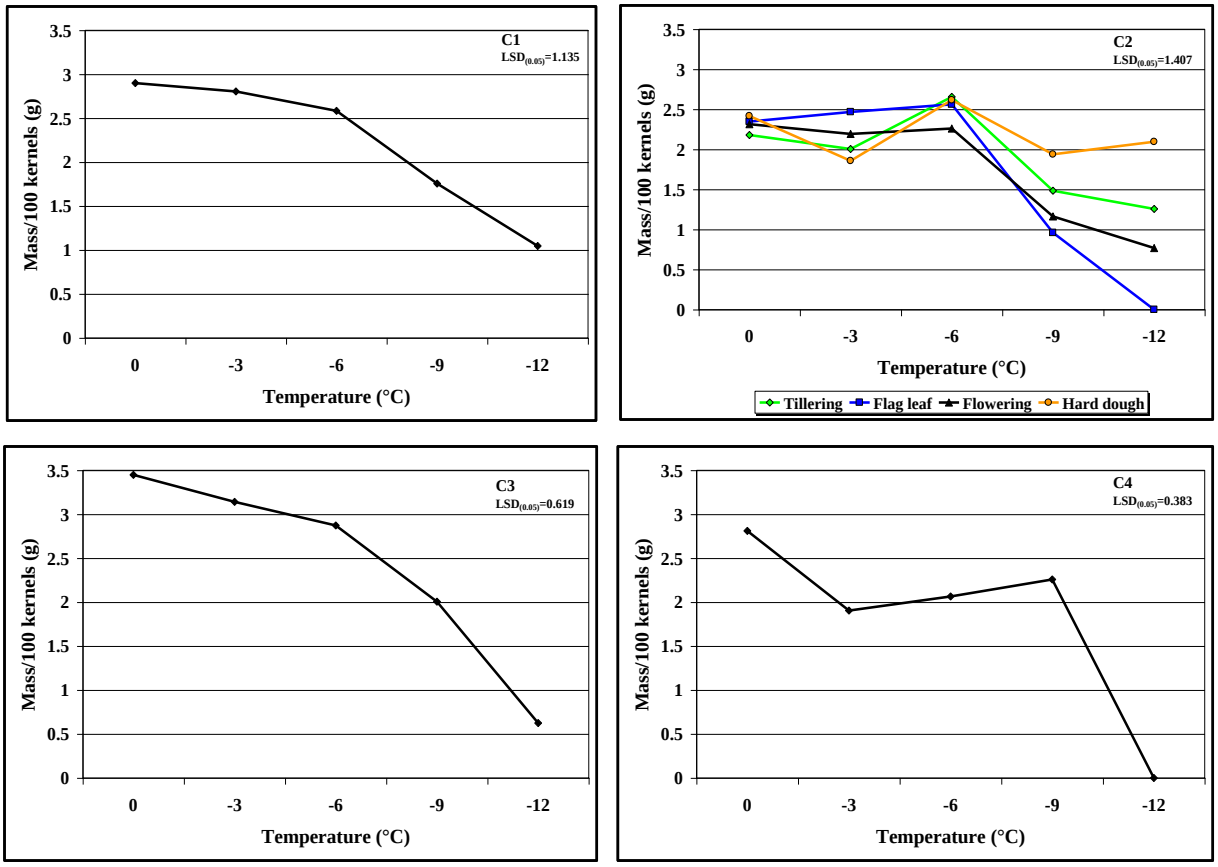


Figure 4.24 Mass per 100 kernels (secondary spike) as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

In cultivar 2 significant differences in the mass per 100 kernels was observed as a result of the treatment combinations (Appendix 4.66 and Figure 4.24 – C2). The mass per 100 kernels produced by the secondary spikes showed no reduction and/or differences for the different growth stages at temperatures of 0 to –6°C. However, a 49.71% reduction in the mass per 100 kernels was obtained in the flowering stage at –9°C compared to the control (average of different growth stages at 0°C). The latter was significantly lower than at –6°C during the tillering and hard dough stages. During the flag leaf stage a reduction of 58.49% was observed at –9°C and this was significantly lower than in the tillering stage at –6°C, the flag

leaf stage at –3 and –6°C and the hard dough stage at 0 and –6°C. Treatment of the flowering stage at –12° resulted in a reduction of 66.81% that was significantly lower than the mass obtained during all growth stages at 0 and –6°C as well as that of the flag leaf and flowering stages at –3°C. The most sensitive growth stage was once more the flag leaf stage as it showed a reduction of 100% at –12°C. This (0g/100 kernels) was significantly lower than all growth stages at 0, –3 and –6°C; the tillering and hard dough stages at –9°C and the hard dough stage at –12°C.

Temperature as a main effect (cultivars 1,3 and 4) or in treatment combinations (cultivar 2) led to a decrease in the mass per 100 kernels of the wheat cultivars under scrutiny (Figure 4.24). This was more evident at temperatures below –6°C. Especially in cultivar 2 the flag leaf stage was the most sensitive growth stage.

4.3.8.3 Mass per 100 kernels produced by both primary and secondary spikes

Mass per 100 kernels (that is of the primary and secondary spikes combined) as parameter showed that treatment combinations had a significant influence (Appendix 4.69 – 4.72). In cultivar 1 the mass per 100 kernels produced during the flag leaf stage at –9°C (62.92% reduction) and the flowering stage at –12°C (63.37% reduction) was significantly lower than that produced during the tillering stage at 0 and –3°C. At –12°C a 100% reduction was observed during the flag leaf stage and this was significantly lower than that of all growth stages at 0 to –6°C. The latter was significantly lower than that of the hard dough stage at –9 and –12°C. Figure 4.25 – C1 illustrates only a slight reduction in the mass per 100 kernels in all the growth stages of cultivar 1at temperatures between 0 and –6°C, but a marked reduction in especially the flag leaf stage at temperatures below –6°.

Cultivar 2 (Figure 4.25 – C2) showed the same tendency as cultivar 1 while cultivars 3 and 4 were similar in their reaction to temperatures below –6°C (Figure 4.25 – C3 and C4). In the latter two cultivars both the tillering and flag leaf stages were most sensitive to cold injury in terms of the reduction in the mass per 100 kernels when the temperature dropped below –6°C. In Cultivar 4 also the flowering stage was sensitive in this regard at –12°C.

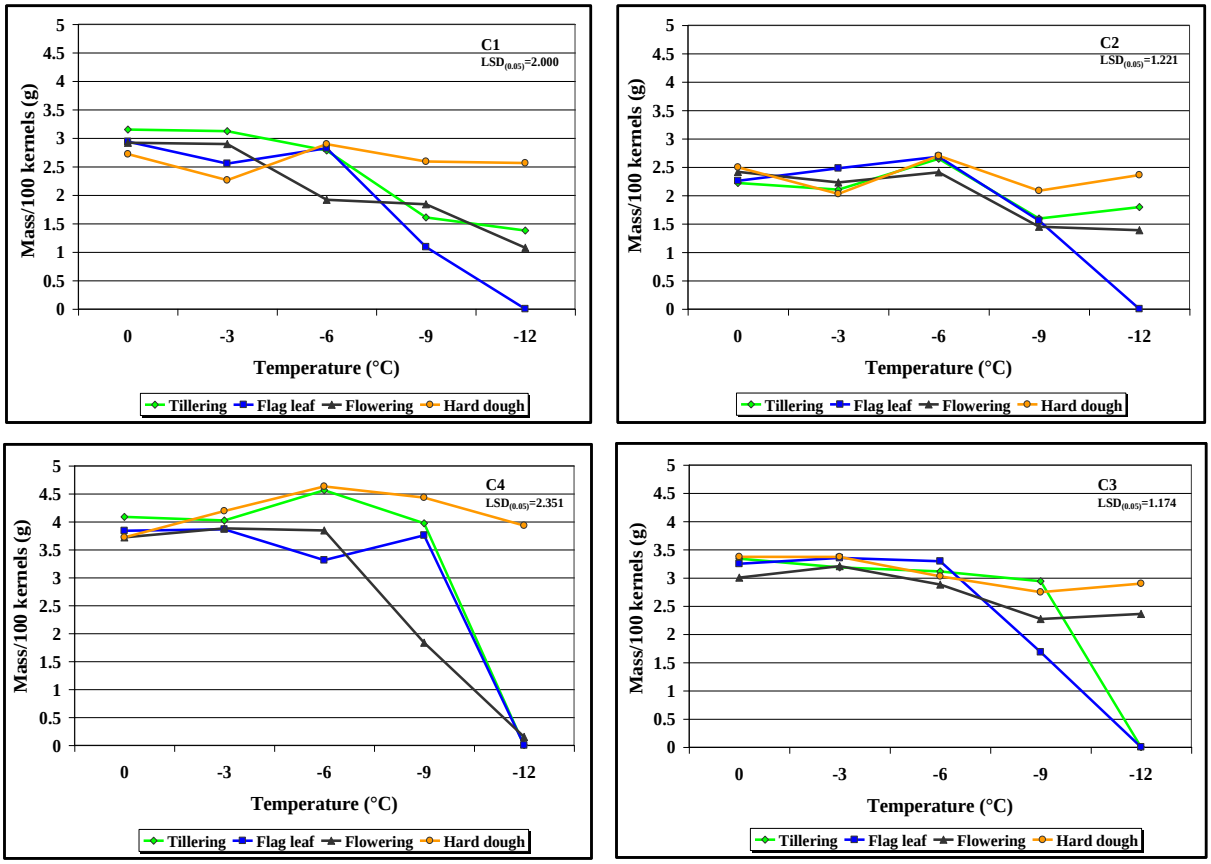


Figure 4.25 Mass per 100 kernels as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

Cultivars 2, 3 and 4 showed that the mass per 100 kernels obtained at temperature of 0 to –6°C for all growth stages were not significantly influenced. The only variation was that of cultivar 4 where the flag leaf and flowering stages were slightly lower than that of the tillering and hard dough stages at –6°C (Figure 4.25 – C3 – 4).

In summary temperatures below –6°C caused a severe reduction in the mass per 100 kernels during the tillering, flag leaf and flowering stages with variation between the cultivars. Cultivar 3 (intermediate type) produced the highest mass per 100 kernels, followed by the spring wheat type (cultivar 4) and then the winter type (cultivars 1 and 2). The South African wheat type showed a slightly lower mass per 100 kernels than the Canadian wheat in the case of the winter cultivars. The mass per 100 kernels produced by the secondary spike showed a

more severe loss in mass than that of the primary spikes. This could be due to the fact that the secondary spikes were less mature than the primary spikes when subjected to frost conditions, making it more sensitive to frost injury.

4.4 DISCUSSION and CONCLUSION

With this study an attempt was made to evaluate the reaction of different growth stages to cold stress in different wheat types (winter, intermediate and spring) by subjecting different growth stages to temperatures in the range of 0 to –12°C with increments of 3°C. Results obtained in this study confirmed, to a large extent, what has previously been found by other researchers all over the world but with some differences. However, in this discussion the emphasis will be placed on practical aspects to illustrate the effect of frost stress under South African conditions with South African cultivars.

Various studies have been conducted in the past on the survival of wheat cultivars under freezing conditions (McKersie & Hunt, 1987; Brule-Babel & Fowler, 1989; Damania & Tahir, 1993). Fowler and Lamin (1987) found, after screening of extensively diverse hexaploid wheat genotypes, that none was to surpass the commercially produced cultivars of North America at that time. Under Finnish conditions Hömmö (1994) observed a wide range of winter hardiness levels for winter wheat cultivars. Furthermore, Brule-Buble and Fowler (1989) described Norstar as one of the most hardy wheat cultivars and for this reason it was included in this study as a control. South African tested cultivars could be divided into three definite classes according to their ability to survive different test temperatures. Norstar, the cold tolerant check, displayed superior tolerance between – 6 and –12°C (Jacobs, 1999) and this also correlated with the findings by other researchers (Brule-Buble & Fowler, 1989).

The different types of cultivars used (winter, intermediate and spring) reacted differently to low temperatures at different growth stages in terms of different parameters employed. Dry matter production in the different cultivars was only severely negatively influenced at temperatures below –6°C. This was more evident for the intermediate and spring cultivar. The winter type cultivars keep their growing points below or close to the soil surface for a longer period than the intermediate and spring types and therefore avoid frost injury. Peel

(1998) reported that once a plant's growing point is above the soil surface, it is more likely to be injured by frost. Furthermore, cold injury is influenced by more than one factor for instance: i) the physiological condition of the plants when cold snap occurs; ii) genetic differences in cultivar hardiness; and iii) moisture conditions during the cold period (Karow, 1998). Therefore researchers found frost damage to occur at different temperatures that ranges from –2.9 to –12°C and even below this (Single, 1966; Metcalfe, Cress & Olein, 1970; Fletcher & Cullis, 1988; Jacobs, 1999). Fowler and Carles (1979) reported that the maximum cold hardiness attained by the hardiest cultivars of each species, when fully acclimated, was –13, –15, –21, and –30°C for oats, barley, wheat and rye, respectively. The difference in cold tolerance has also been related to specific growth stages as well as specific plant organs. Marcellos and Burke (1979) found that leaves of several wheat cultivars were able to tolerate temperatures as low as –7 to –9°C while Chen *et al*. (1983) observed that Norstar leaves could tolerate temperatures of –18°C.

In this study the dry matter production was found to be severely affected during the early growth stages when the temperature dropped below –6°C. This reduction was more evident during the tillering and flag leaf stages of which the latter was more pronounced. During the flag leaf stage the growth point has moved above the soil surface and it is therefore not as isolated and protected as it was when it was still below the soil surface. The position of the growth point within the stem is determined by the cultivar and this has an influence on the degree of frost injury. This corresponds with the findings of Fujita *et al*. (1992) where tiller avoidance is determined by the developmental stage of wheat. The winter and intermediate wheat types showed the same tendency, but the spring type was more sensitive during the tillering stage and this is attributed to the fact that the spring type has pushed its growth point higher during this stage. This is supported by Fujita *et al*. (1992), who found that tiller frost avoidance was determined by the position of shoot apexes, since the apices growing under ground are protected from frost injury. Contrary to this, the winter types tend to keep their growth points under or close to the soil surface and are therefore more tolerant to frost stress during the tillering stage. The flowering and hard dough stages have not shown severe injury to frost stress indicating that plants that have completed their vegetative growth is more

tolerant to low temperature exposure, especially after anthesis, except for kernels that might still be prone to injury.

The number of spikes produced by the different cultivars or growth types was not significantly influenced by low temperature during the flowering and hard dough stages as spikes have already emerged at these growth stages and therefore the number could not be influenced. However, during the tillering and flag leaf stages a severe reduction in the number of spikes per plant was observed and this was more pronounced in the winter type cultivars at temperatures below –6°C. The reduction was more pronounced at the flag leaf stage than the tillering stage. The reason for this may be that the winter type cultivars have the ability to produce more leaves and therefore more tillers than the intermediate and especially the spring type cultivars. Tiller initiation and its appearance is closely related to leaf emergence in the absence of any restriction of assimilates (Miralles & Slafer, 1999). Secondary tillers may appear from the axillary buds developed in each tiller phytomer and the relationship is similar to that of the main or primary tiller. Therefore, the pattern of potential tiller emergence follows a Fibonacci series (Malse, 1985). According to Miralles and Slafer (1999) most wheat crops grow for a short period with virtually unlimited resources and therefore the above relationship only holds for a short period. With the limitation of resources not all the tillers that were potentially expected to appear do so, and the rate of tiller appearance slow down, though still positive. Later, the resources become increasingly limited and the ability to maintain growth of all tillers decreases and some die, in reverse of the order they appeared. No mechanistic relationship exists between the onset of tiller mortality and development progress, but it generally coincides with the beginning of stem elongation when a sharp increase in the demand of assimilates by the elongating vegetative internodes are required. During this period of growth and development the growth point emerges above the soil and is more prone to environmental stress factors. With the onset of the flag leaf stage the growth points are extremely susceptible to frost injury and therefore the reduction in tiller number could be higher at this stage than at the tillering stage.

The primary stem, and therefore the primary spike, was used as an indicator to determine the growth stage of the plants as this had an influence on the degree of frost injury sustained.

For this purpose a distinction was made between primary spikes and secondary spikes. Subsequently, data collected on primary and secondary spikes were analysed separately after which it was combined to provide a true reflection of the total effect. According to Pinthus (1967) one way in which wheat yield could be modified is through the influence of environmental factors on the number of spikelets in a wheat ear as this affects the grain number. Factors such as vernalisation, photoperiod and temperature on the number of spikelets in a wheat ear have been demonstrated (Davidson, Christian, Jones & Bremner, 1985; Rawson, 1971) and therefore subzero temperatures could also have an effect on spikelet number. In terms of the number of spikelets per primary spike the flag leaf stage was the most vulnerable growth stage followed by the tillering stage at temperatures below –6°C. In this regard the flowering and hard dough stages were not influenced by frost injury at any of the temperatures, in the range of 0 to –12°C. The number of spikelets per secondary spike were also significantly reduced during the flag leaf stage for cultivars 1 to 3 and also at temperatures below –6°C while the average number of spikelets per spike showed similar tendencies. From this it could be concluded that cultivars 1 to 3 were most vulnerable during the flag leaf stage at temperatures below –6°C. Cultivar 4, the spring type, only showed a reduction in the number of spikelets when the temperature dropped below –9°C.

Additionally, the number of kernels produced by the primary spike was severely reduced when the plants were exposed to frost conditions during the flag leaf stage at temperatures below –6°C. This was once again applicable to cultivars 1 to 3, but cultivar 4 was more sensitive during the tillering stage at temperatures below –6°C and at temperatures lower than –9°C its reaction corresponded with that of the other cultivars. A significant reduction in the number of kernels produced by the secondary spikes were observed for cultivars 1 and 2 (winter types) during the flag leaf stage and to a lesser extend the tillering stage at temperatures below –6°C. Cultivars 3 (intermediate type) and 4 (spring type) showed no significant differences in the number of kernels produced by the secondary spikes with regard to the different growth stages. Therefore only temperature (main factor) had a significant influence on the number of kernels produced by the secondary spikes, where temperatures below –6°C severely reduced the number of kernels. The total number of

kernels produced by the primary and secondary spikes of cultivars 1 to 3 showed that the flag leaf growth stage was highly sensitive and that the reduction occurred at temperature below –6°C. Cultivar 4 was more vulnerable during flowering at –6°C, but did not react differently at –12°C.

When the ratio of number of kernels produced by the primary spike (average of the different growth stages) was compared to that produced by the secondary spikes (average of the different growth stages) it was clear that in the winter type cultivars a contribution of approximately 50% was low compared to intermediate (60%) and spring (70%) types. The higher contribution by the latter could be ascribed to the growth habit of different cultivars where the winter type cultivars produced more tillers than the intermediate and spring type cultivars. As the ratio of the number of secondary tillers decreased from winter to spring type cultivars, the importance of the primary spike production became more evident.

The number of kernels produced per primary and secondary spike as well as the average showed similar tendencies. Temperatures between 0 and –6°C did not have an adverse effect on the kernel weight produced by the primary spikes during the different growth stages. At temperatures lower than –6°C the flag leaf growth stage experienced a severe reduction in kernel weight. This reduction was clearly shown by cultivars 1 to 3. Cultivar 4 (spring type) was once more, sensitive during the flowering stage, but not as previously noted at temperatures below –9°C, but now at temperatures of below –6°C. Therefore kernel weight seem to be more sensitive to temperature than the previously mentioned parameters. The weight of kernels produced by the secondary spikes was severely reduced in all cultivars at temperatures below –6°C. This was especially true for cultivars 1 to 3 at the flag leaf stage which was the most sensitive growth stage as was the flowering stage for cultivar 4. Although the growth stages differed with regard to their sensitivity to the temperature at which severe reductions was clearly visible, this was more pronounced at temperatures below –6°C.

The weight of kernels produced per spike also provides information with regard to how the primary and secondary spikes reacted to frost injury, especially during the grain filling

period. Kernel weight per primary spike has been discussed in the previous paragraph. However, as there is only one primary spike per plant the kernel weight per secondary spike needs to be considered more comprehensively as there is more than one secondary spike per plant. Cultivars 1 and 2 showed similar tendencies in the reduction of kernel weight per secondary spike at temperatures below –6°C, while the flag leaf stage was the most sensitive growth stage. Cultivars 3 and 4 showed that only temperature (therefore different growth stage had no effect on kernel weight) had an effect on kernel weight per secondary spike and the reduction commenced at temperatures below –6°C. When the primary and secondary spikes' kernel weight were combined it had the same tendency than what have been discussed with regard to the kernel weight per secondary spike, with the exception of cultivar 1 that did not show an interaction between the two main factors. Though there was no interaction, the main factors also showed that the flag leaf stage was the most sensitive growth stage and that temperatures below –6°C had a negative effect on kernel weight per spike for this cultivar. The mass per 100 kernels showed similar tendencies as previously discussed parameters.

The local climatic environment and the degree of how wheat has adapted to it, largely determine the yields from wheat cultivars. Grain number, as discussed above, can be an important determinant of wheat yields. Gifford, Bremner and Jones (1973) stated that there is a balance between source and sink limitation of yield in barley. Therefore, if any organs or parts of organs and translocation pathways were damaged due to frost injury after anthesis the growth of kernels would either be inhibited or ceased, leading to shrinked or shrivelled and light kernels (Afanasiev, 1966). Thus, the quality of the grain measured in hectolitre would be lowered and downgraded during grading or could even be unacceptable by the standard of wheat buyers.

According to Single (1988) frost damage to cereals in the stage of stem elongation and spike emergence is largely confined to areas where heat and drought during summer restrict the main growing period to late winter and early spring, when temperatures during the day are ideal for growth, but at night fall to sub zero and consequently damaging levels. This correspond with the findings of this study where the flag leaf and flowering stages showed to

be the most sensitive. Though researchers have reported that the flowering stage is extremely sensitive to frost damage this study has indicated that the flag leaf stage seemed to be the most sensitive growth stage.

Generally, for cultivars 1 to 3 (winter and intermediate types) the flag leaf stage was the most sensitive growth stage while the flowering stage was the most sensitive growth stage for cultivar 4 (spring type). Though the growth stages differed in terms of sensitivity to cold stress it is generally concluded that temperatures below –6°C led to an inhibition or reduction of growth and development and subsequently a severe reduction of the mentioned parameters. Finally, it was noted that the growth habit of the cultivars had a significant influence on their reaction to frost injury, with the winter types being more tolerant than the spring types. This is in agreement with the findings of Jacobs (1999) as well as that of Fowler and Carles (1979); Brule-Babel and Fowler (1988) and Roberts (1990).

CHAPTER 5

ASSESSMENT OF FROST STRESS ON QUALITY ASPECTS IN SOUTH AFRICAN WINTER, INTERMEDIATE AND SPRING WHEAT

5.1 INTRODUCTION

Wheat quality, as dictated by the wheat grading system, is used to come to an agreement on the price of wheat between wheat buyers and sellers. This system is crucial when wheat consignments are delivered to silo's by producers/farmers as the experienced grain grader has to apply the grading system in accordance to specifications based on both objective and subjective evaluations defining the grade (Department of Agriculture, 1990).

The visual system used in South Africa is a simple and effective way of assessing if frost damage occurred especially on kernels during the milk to soft dough stage as defined with the term "heavily frost-damaged wheat" which is characterised by: a) the kernels being fairly plump but covered entirely with small blisters extending into the crease. This excludes kernels where the blisters are confined to the back of the kernel as well as immature wrinkled kernels in which wrinkling has been caused by frost while the kernels were still immature and b) wheat kernels which have a slightly-off bran coat due to frost damage; provided that the bran coat had not been rubbed off as a result of handling and that evidence of frost damage is present (Department of Agriculture, 1990).

This visual system of assessment is usually in dispute when producers deliver wheat consignments at silo's and grain graders observe wrinkled kernels, making the assumption that frost damage occurred during the growing season. This leads to confusion between the producer and insurance companies as wrinkled kernels could have been the result of various stress factors (high temperatures, low/freezing temperatures, drought, salinity, water logging, etc.) during the growing season (Giunta, Motzo & Deidda, 1993; Cromey, Wright & Boddington, 1998; Saqib *et al.,* 2004).

Other quality parameters also used to evaluate the effect of frost stress on yield quality include hectolitre mass, protein content, stirring number (correlated to falling number), flour yield, flour colour, etc. (Tipples, 1980; Dexter, Martin, Preston, Tipples & MacGregor, 1985).

As previously mentioned (Chapter 4; 4.1) one of the frequently asked questions by producers is: "Does frost damage have an effect on wheat quality". The aim of this study was to determine if sub-zero temperatures influenced wheat quality at different growth stages.

5.2 MATERIALS and METHODS

See Chapter 3, section 3.1.1, 3.2 and 3.3.2.

5.3 RESULTS

Water-soluble protein (µg/g), total protein content (%) as well as the stirring number (SN) of the seed obtained in Experiment 1 was used to evaluate qualitative aspects of frost stress on wheat growth. The data obtained was not statistically analysed for all the treatment combinations did not yield seed or the available weight was insufficient which in turn restricted the analyses of protein content and SN. This was specifically the case at temperatures of –9°C at the flag leaf stage and especially at –12°C during the tillering, flag leaf and flowering stages. Averages were however, calculated for available data and omitted where insufficient material was available. Results obtained for the water-soluble protein, total protein content and the SN are represented in Figures 5.1 – 5.3.

5.3.1 Water- soluble protein (µg/g) in kernels

The water-soluble protein measured for the different cultivars at 0°C showed no variation at the different growth stages. Despite of this cultivar 3 (2.679 µg/g) had the highest water-soluble protein content, compared to the average of the control, followed by cultivar 2, 4 and 1 with a water-soluble protein content of 2.408, 2.338 and 2.321 µg/g, respectively.

In cultivar 1 a slight decreasing tendency in the water-soluble protein content was observed at the tillering stage at both –3 (2.00%) and –6°C (4.69%) compared to the control at 0°C (2.321 µg/g). The same tendency was observed at the flag leaf stage with a reduction of 3.35 and 2.45% at –3 and –6°C, respectively. Exposure of plants to low temperature at the flowering stage resulted in the highest water-soluble protein decrease (5.82%) at –3°C but further cooling did not exaggerate the situation. The predominant decrease in water-soluble proteins of cultivar 1 was observed at –6°C at the hard dough stage (Figure 5.1 – C1). However, no significant differences in water-soluble protein content were observed for growth stage as a main effect.

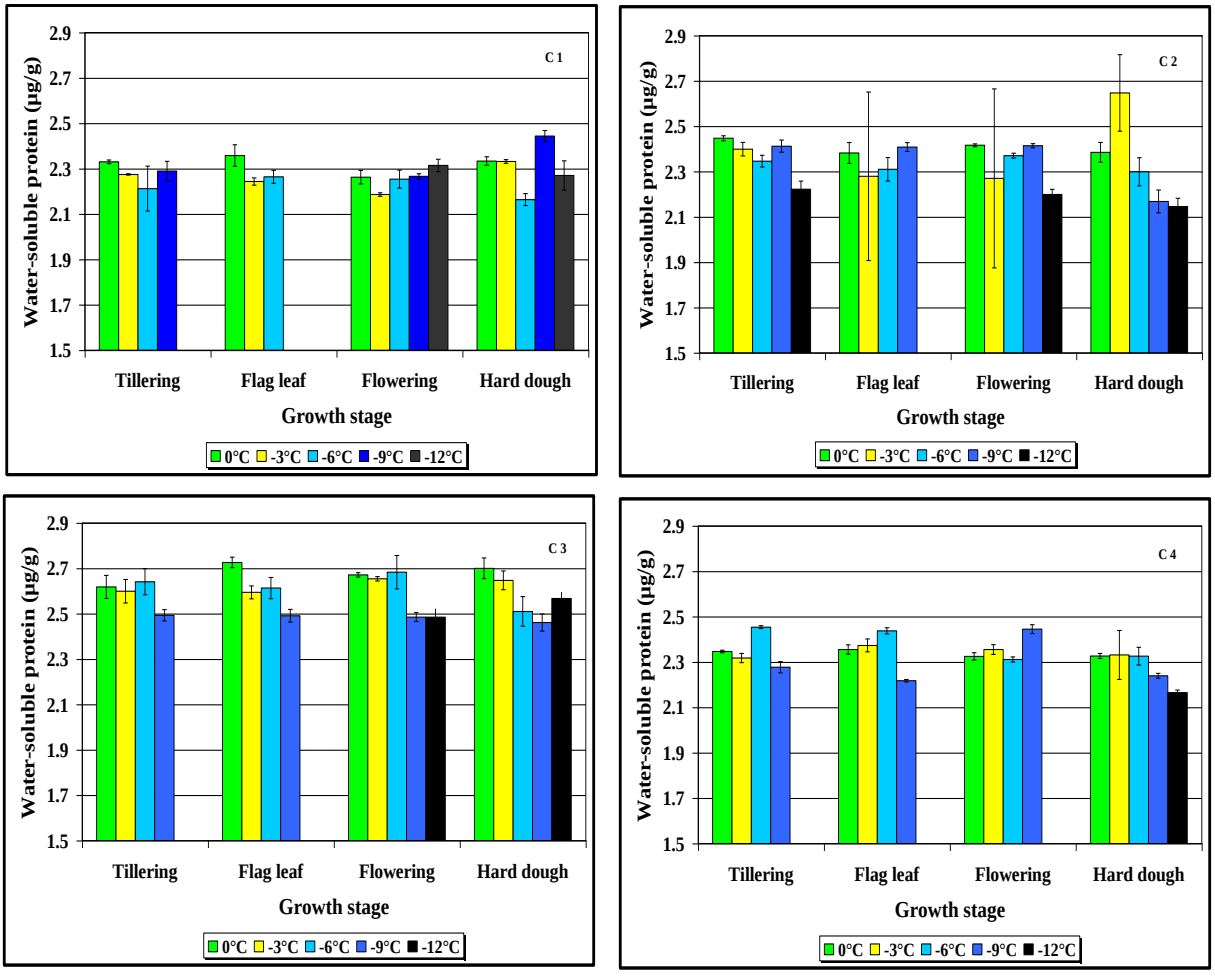


Figure 5.1 **Water-soluble protein content as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).**

In cultivar 2 a reduction in water-soluble protein content was observed at the tillering, flowering and hard dough growth stages and especially after exposing plants to –12°C (Figure 5.1 – C2). The highest water-soluble protein content in cultivar 2 was obtained at –3°C (2.647 µg/g; therefore 9.93% higher than that of the control average of 2.408 µg/g) at the hard dough stage whereas the largest reduction was obtained at –12°C (10.88%) at the same growth stage (Figure 5.1 – C2). No significant differences were observed in water-soluble proteins for growth stage as a main effect (tillering = 2.365, flag leaf = 2.345, flowering = 2.334 and hard dough stage = 2.329 µg/g) but a decrease in temperature showed a decreasing tendency in water-soluble proteins with the exception of –9°C (0 = 2.408, –3 = 2.399, –6 = 2.331, –9 = 2.351 and –12°C = 2.189 µg/g).

In cultivar 3 (Figure 5.1 – C3) the tendency to decrease the water-soluble protein content was observed for all growth stages when temperature was decreased from 0 to –12°C (0 = 2.679, –3 = 2.624, –6 = 2.612, –9 = 2.483 and –12°C = 2.526 µg/g). The largest reduction was at –9°C (8.14%) at the hard dough stage but no significant differences were obtained in water-soluble proteins for growth stage as a main effect (tillering = 2.588, flag leaf = 2.606, flowering = 2.596 and hard dough stage = 2.577 µg/g).

In cultivar 4, the spring type, only the tillering, flag leaf and hard dough growth stages showed a reduction in the water-soluble protein content at a temperature of –9°C and these reductions were 2.61%, 6.86% and 4.23%, respectively. This reduction was accentuated at –12°C at the hard dough growth stage (Figure 5.1 – C4). With the exception of the hard dough stage that showed a slight reduction in water-soluble protein content, no significant differences were obtained in water-soluble proteins for growth stage as a main effect (tillering = 2.349, flag leaf = 2.346, flowering = 2.359 and hard dough stage = 2.277 µg/g). Only temperatures below –6°C showed a reduction in water-soluble protein content (0 = 2.338, –3 = 2.344, –6 = 2.382, –9 = 2.295 and –12°C = 2.165 µg/g).

In summary, although cultivars differed slightly in terms of the water-soluble protein content in kernels their response to cold treatment was significantly different. Except for cultivar 1, the other three cultivars showed a slight reduction in water-soluble protein content at one or

the other growth stage as temperature decreased but especially when exposed to the lower temperature range.

5.3.2 Total protein content (%)

In cultivar 1 a slight decrease in the total protein content was observed at the tillering stage at –3 (3.95%) and –6°C (6.37%) compared to the control (0°C = 17.91% total protein). A reduction was also observed at the flowering stage at –9 (6.93%) and –12°C (3.58%). At the hard dough stage, decreasing temperatures did not have a negative effect on the total protein content of the kernels (Figure 5.2 – C1).

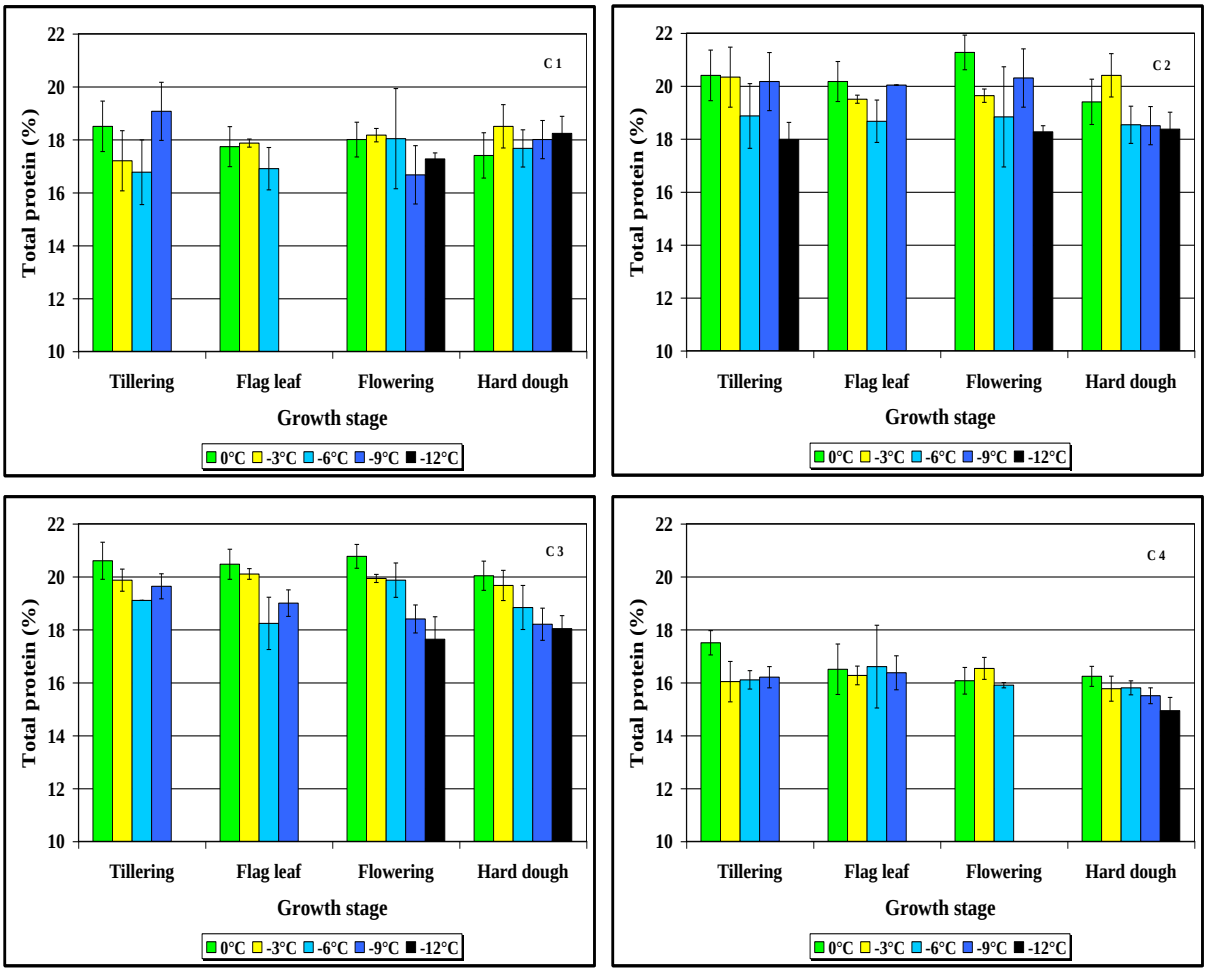


Figure 5.2 **Total protein content as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).**

In cultivar 2 a reducing tendency in the total protein content was observed in all growth stages as temperatures decreased with the exception of the tillering, flag leaf and flowering stages at –9°C as well as the hard dough growth stage at –3°C (Figure 5.2 – C2). The largest reduction was obtained at –12°C at the tillering, flowering and hard dough growth stages, with respective reductions of 11.53, 10.05 and 9.56%. Growth stage as a main factor had no effect on protein content but the decreasing temperature slightly decreased the protein content of wheat kernels (0°C = 20.31%, –3°C = 19.97%, –6°C = 18.73% –9°C = 19.75% and –12°C = 18.32%).

In cultivar 3 a decrease in total protein content was observed in all growth stages as temperature decreased (Figure 5.2 – C3). Though this cultivar had the highest total protein content (20.47% with regard to the control – 0°C at all growth stages) compared to other cultivars, it also showed the largest degree of reduction under the influence of low temperature. This was marked at especially the flowering stage at –12°C (13.85%) as well as the hard dough growth stage (18.95%). As temperature decreased a linear reducing response in terms of the total protein content was observed in cultivar 3 (0°C = 20.47%, –3°C = 19.89%, –6°C = 19.01%, –9°C = 18.81% and –12°C = 17.83%).

In cultivar 4 the total protein content was only slightly reduced with decreasing temperatures at the tillering and hard dough growth stages (Figure 5.2 – C4) with the largest reduction (9.90%) at the hard dough growth stage at –12°C compared to the control.

In summary, the total protein content did not differ significantly at the different growth stages for cultivars 1, 2 and 3 compared to the control (0°C). However, in cultivar 4 the total protein content measured at the flowering and hard dough stages was slightly lower than that of the tillering and flag leaf stages. Although there were no significant differences between the growth stages in terms of the total protein content, cultivar 3 (20.47%) had the highest total protein content compared to the average of the control (0°C), followed by cultivars 2, 1 and 4 (20.31, 17.91 and 16.57%, respectively).

5.3.3 Stirring number

In cultivar 1 a reduction in stirring number was observed in all growth stages with a decrease in temperature and this was marked (27.67%) in the hard dough stage at –12°C (Figure 5.3 – C1) compared to the control (0°C). Both growth stage and temperature as main factors had a decreasing effect on the stirring number in cultivar 1.

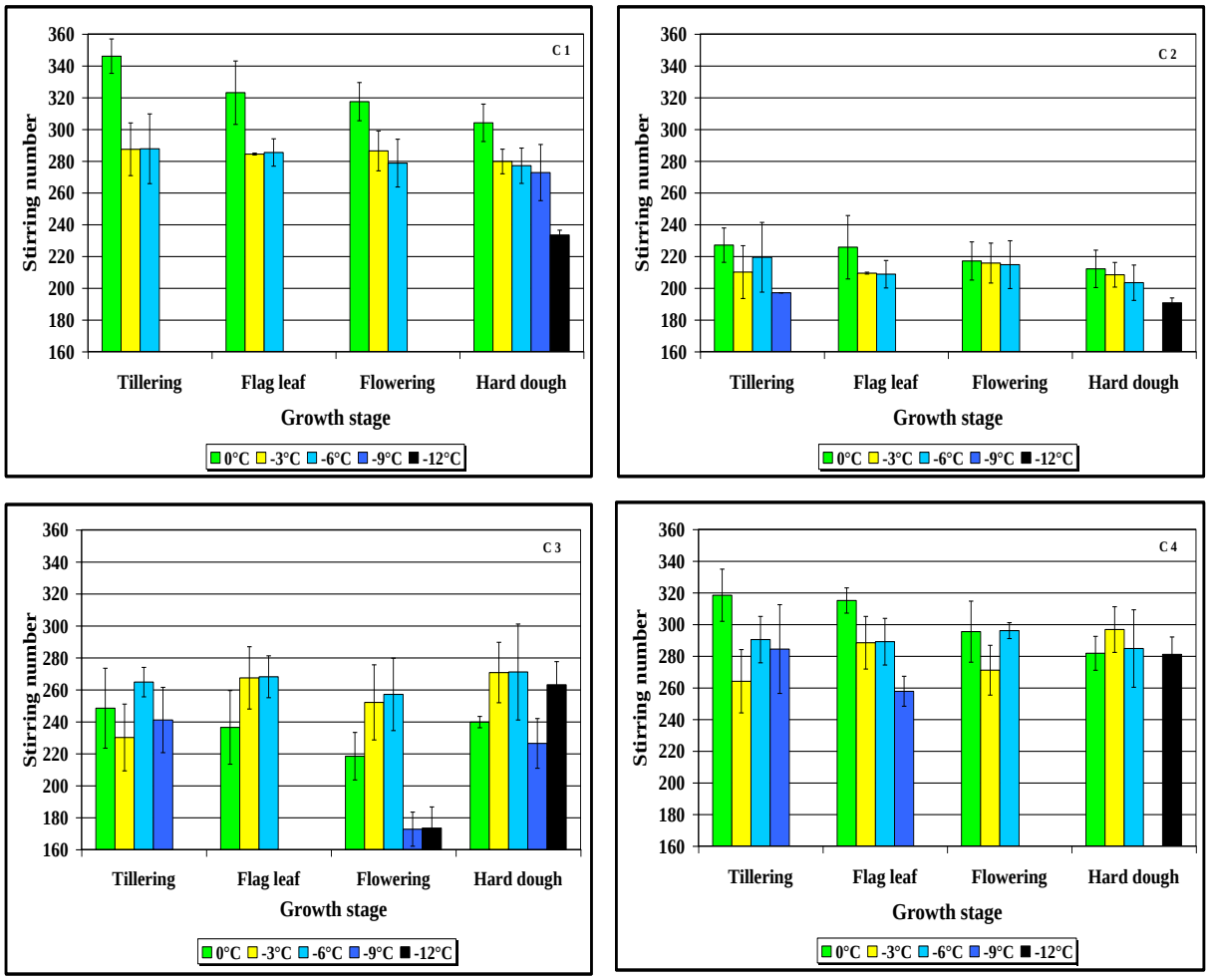


Figure 5.3 Stirring number as affected by different temperatures at different growth stages for different cultivars (C1 and C2 – winter type, C3 – intermediate type and C4 – spring type).

Although the stirring number measured in cultivar 2 was far lower than that of cultivar 1, the tendency for stirring number to drop at virtually all growth stages in both cultivars was

similar (Figure 5.3 C1 and 2). Especially at the hard dough stage this tendency was more evident (-13.5%) and at the low temperature range.

In cultivar 3 a slight increase in the stirring number was observed at all growth stages at a temperature range between 0 and –6°C, with the exception of the tillering stage (Figure 5.3 – C3). At –9 and –12°C marked reductions were observed during the flowering stage.

In cultivar 4 a drop in stirring number was only observed at the tillering and flag stages as temperatures decreased (Figure 5.3 – C4). The largest reduction (14.8%) was obtained at –9°C at the flag leaf stage. At the flowering and hard dough stages no significant reduction in the stirring number was observed.

In summary, the winter wheat types (cultivars 1 and 2) seemed to be most sensitive to a drop in temperature in terms of stirring number reductions and this was the case at all growth stages. However, in both cultivars the hard dough stage was most sensitive, especially at –12°C. In terms of a decrease in stirring number cultivar 3 was highly sensitive at the flowering stage at extremely low temperatures (–9 and –12°) while in cultivar 4 a reduction was observed at the tillering and flag leaf stages.

5.4 DISCUSSION and CONCLUSION

With this study an attempt was made to evaluate grain quality obtained from Experiment 1. Both water-soluble and total protein content as well as the stirring number of kernels was used as quality parameters. Protein content is recognised as one of the most important quality parameters (Weegels *et al*., 1996) and this is depicted in the price fetched by the producer. Likewise, stirring number or for that matter falling number is also regarded as an informative quality parameter as it is indicative of the milling quality of wheat kernels (Tipples, 1980). Unfortunately, due to a lack of material, these parameters could not be measured for all treatments. However, sufficient information was obtained for all growth stages to observe tendencies in terms of kernel quality. The results obtained in this study confirmed, to a large extent, what other researchers have previously reported.

According to Tipples (1980) the ash content of frost damaged and immature kernels affected milling performance and reduced flour colour. Not only are the milling performance affected, but the milling process is also affected. Frost damage increases kernel hardiness and therefore more energy is needed during the milling process (Dexter *et al*., 1985). An increased difficulty in separating endosperm from the bran resulted in a higher proportion of shorts (that is bran material) in frost damaged wheat kernels compared to sound kernels (Preston, Kilborn, Morgan & Babb, 1991). According to Preston *et al*. (1991) the effect of frost damage on the end use quality of wheat is dependant on the stage of crop development (maturity) as well as the duration and degree of frost. This was also observed during this study.

The average result of the parameters obtained at each of the growth stages measured before grain filling commenced (tillering, flag leaf and flowering stages) showed no differences in cultivars 2,3 and 4 with the exception of the flowering stage in cultivar 3. The only marked reduction in water-soluble and total protein content as well as stirring number was obtained at the hard dough growth stage of these cultivars. Furthermore, this tendency was more pronounced for the total protein content.

Protein content is recognised as one of the most important components governing bread-making quality (Weegels *et al*., 1996). Protein quality is also an important consideration. The end-use quality of wheat is uniquely affected by the protein fractions that constitute gluten protein, namely gliadin and glutenin (Stone & Savin, 1999). Glutenin is a polymeric protein that forms strong bonds, reduces dough extensibility and is the protein fraction responsible for dough strength (Stone & Savin, 1999; Wieser & Kieffer, 2001). Conversely, gliadins are responsible for the viscous properties of dough during mixing. The gliadin fraction is the first storage protein fraction to accumulate in quantity and is synthesised most rapidly during the mid development of the wheat kernel. Conversely, the glutenin fraction is not present in large quantities in the kernel until the latter half of the grain filling period (Stone & Savin, 1999). Stone, Gras and Nicolas (1997) determined that high temperature stress reduced the grain-filling period, thus reducing the glutenin synthesis and therefore reducing the dough strength. Moreover, conditions during the grain filling period are likely

to have and effect on protein quality. Dexter *et al*. (1985), Preston *et al*. (1991) and Tipples (1980) reported on the poor bread-making quality of frost damaged wheat and the alteration in the composition of the proteins could partially explain this phenomenon.

From this study it is concluded that frost damage affects wheat quality and it is of utmost importance to the producer and consumer. A comprehensive study in this regard has to be envisaged for South African conditions to determine the extent frost damage has on wheat quality in order to overcome the short falls of this study, namely a lack of sufficient material to conduct a comprehensive study on the effect of frost stress on wheat quality.

CHAPTER 6

ASSESSMENT OF FROST STRESS TOLERANCE IN SOUTH AFRICAN WHEAT DURING THE FLAG LEAF AND FLOWERING STAGES

6.1 INTRODUCTION

Chapter 4 showed that the reproductive tissues of the developing wheat ear are extremely susceptible to freezing as a result of frost damage and the only way it can avoid or escape injury at subzero temperatures is through supercooling (Single and Marcellos, 1974). Marcellos (1977) reported that wheat crops in Australia frequently encounter overnight radiation frost at susceptible stages of growth in late winter and early spring. Plants are also subjected to temperatures of 0 to –7°C and at these temperatures may experience freezing (at night) followed by thawing during the day at temperatures as high as 20°C. Single (1971) reported that histological damage to stems and leaves as well as the death of reproductive organs may occur depending on the severity and timing of frost and in many instances freezing was tolerated without any visible effects.

The risk of spring frost precludes early sowing of wheat. The delay in sowing of wheat can reduce yield by shortening the grain filling period, before summer drought and when high temperature becomes a limiting factor (Marcellos & Single, 1972; Doyle & Marcellos, 1974). Halse and Weir (1974) reported that the differences in heading date of Australian produced wheat cultivars could be determined by differences in vernalisation or response to day length as well as differences in basic development rate. Cultivars are enabled by these mechanisms to be sown so that flowering and grain filling can occur under satisfactory conditions. Therefore the crops growth and development has to be delayed to avoid frost injury and yet the delay should be not so late as to have grain filling coincide with the onset of summer drought (Fletcher, 1988).

If resistance to freezing injury was available in commercial wheat cultivars, the crop could flower earlier in many Australian wheat growing areas (Fletcher, 1988) and this is also valid under South African conditions. This could lead to a longer grain filling period since temperatures would be lower and moisture more available. Genetic variability is accountable for resistance to freezing injury since Single (1966) reported variability in resistance to injury during stem elongation.

The aim of this study was to investigate if there was a difference between the reaction of winter and intermediate types of cultivars to frost injury during the flag leaf and flowering stages. Furthermore, these two growth stages were divided into an early flag leaf, a flag leaf and emergence of the awns stages for the flag leaf stage and during the flowering stage it was divided into a 0, 50 and 100% flowering stages. This was done to determine if there were differences at these stages to the degree of frost injury sustained.

6.2 MATERIAL and METHODS

See Chapter 3, section 3.1.2, 3.1.3, 3.2, 3.3.1 and 3.4

6.3 RESULTS

Plant dry matter has been used to evaluate the growth analysis. Different plant and yield components have also been used to evaluate the influence of frost stress on wheat production for three different sub zero temperatures (–5, –7 and –9°C) at different growth stages and are presented in Figures 6.1 – 6.9 and Tables 6.1 – 20. For all parameters the different cultivars were grouped and the different cultivars were annotated as follows: Winter type – C1 and intermediate type – C2. Analysis of variance for the different parameters is presented in Appendix 6.1 – 6.62.

Some of the parameters were fractionated in three different components (primary, secondary and total) where applicable. This was done where spike components were used. The reason for this was that the primary spike was used as indicator of the specific growth stage and therefore not all spikes were at the same stage of growth and development. The importance

to distinguish between primary and secondary parameters was imperative to show differences in reaction to frost injury between the two cultivars at the different growth stages.

6.3.1 The reaction of different flag leaf stages to frost injury

The winter wheat cultivar showed no significant difference between temperature and growth stage for all measured parameters with the exception of the number of spikelets per primary spike, the number of spikelets per secondary spike and the number of spikelets per spike produced. The intermediate wheat type showed a higher degree of significant interaction between temperature and growth stage than the winter wheat type and this could be an indication of a higher level of sensitivity to frost injury.

6.3.1.1 Dry matter

Although a reduction in dry matter production occurred for both cultivars (Figure 6.1), only cultivar 2 showed a significant reduction as a result of decreasing temperatures (Appendix 6.1 – 6.2). Cultivar 1 was more tolerant to frost injury than cultivar 2 and this confirmed the results outlined in Chapter 4. Therefore, the winter type was found to be more tolerant than the intermediate type and this was probably a result of the growth pattern difference between these two cultivars. The intermediate wheat type (C 2) has a shorter growing season and also produces fewer tillers than the winter wheat type (C 1). Therefore the variation in age of the tillers are less for the intermediate type and if damage or stress should occur, the degree of injury will be greater for the intermediate wheat type.

The winter wheat type has a longer growing season and produces more tillers. These tillers vary in age and the younger tillers are more tolerant to frost injury than the older tillers. This is possible for the growth points are protected in the crown beneath the soil surface. If some of the older tillers were to be damaged by frost, the younger tillers would compensate for the loss of tillers. The degree of compensation can be seen in the reduction of dry matter between the cultivars. Cultivar 1 showed a reduction of 10.3 and 12.4% at –7 and –9°C respectively. Cultivar 2 showed a reduction of 20.7 and 53.2% at the respective temperatures, thus showing the sensitivity of cultivar 2 and the inability to compensate.

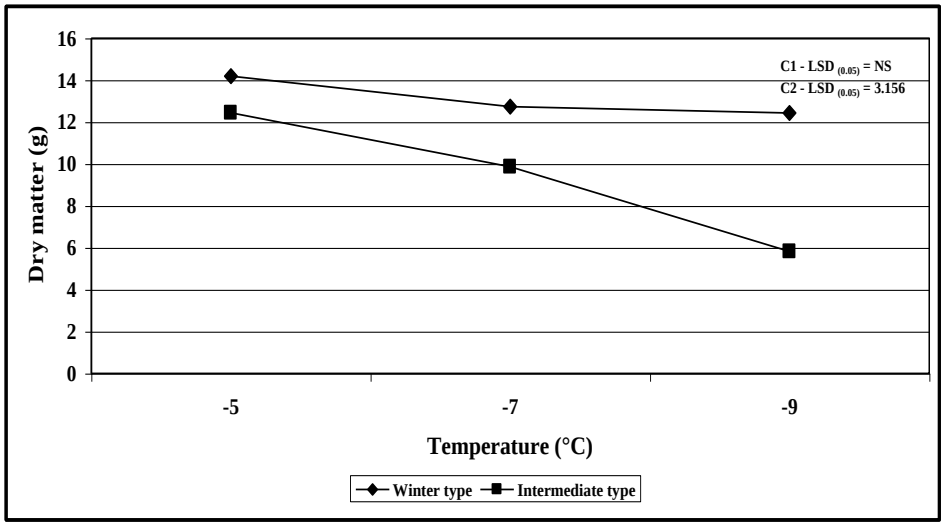


Figure 6.1 Dry matter production as affected by different temperatures for different cultivars (C1 – winter type and C2 – intermediate type).

6.3.1.2 Spikes per plant

The number of spikes per plant was significantly influenced by the main effects, temperature (both cultivars) and growth stage (intermediate cultivar) (Appendix 6.3 – 6.4). The number of spikes per plant for the winter type was reduced by 49.5% at –9°C, while that of the intermediate cultivar was reduced by 29.1 and 25.3% at –7 and –9°C, respectively. The winter type was found to be more tolerant to frost injury than the intermediate type with no reduction in the number of spikes per plant between –5 and –7 °C, while the intermediate cultivar was found to be sensitive at –7 °C (Figure 6.2).

Only the fully developed spikes were counted and not all the tillers that initially sprouted. This means that some of the tillers died as a result of frost injury and others survived, depending on their stage of development. Therefore, the winter type cultivar that usually initiates more secondary tillers than the intermediate cultivar had a higher compensation ability.

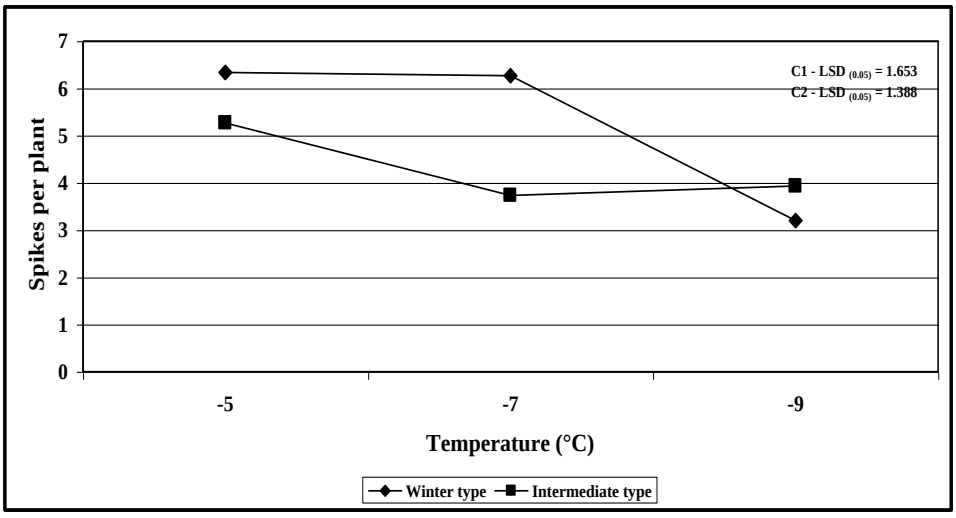


Figure 6.2 **Number of spikes as affected by different temperatures for different cultivars (C1 – winter type and C2 – intermediate type).**

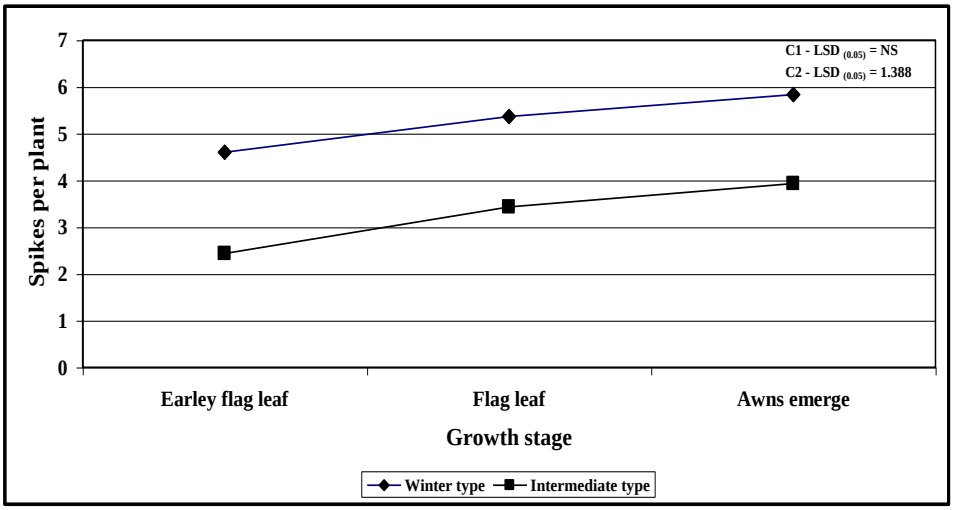


Figure 6.3 **Number of spikes as affected at different growth stages by frost injury for different cultivars (C1 – winter type and C2 – intermediate type).**

No significant differences exist for the winter type wheat, but the cumulative effect of temperature at the different flag leaf stages of the intermediate wheat type show a significant increase in the number of spikes per plant. This also indicates that the plants became more tolerant to frost injury as the plant progresses through its development with regard to specific organs, in this case the number of spikes. Therefore more spikes died as a result of frost

injury during the early flag leaf stage than after the awns have emerged (Figure 6.3). This is valid for the intermediate wheat type. Although the winter type showed no significant differences between the different flag leaf growth stages as observed in the intermediate type the same tendency revealed itself in the winter type making this cultivar more tolerant and stable.

6.3.1.3 Spikelets per spike

6.3.1.3.1 Spikelets per primary spike

Data for the number of spikelets per primary spike showed that the cultivars reacted differently to frost injury during the different categories of flag leaf stage development and that significant differences were obtained in the number of spikelets per primary spike as a result of the interaction between temperature and growth stage (Appendix 6.5 – 6.6).

In cultivar 2 a significant reduction in the number of spikelets per primary spike was observed. The reduction during the early flag leaf stage was 58.1% at –7°C and 100% at –9°C (Figure 6.4). Cultivar 1 showed a significant reduction of 63% during the early flag leaf stage at –9C. This also indicated that cultivar 1 was more tolerant to frost injury than cultivar 2 and that cultivar 2 could not withstand temperatures lower than –5°C.

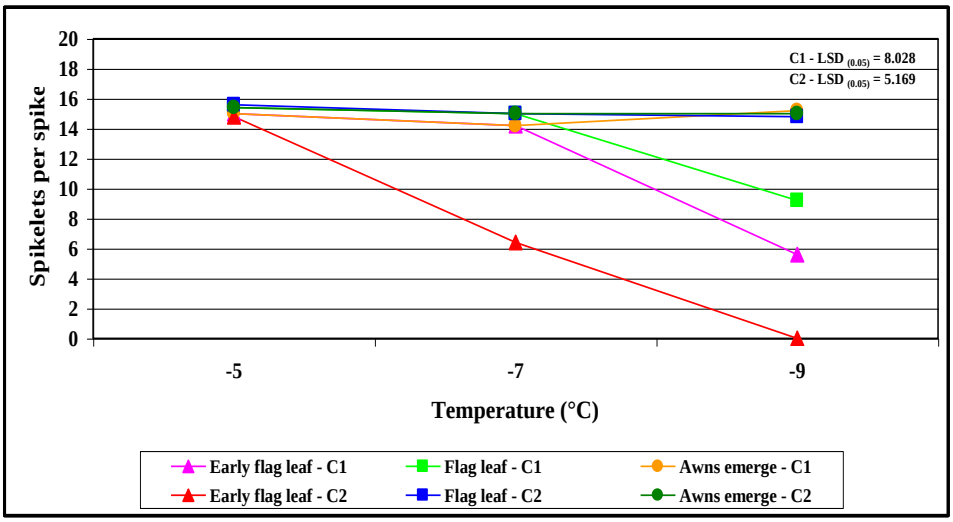


Figure 6.4 Number of spikelets per primary spike as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type).

6.3.1.3.2 Spikelets per secondary spike

Both cultivars showed a slight reduction in the number of spikelets per secondary spike at –7°C compared to the average number of spikelets at –5°C (Figure 6.5). In cultivar 1 a reduction of 44.8% at –9°C was obtained during the early flag leaf stage, but still was not significantly lower than the average of the different growth stages at –5°C (Appendix 6.7). At –9°C a significant reduction occurred where cultivar 2 experienced a 100% reduction in spikelet number during all the flag leaf growth stages and therefore only temperature had a significant influence on the number of spikelets produced per secondary spike (Appendix 6.8). The $LSD_{(0.05)}$ of 2.602 is only valid for the main factor namely temperature for cultivar 2 for only temperature had a significant influence on spikelet number.

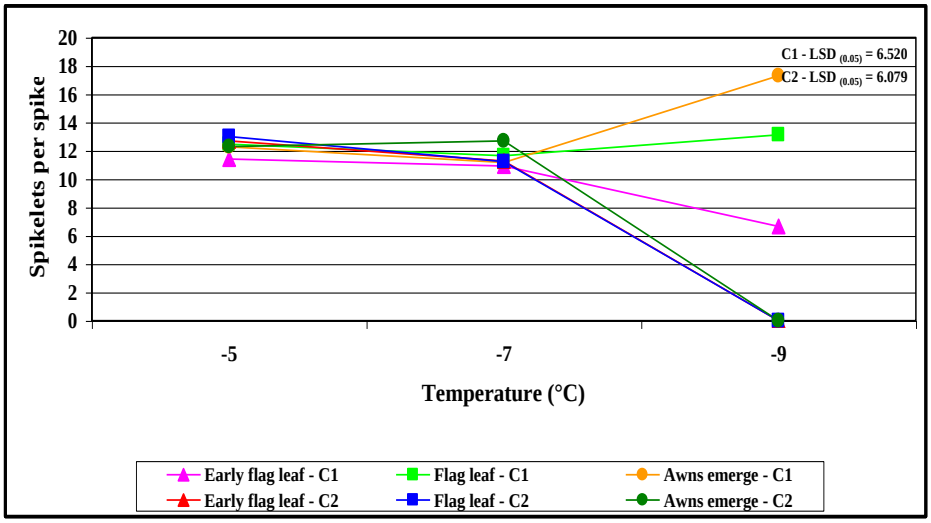


Figure 6.5 Number of spikelets per secondary spike as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type).

6.3.1.3.3 Average number of spikelets per spike

Significant differences were obtained for the average number of spikelets per spike as affected by the interaction of temperature and growth stage for cultivar 1 and 2 (Appendix 6.9 – 6.10). The combination of the number of spikelets per primary and secondary spike resulted in a slightly different picture that can be seen from the data presented in Figure 6.6.

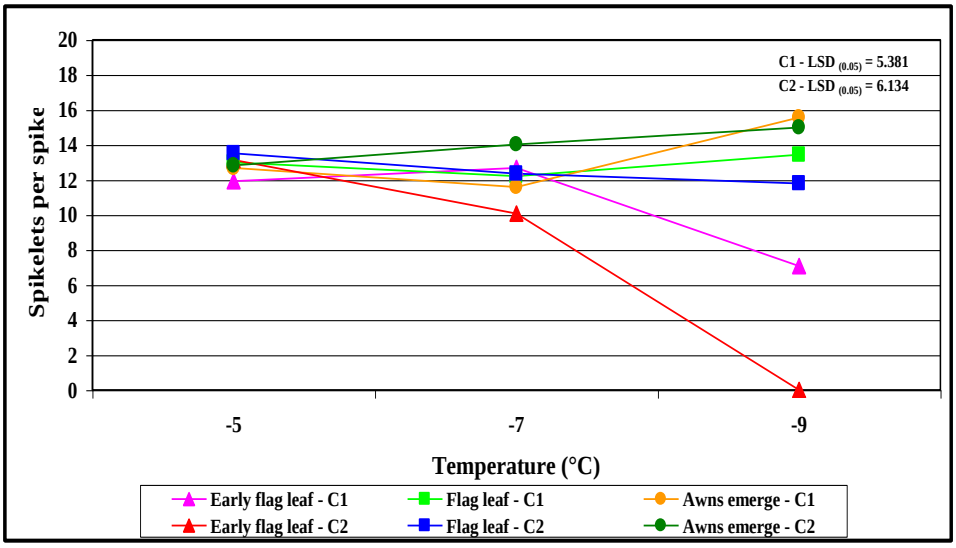


Figure 6.6 **Average number of spikelets per spike as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type).**

No significant differences in the average number of spikelets per spike were obtained for both cultivars at –5 and –7°C and for all the growth stages. At –7°C the early flag leaf stage of cultivar 2 showed a reduction of 23.5% and this reduction reached its ultimate peak at –9°C with 100 %. In cultivar 1 a reduction 43.5% with regard to the average number of spikelets of this cultivar at –5°C, was observed at –9°C.

It is clear that cultivar 2 is more prone to frost injury than cultivar 1 and that the early flag leaf stage showed to be the most sensitive growth stage. At –5°C no damage was sustained by any of the growth stages for both cultivars. This was not the case at –7°C where the early flag leaf stage was the most sensitive growth stage, especially for cultivar 2 with regard to primary spikes. The spikelets of the secondary spikes, though not significantly, showed a slight reduction in the average number of spikelets per spike at –7°C. All the growth stages of cultivar 2 were very sensitive to frost injury compared to the primary spikes and this confirmed that the intermediate wheat type was more sensitive to frost damage than the winter wheat type.

6.3.1.4 Kernel count

The number of kernels produced by the primary and secondary spikes could, as a parameter, provide an indication of the effect of subzero temperatures at the different flag leaf growth stages and also confirm the damage that the spikelets encountered.

6.3.1.4.1 Number of kernels produced by primary spikes

The number of kernels produced by the primary spikes showed no significant difference in cultivar 1 as a result of the interaction between temperature and growth stage (Appendix 6.11). The main factor, namely temperature, had a significant influence on the number of kernels produced and the number of kernels (7.133) at –9°C was significantly lower than that at –5 (28.167) and –7°C (22.200) with a LSD $_{0.05}$ of 11.427.

The number of kernels produced by the primary spikes of cultivar 2 differed significantly as a result of the interaction between temperature and growth stage (Appendix 6.12). At –7°C the reduction in the number of kernels produced compared to the average number of kernels at –5°C (28.667) was reduced by 69.0, 78.0 and 87.4% during the early flag leaf, flag leaf and awns emerging growth stages, respectively. At –9°C the reduction was 100% for all growth stages of cultivar 2 (Figure 6.7).

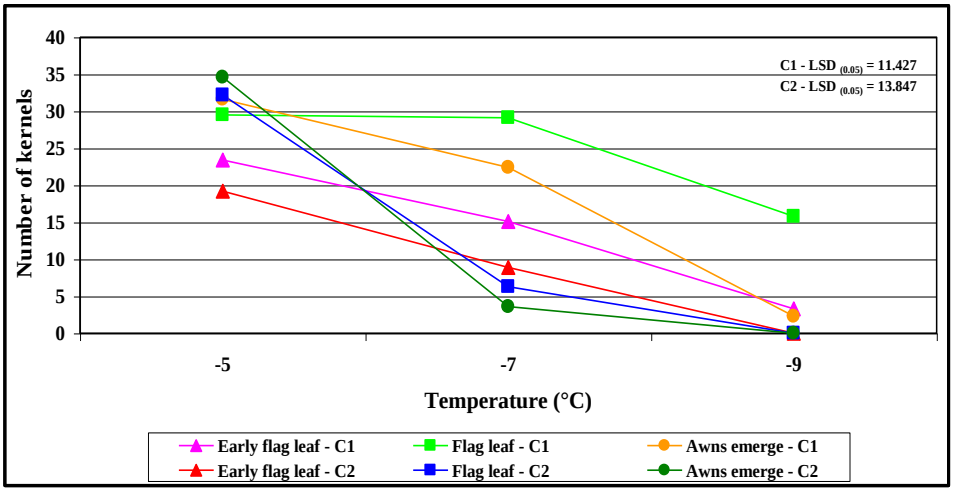


Figure 6.7 Number of kernels produced by the primary spike as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type).

This reduction in the number of kernels is contradictory to the number of spikelets produced per primary spike. This may indicate that the flowering male and female parts become more sensitive to frost injury as the plant's development progresses and gets closer to flowering.

6.3.1.4.2 Number of kernels produced by secondary spikes

No significant differences in the number of kernels produced by the secondary spikes were found for cultivar 1, but cultivar 2 showed significant differences as a result of temperature (Appendix 6.13 – 6.14).

Figure 6.8 clearly show that both cultivars experienced a reduction in the number of kernels produced by the secondary spikes. In cultivar 1 a severe reduction was obtained at –9°C, though not significant. Cultivar 2, the more sensitive cultivar, showed a reduction of 35.7% at –7°C and a 100% at –9°C. The number of kernels produced at –7 and –9°C were both significantly lower than that at –5°C. This also showed that cultivar 2 was more sensitive to frost injury than cultivar 1.

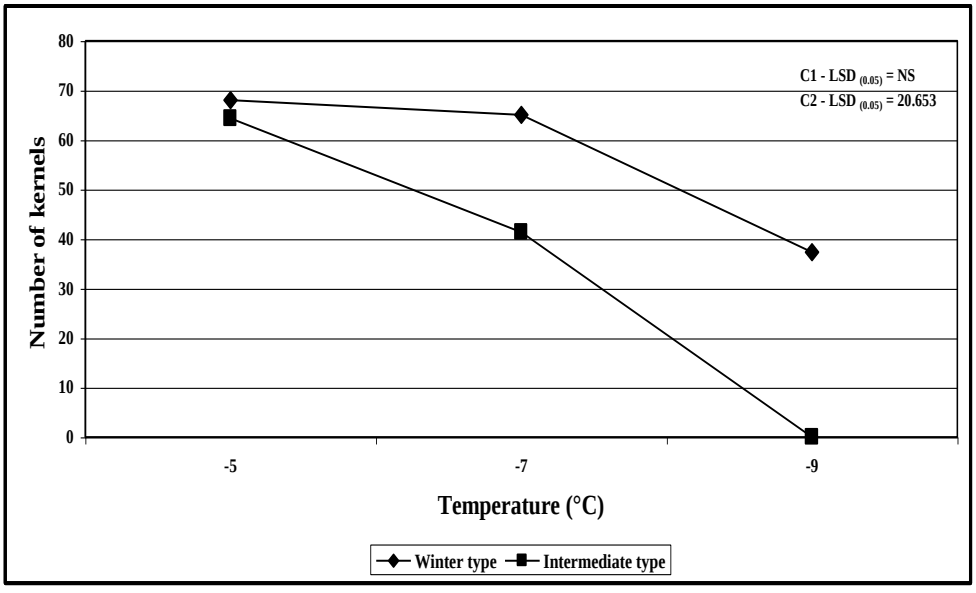


Figure 6.8 Number of kernels produced by secondary spikes as affected by different temperatures for different cultivars (C1 – winter type and C2 – intermediate type).

6.3.1.4.3 Total number of kernels produced by primary and secondary spikes

The total number of kernels produced by both cultivars showed significant differences as a result of the decrease in temperature (Appendix 6.15 – 16). Cultivar 1 showed a slight reduction (9.3%) in the total number of kernels produced at –7°C compared to that at –5°C, but at –9°C the reduction was 53.8% (Figure 6.9). This was significantly lower than the number of kernels produced at –5 and –7°C.

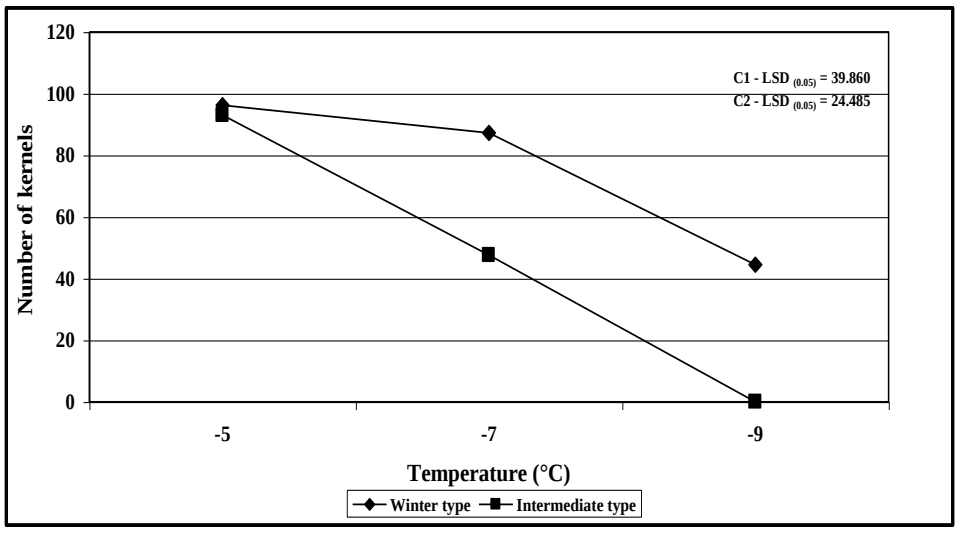


Figure 6.9 Total number of kernels as affected by different temperatures for different cultivars (C1 – winter type and C2 – intermediate type).

The total number of kernels produced by cultivar 2 was severely reduced at –7°C (48.8%) and at –9°C (100%). This shows that cultivar 2 is definitely more sensitive to frost injury than cultivar 1 and that the primary spikes were also more sensitive to frost injury than the secondary spikes. This phenomenon is attributed to the fact that the secondary spikes are younger than the primary spike and has not yet developed into the more sensitive growth stage as the primary spike has done and are therefore less sensitive to frost injury.

6.3.1.5 Number of kernels per spike

The number of kernels produced per spike could, as a parameter, provide information on the sensitivity and reaction of primary and secondary spikes at different growth stages to declining temperatures.

6.3.1.5.1 Number of kernels per primary spike

The number of kernels produced by the primary spikes of cultivar 1 showed no significant difference as a result of the interaction between temperature and growth stage (Appendix 6.11). The main factor namely temperature had a significant influence on the number of kernels produced and the number of kernels was significantly lowered with decreasing temperatures. The number of kernels produced by the primary spikes of cultivar 2 differed significantly as a result of the interaction between temperature and growth stage (Appendix 6.12). The data is presented in Figure 6.7. The discussion on this parameter is exactly the same as that presented in 6.3.1.4.1. The reasons for this is simply because there can only be one primary spike and therefore the number of kernels produced by the primary spike is equal to the number of kernels per primary spike. This is not true for the secondary spikes as well as the total number of kernels per spike and therefore the number of kernels per secondary spike will be discussed comprehensively.

6.3.1.5.2 Number of kernels per secondary spike

The number of kernels produced by the secondary spikes of both cultivars showed no significant differences as a result of the interaction between temperature and growth stage (Appendix 6.17 – 6.18). Both the main factors, temperature and growth stage showed no significant effect on the number of kernels produced by the winter type, cultivar 1. In cultivar 2, the intermediate type, decreasing temperature negatively affected the number of kernels produced by the secondary spikes and the reduction in kernel number per spike was 12.9% at –7 °C and 100% at –9°C (Table 6.1).

This again showed that cultivar 2 was less tolerant than cultivar 1 to frost injury. The reason for this major reduction is that the secondary spikes of the intermediate wheat type develop at a faster rate than the winter type. Therefore the difference in age between the primary spike

and secondary spikes of cultivar 2 is restricted, which means that the secondary spikes are just as vulnerable as the primary spike. This is very important with regard to frost avoidance and recommendations of planting dates choice of cultivar.

Table 6.1 Number of kernels per secondary spike as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage			
		Early flag leaf	*Flag leaf*	*Awns emerge*	*Average*
Winter type	**-5**	11.1	14.5	12.8	***12.811***
	-7	10.3	16.2	9.1	***11.859***
	-9	13.1	14.2	5.6	***10.988***
	Average	***11.482***	***14.971***	***9.205***	***11.886***
Intermediate type	**-5**	12.1	18.8	18.1	***16.340***
	-7	14.4	15.4	12.9	***14.235***
	-9	0	0	0	***0***
	Average	***8.845***	***11.406***	***10.324***	***10.192***

Winter type - T = LSD $_{0.05}$ = NS; G = LSD $_{0.05}$ = NS; T x G = LSD $_{0.05}$ = NS

Intermediate type - T = LSD $_{0.05}$ = 7.093; G = LSD $_{0.05}$ = NS; T x G = LSD $_{0.05}$ = NS

T = Temperature G = Growth stage T x G = Temperature, growth stage interaction NS = Not significant

6.3.1.5.3 Average number of kernels per spike

Results on the average number of kernels produced per spike, which include primary and secondary spikes, are presented in Appendix 6.19 – 6.20. Treatment combinations for the different cultivars, growth stages and temperature, did not have a significant influence on the average number of kernels produced per spike. Though not significant, cultivar 1 showed a severe reduction in the average number of kernels produced per spike when the awns emerged at –9°C (Table 6.2). Neither growth stage nor temperature, as main factors, significantly influenced the number of kernels produced per spike for cultivar 1.

Cultivar 2 showed a reduction in the average number of kernels produced per spike at –7°C of 32.5% and a 100% reduction occurred at –9°C. The latter was significantly lower than the number produced at –5 or –7°C.

Table 6.2 Average number of kernels per spike as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage			
		Early flag leaf	*Flag leaf*	*Awns emerge*	*Average*
Winter type	**-5**	13.0	17.3	15.8	***15.365***
	-7	11.5	17.7	11.0	***13.397***
	-9	12.5	14.6	5.0	***10.681***
	Average	***12.307***	***16.542***	***10.594***	***13.148***
Intermediate type	**-5**	13.9	21.6	20.6	***18.712***
	-7	13.0	12.4	12.5	***12.623***
	-9	0	0	0	***0***
	Average	***8.952***	***11.349***	***11.034***	***10.445***

Winter type - $T = LSD_{0.05} = NS$; $G = LSD_{0.05} = NS$; $T \times G = LSD_{0.05} = NS$

Intermediate type - $T = LSD_{0.05} = 6.432$; $G = LSD_{0.05} = NS$; $T \times G = LSD_{0.05} = NS$

From the previous discussion it is clear that the primary spikes were affected severely by frost injury and that cultivar 2, the intermediate cultivar, was more sensitive to frost injury than cultivar 1. The secondary spikes of cultivar 2 also seemed to be more sensitive to frost injury than that of cultivar 1. The degree of grain set of cultivar 1 was more stable than cultivar 2 and in this regard cultivar 1, representative of the winter wheat type, would be the more favourable cultivar choice in areas where frost occur or the planting date of cultivar 2 should be adjusted accordingly.

6.3.1.6 Kernel weight

6.3.1.6.1 Primary kernel weight

The kernel weight produced by the primary spike showed no significant differences for the treatment combinations for cultivar 1, but in the case of cultivar 2 the treatment combinations lead to a significant difference in kernel weight (Appendix 6.21 – 6.22). Table 6.3 clearly show that the kernel weight of cultivar 1 was reduced from –5 to –9°C. The reduction at –7°C was 21.8% and at –9°C 72.6%, but only the latter was significantly lower than the kernel weight obtained at –5 and –7°C.

Table 6.3 Kernel weight produced by primary spikes as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage: *Early flag leaf*	*Flag leaf*	*Awns emerge*	*Average*
Winter type	**-5**	0.462	0.682	0.604	***0.583***
	-7	0.293	0.541	0.534	***0.456***
	-9	0.098	0.334	0.048	***0.160***
	Average	***0.284***	***0.519***	***0.395***	***0.400***
Intermediate type	**-5**	0.560	1.305	1.121	***0.995***
	-7	0.282	0.293	0.116	***0.230***
	-9	0	0	0	***0***
	Average	***0.281***	***0.533***	***0.412***	***0.409***

Winter type - $T = LSD_{0.05} = 0.245$; $G = LSD_{0.05} = NS$; $T \times G = LSD_{0.05} = NS$

Intermediate type - $T = LSD_{0.05} = 0.197$; $G = LSD_{0.05} = 0.197$; $T \times G = LSD_{0.05} = 0.461$

The kernel weight obtained in cultivar 2 was significantly affected by the treatment combination of growth stage and temperature. No explanation for the reduction in kernel weight of the early flag leaf stage at –5°C, which was significantly lower than that of the flag leaf and emergence of the awns at –5°C is ventured as deviations do occur naturally in

biological systems and are not always explainable. In terms of kernel weight the growth stages showed significant reductions at –7 and –9°C than at –5°C, with the exception of the early flag leaf stage, indicating that this cultivar was particularly sensitive to frost injury at temperatures below –5°C. A 100% reduction was obtained at –9°C for all the growth stages.

6.3.1.6.2 Secondary kernel weight

The weight of kernels produced by secondary spikes showed no significant differences as a result of the treatment combinations for cultivar 1 and 2 (Appendix 6.23 – 6.24). Cultivar 2 showed that temperatures below –7°C reduced the kernel weight with 100%, meaning that temperatures below –7°C were fatal to seed set (Table 6.4).

Table 6.4 Kernel weight produced by secondary spikes as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage			
		Early flag leaf	***Flag leaf***	***Awns emerge***	***Average***
Winter type	**-5**	1.165	1.625	1.414	***1.401***
	-7	0.992	1.597	1.437	***1.342***
	-9	0.466	1.474	0.515	***0.818***
	Average	***0.875***	***1.565***	***1.122***	***1.187***
Intermediate type	**-5**	1.215	2.460	2.191	***1.955***
	-7	0.932	1.250	1.234	***1.139***
	-9	0	0	0	***0***
	Average	***0.716***	***1.237***	***1.142***	***1.031***

Winter type - T = LSD $_{0.05}$ = NS
G = LSD $_{0.05}$ = NS
T x G = LSD $_{0.05}$ = NS

Intermediate type - T = LSD $_{0.05}$ = 0.594
G = LSD $_{0.05}$ = NS
T x G = LSD $_{0.05}$ = NS

6.3.1.6.3 Total kernel weight

No significant differences in total kernel weight were observed as a result of treatment combinations (Appendix 6.25 – 6.26). Cultivar 1 showed a reduction of 48.7% in kernel weight when the temperature decreased from –5 to –9°C and this reduction was also significantly lower than the kernel weight at –5°C (Table 6.5).

Table 6.5 Total kernel weight as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage			
		Early flag leaf	*Flag leaf*	*Awns emerge*	*Average*
Winter type	**-5**	1.638	2.307	2.018	***1.984***
	-7	1.285	2.138	1.971	***1.798***
	-9	0.564	1.808	0.563	***0.978***
	Average	***1.159***	***2.084***	***1.517***	***1.587***
Intermediate type	**-5**	1.775	3.765	3.312	***2.951***
	-7	1.214	1.543	1.350	***1.369***
	-9	0	0	0	***0***
	Average	***0.996***	***1.769***	***1.554***	***1.440***

Winter type - $T = LSD_{0.05} = 0.984$; $G = LSD_{0.05} = NS$; $T \times G = LSD_{0.05} = NS$

Intermediate type - $T = LSD_{0.05} = 0.737$; $G = LSD_{0.05} = 0.737$; $T \times G = LSD_{0.05} = NS$

Decreasing temperatures significantly reduced the kernel weight in cultivar 2. The kernel weight at –7°C was reduced by 53.6%, which was significantly lower than that at –5°C and at –9°C where a 100% reduction occurred. This was significantly lower than that of both the mentioned temperatures. A larger degree of reduction at –7°C occurred and this could mainly be ascribed to the contribution of the kernel weight produced by the primary spikes which were severely negatively affected by temperatures lower than –5°C.

6.3.1.7 Kernel weight per spike

6.3.1.7.1 Kernel weight per primary spike

Kernel weight per primary spike showed no significant differences for the treatment combinations for cultivar 1, but in the case of cultivar 2 the treatment combinations lead to a significant difference in kernel weight (Appendix 6.21 – 6.22). The data is presented in Table 6.3 and the discussion on this parameters is exactly the same as that presented in section *6.3.1.6.1*.

6.3.1.7.2 Kernel weight per secondary spike

The kernel weight produced per secondary spike was found to be influenced significantly by the growth stages for cultivar 1 and by temperature for cultivar 2 (Appendix 6.27 – 6.28). In cultivar 1 the early flag leaf stage and emergence of the awns produced a significant lower kernel weight per spike than the flag leaf stage (Table 6.6).

Table 6.6 Kernel weight per secondary spike as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage			
		Early flag leaf	***Flag leaf***	***Awns emerge***	***Average***
Winter type	**-5**	0.209	0.339	0.244	***0.264***
	-7	0.210	0.313	0.198	***0.240***
	-9	0.157	0.356	0.106	***0.206***
	Average	***0.192***	***0.336***	***0.183***	***0.237***
Intermediate type	**-5**	0.310	0.630	0.540	***0.493***
	-7	0.372	0.509	0.330	***0.404***
	-9	0	0	0	***0***
	Average	***0.227***	***0.380***	***0.290***	***0.299***

Winter type - T = $LSD_{0.05}$ = NS; G = $LSD_{0.05}$ = 0.148; T x G = $LSD_{0.05}$ = NS

Intermediate type - T = $LSD_{0.05}$ = 0.233; G = $LSD_{0.05}$ = NS; T x G = $LSD_{0.05}$ = NS

A decrease in temperature reduced the kernel weight produced by the secondary spikes of cultivar 2. At –9°C the reduction in kernel weight was 100% and this was significantly lower than that produced at –5 and –7°C. The reduction between –5 and –7°C was only 18% and this was less than the reduction that occurred in the primary spike at the same temperatures. This indicates that the secondary spikes are more tolerant to frost injury when frost occurs than the primary spikes are during this specific growth stage.

6.3.1.7.3 Average kernel weight per spike

The average kernel weight per spike, where the primary and secondary spikes were combined, showed nearly the same tendencies as that of the secondary spikes (Appendix 6.29 – 6.30). Once more the different growth stages showed a significant difference in average kernel weight for cultivar 1 where the flag leaf stage produced a significantly higher average kernel weight per spike than the early flag leaf and emerging awns growth stages (Table 6.7).

Table 6.7 Total kernel weight per spike as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage			
		Early flag leaf	*Flag leaf*	*Awns emerge*	*Average*
Winter type	**-5**	0.247	0.404	0.299	***0.317***
	-7	0.231	0.337	0.243	***0.271***
	-9	0.175	0.343	0.096	***0.205***
	Average	***0.218***	***0.361***	***0.213***	***0.264***
Intermediate type	**-5**	0.372	0.768	0.632	***0.591***
	-7	0.350	0.435	0.323	***0.369***
	-9	0	0	0	***0***
	Average	***0.241***	***0.401***	***0.318***	***0.320***

Winter type - $T = LSD_{0.05} = NS$; $G = LSD_{0.05} = 0.143$; $T \times G = LSD_{0.05} = NS$

Intermediate type - $T = LSD_{0.05} = 0.209$; $G = LSD_{0.05} = NS$; $T \times G = LSD_{0.05} = NS$

Decreasing temperatures significantly reduced the average kernel weight in cultivar 2. The kernel weight at –7°C was reduced by 37.6%, which was significantly lower than at –5°C and at –9°C where a 100% reduction occurred. This was significantly lower than that at both the mentioned temperatures. A larger degree of reduction occurred at –7°C and this was mainly ascribed to the contribution of the kernel weight of the primary spikes which were severely negatively influenced by temperatures lower than –5°C.

6.3.1.8 Mass per 100 kernels

6.3.1.8.1 Mass per 100 kernels produced by primary spikes

The primary spikes of cultivar 1 showed no significant differences in the mass per 100 kernels for the treatment combinations, but in the case of cultivar 2 the treatment combinations led to significant differences (Appendix 6.31 – 6.32). Table 6.8 show that the mass per 100 kernels was reduced from –5 to –9°C in cultivar 1. The reduction at –7°C was only 10.1% and 71.3% at –9°C, but only the latter was significantly lower than that at –5 and –7°C.

The mass per 100 kernels was significantly influenced by the treatment combinations (growth stage and temperature) in cultivar 2. This was significantly lower at –7 and –9°C for all growth stages than at –5°C indicating that this cultivar was particularly sensitive to frost injury at temperatures below –5°C. A 100% reduction occurred at –9°C for all growth stages. At –7°C the mass per 100 kernels was significantly lower that that at –5°C in the early flag leaf and emerging awns growth stages.

The data in Table 6.8 show that the flag leaf stage at –5 and –7°C produced the heaviest mass per 100 kernels for cultivar 2.

Table 6.8 Mass per 100 kernels produced by the primary spikes as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage: *Early flag leaf*	*Flag leaf*	*Awns emerge*	*Average*
Winter type	**-5**	1.982	2.582	1.910	***2.158***
	-7	1.612	1.838	2.372	***1.940***
	-9	0.594	0.848	0.417	***0.620***
	Average	***1.396***	***1.756***	***1.566***	***1.573***
Intermediate type	**-5**	2.863	4.117	3.276	***3.419***
	-7	1.267	4.345	1.435	***2.349***
	-9	0	0	0	***0***
	Average	***1.377***	***2.821***	***1.570***	***1.923***

Winter type - T = LSD $_{0.05}$ = 0.743
G = LSD $_{0.05}$ = NS
T x G = LSD $_{0.05}$ = NS

Intermediate type - T = LSD $_{0.05}$ = 1.023
G = LSD $_{0.05}$ = 1.023
T x G = LSD $_{0.05}$ = 2.389

6.3.1.8.2 Mass per 100 kernels produced by secondary spikes

The mass per 100 kernels produced by the secondary spikes did not differ significantly for the treatment combinations in both cultivars (Appendix 6.33 – 6.34). Significant differences in mass per 100 kernels were obtained as a result of the main factors, growth stages and temperature for both cultivars (Table 6.9).

The reduction in mass per 100 kernels obtained at –7°C in cultivar 1 was only 6%, but a significant reduction of 44.7% was obtained at –9°C. This was significantly lower than that obtained at both –5 and –7°C. The mass per 100 kernels was reduced by 19.1% at –7°C in cultivar 2, but this reduction was not significant (Table 6.9). A reduction of 100% occurred at –9°C and this was significantly lower than the masses obtained at both –5 and –7°C. Growth stages also had a significant effect on the mass per 100 kernels in cultivar 2. During the early flag leaf stage this was significantly lower than that obtained at the older growing

stages (flag leaf and awns emerging) indicating that this growth stage was more sensitive to frost injury.

Table 6.9 Mass per 100 kernels produced by the secondary spikes as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage: *Early flag leaf*	*Flag leaf*	*Awns emerge*	*Average*
Winter type	**-5**	1.891	2.295	1.913	***2.033***
	-7	1.996	1.869	1.870	***1.912***
	-9	0.757	2.020	0.595	***1.124***
	Average	***1.548***	***2.061***	***1.460***	***1.690***
Intermediate type	**-5**	2.503	3.443	3.010	***2.985***
	-7	1.4922	3.151	2.598	***2.414***
	-9	0	0	0	***0***
	Average	***1.332***	***2.198***	***1.869***	***1.800***

Winter type - $T = LSD_{0.05} = 0.602$; $G = LSD_{0.05} = 0.602$; $T \times G = LSD_{0.05} = NS$

Intermediate type - $T = LSD_{0.05} = 0.696$; $G = LSD_{0.05} = 0.696$; $T \times G = LSD_{0.05} = NS$

6.3.1.8.3 Mass per 100 kernels produced by both primary and secondary spikes

The mass per 100 kernels (primary and secondary spikes combined) did not differ significantly for the treatment combinations in both cultivars (Appendix 6.35 – 6.36). Significant differences in the mass per 100 kernels were however obtained as a result of temperature in cultivar 1 and both main factors, growth stages and temperature, in cultivar 2 (Table 6.10).

In cultivar 1 the mass per 100 kernels was slightly reduced at –7°C (3.1%). At –9°C the reduction obtained were 43.5% and this was significantly lower than the mass per 100 kernels obtained at bot –5 and –7°C (Table 6.10).

The mass per 100 kernels was reduced by 19.7% at –7°C in cultivar 2, but this reduction was not significant (Table 6.10). A reduction of 100% was obtained at –9°C and this was significantly lower than the masses obtained at both –5 and –7°C. The growth stages also had a significant influence on the mass per 100 kernels. During the early flag leaf stage, this was significantly lower than that obtained at the older growing stages (flag leaf and awns emerging) indicating that this growth stage was more sensitive to frost injury. The mass per 100 kernels was also significantly lower when the awns emerged than that of the flag leaf stage.

Table 6.10 Mass per 100 kernels produced by the primary and secondary spikes as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage: *Early flag leaf*	*Flag leaf*	*Awns emerge*	*Average*
Winter type	**-5**	1.916	2.350	1.898	***2.055***
	-7	1.980	1.869	2.124	***1.991***
	-9	0.922	1.964	0.596	***1.161***
	Average	***1.606***	***2.061***	***1.539***	***1.736***
Intermediate type	**-5**	2.636	3.626	3.093	***3.118***
	-7	1.564	3.324	2.620	***2.503***
	-9	0	0	0	***0***
	Average	***1.400***	***2.319***	***1.904***	***1.874***

Winter type - T = $LSD_{0.05}$ = 0.555; G = $LSD_{0.05}$ = NS; T x G = $LSD_{0.05}$ = NS

Intermediate type - T = $LSD_{0.05}$ = 0.670; G = $LSD_{0.05}$ = 0.670; T x G = $LSD_{0.05}$ = NS

This section dealt with the influence of temperature and different growth or developing stages and the reaction of two wheat cultivars to determine the effect of these main factors and in combination on the yield components of the crop during the flag leaf stage. All parameters showed that cultivar 1 (winter wheat) were more tolerant than cultivar 2

(intermediate wheat). These cultivars differ in their growth patterns. Cultivar 1 has a longer growth period, a longer vernalisation period and it has the ability to form more tillers than cultivar 2. These and other factors support this cultivar's ability to tolerate temperatures that may cause frost injury.

Cultivar 1 showed the ability to withstand temperatures as low as –7°C without significant negative effects. In contrast, cultivar 2 encountered severe frost injury at temperatures lower than –5°C. This usually coincided with the early flag leaf stage. During the flag leaf stage and emergence of the awns both cultivars showed to be more tolerant to frost injury than the early flag leaf stage. The primary spike usually experiences a higher degree of frost injury compared to the secondary spikes. This was also more evident for cultivar 2 at –7°C, indicating that cultivar 2 is more sensitive than cultivar 1. The highest degree of frost injury observed was in terms of the number of spikelets produced during the early flag leaf stage. This injury affected all other yield components that were dependent on the number of spikelets produced and, thus, having a major influence on the yield.

6.3.2 The reaction of different flowering stages to frost injury

Dry matter production, number of spikes and the number of spikelets (primary, secondary and combined) are parameters that have already been set at the time of flowering and no significant differences were encountered as a result of the applied treatments. The main emphasis of this section will be on seed set (number of seeds and seeds per spike) as well as on the weight of these seeds and the influence of temperature and growth stage on these parameters.

6.3.2.1 Kernel count

The number of kernels produced by the primary and secondary spikes separately as well as the total number of kernels (combination of the spikes) showed no significant differences as a result of the treatment combinations, of temperature and growth stages, in both cultivars (Appendix 6.37 – 6.42). Only temperature contributed to significant differences in the number of kernels produced (Table 6.11).

In cultivar 1 a smaller reduction in the number of kernels produced by the primary spike was observed compared to that of the secondary spikes. The reduction (52.3%) for the primary spike was only significant between –5 and –9°C. A reduction of 27.1% was obtained at –7°C but this was not significantly lower than that at –5°C. Both the number of kernels produced by the secondary spikes and that of the primary and secondary spikes combined showed reductions of 42.1 and 38.0% at –7°C and 49.4 and 50.0% at –9°C, respectively which were significantly lower than that of –5°C.

Table 6.11 Kernel count as affected by temperature (C1 – winter type and C2 – intermediate type)

Wheat type	Temperature (°C)	Kernel count		
		Primary Spike	***Secondary Spike***	***Total***
Winter type	**-5**	26.067	69.367	95.433
	-7	19.000	40.167	59.167
	-9	12.367	35.100	47.467
	LSD $_{(0.05)}$ =	*10.383*	*25.282*	*32.860*
Inter-mediate type	**-5**	29.367	40.033	69.400
	-7	19.667	25.700	45.367
	-9	16.533	19.567	36.100
	LSD $_{(0.05)}$ =	*10.361*	*13.341*	*21.030*

Cultivar 2 showed similar tendencies than cultivar 1. The only difference was that the reduction of the number of kernels produced by the primary spike in cultivar 2 was higher (33.0%) compared to that of cultivar 1 (27.1%). The opposite occurred in the secondary spikes where cultivar 2 only showed a reduction of 35.8% compared to 42.1% of cultivar 1. This emphasised the contribution of the secondary spikes and the importance of this contribution to the final yield of a specific cultivar.

6.3.2.2 Number of kernels per spike

The number of kernels per spike produced by the primary spikes has already been discussed in section 6.3.2.1. The number of kernels produced per secondary spike was approximately 50% less than that of the primary spikes. Both cultivars showed a significant reduction in the number of kernels per secondary spike when temperatures dropped below –5°C where the reduction of cultivar 1 and 2 were 30.3% and 42.6%, respectively at –7°C (Appendix 6.43 – 6.44). This reduction in cultivar 2 was more severe than in cultivar 1 (Table 6.12). This was probably as a result of the fact that the secondary spikes of cultivar 2 were in a more advanced stage of development making them less tolerant to frost injury than the secondary spikes of cultivar 1 at this stage. No significant differences or reductions were obtained between –7 and –9°C.

Table 6.12 Kernels per spike as affected by temperature (C1 – winter type and C2 – intermediate type)

Wheat type	Temperature (°C)	Kernels per spike		
		Primary Spike	*Secondary Spike*	*Average*
Winter type	**-5**	26.067	11.551	13.548
	-7	19.000	8.053	9.839
	-9	12.367	5.303	6.211
	LSD $_{(0.05)}$ **=**	*10.383*	*3.253*	*3.821*
Inter-mediate type	**-5**	29.367	14.469	18.517
	-7	19.667	8.304	10.793
	-9	16.533	7.133	9.114
	LSD $_{(0.05)}$ **=**	*10.361*	*4.735*	*4.979*

The total number of kernels produced per spike was similar to that measured per secondary spike (Appendix 6.45 – 6.46). Even the degree of reduction correlated. No significant differences in the number of kernels produced as a result of growth stage occurred between cultivar 1 and cultivar 2 but the latter was sensitive with regard to growth stage (Appendix 6.37– 6.38 and 6.43 – 46). The least amount of injury in terms of the number of kernels produced per primary and secondary spikes as well as the average, occurred at a 100% flowering, indicating that this growth stage was the most tolerant of the three flowering stages (cultivar 2).

Table 6.13 Kernels per spike as affected by growth stage (C1 – winter type and C2 – intermediate type)

Wheat type	Flowering stage (%)	Kernels per spike		
		Primary Spike	***Secondary Spike***	***Average***
Winter type	**0**	21.000	8.221	10.099
	50	17.933	8.528	9.676
	100	18.500	8.158	9.823
	LSD $_{(0.05)}$ =	*NS*	*NS*	*NS*
Inter-mediate type	**0**	11.633	6.794	7.963
	50	25.700	10.568	14.582
	100	28.233	12.544	15.880
	LSD $_{(0.05)}$ =	*10.361*	*4.735*	*4.979*

The difference in number of kernels produced during 50 and 100% flowering was insignificant, but at 0% flowering and the latter two stages significant differences were observed in cultivar 2 (Table 6.13).

6.3.2.3 Kernel weight

The weight of kernels produced by the primary and secondary spikes as well as the total (combination of the spikes) of both cultivars showed no significant differences as a result of the interaction between temperature and growth stage (Appendix 6.47 – 6.52). However, these two main factors contributed to significant differences in the kernel weight when analysed separately (Table 6.14 – 6.15).

Table 6.14 Kernel weight as affected by temperature (C1 – winter type and C2 – intermediate type)

Wheat type	Temperature (°C)	Kernel weight		
		Primary Spike	***Secondary Spike***	***Total***
Winter type	**-5**	0.626	1.633	2.259
	-7	0.359	0.844	1.203
	-9	0.215	0.680	0.895
	LSD $_{(0.05)}$ =	*0.251*	*0.610*	*0.792*
Inter-mediate type	**-5**	0.968	1.454	2.422
	-7	0.600	0.755	1.355
	-9	0.403	0.450	0.853
	LSD $_{(0.05)}$ =	*0.350*	*0.485*	*0.694*

Reduction in kernel weight produced by the primary and secondary spikes as well as the total was closely correlated between the cultivars. Significant reductions occurred when the temperature decreased below –5°C. The average reduction was 46.7 and 60.4% for cultivar 1 and 44.0 and 64.8% for cultivar 2 at –7 and –9°C, respectively. From this it was clear that the degree of reduction in kernel weight did not differ between the cultivars and this was in contrast to previously discussed yield component parameters (Table 6.14).

In cultivar 1 growth stage had no effect on the kernel weight. However, in cultivar 2 significant differences was observed between 0, 50 and a 100% flowering for the primary, secondary spike as well as the total kernel weight, with the exception of 50 and 100%

flowering for the kernel weight of the secondary spikes. Table 6.15 show that the 100% flowering stage was the least affected by frost injury and that early flowering was more sensitive.

Table 6.15 Kernel weight as affected by growth stage (C1 – winter type and C2 – intermediate type)

Wheat type	Flowering stage (%)	Kernel weight		
		Primary Spike	*Secondary Spike*	*Total*
Winter type	**0**	0.481	1.129	1.610
	50	0.381	1.184	1.564
	100	0.338	0.845	1.182
	LSD $_{(0.05)}$ =	*NS*	*NS*	*NS*
Inter-mediate type	**0**	0.330	0.588	0.918
	50	0.794	0.935	1.815
	100	0.847	1.136	1.896
	LSD $_{(0.05)}$ =	*0.350*	*0.485*	*0.694*

6.3.2.4 Kernel weight per spike

In terms of kernel weight per spike the treatment combinations had no significant effect (Appendix 6.47 – 6.48 and 6.53 – 6.56). Both cultivars showed a significant decrease (on average more than 38.4%) in kernel weight per spike as the temperature decreased from –5 to –7°C (Table 6.16). The decrease in kernel weight per spike from –7 to –9°C was, however, not significant for either cultivar. A higher degree of reduction in kernel weight per secondary spike occurred at –7°C in cultivar 2 than in cultivar 1 indicating that the secondary spikes of cultivar 2 were more sensitive to frost injury than that of cultivar 1.

Table 6.16 Kernel weight per spike as affected by temperature (C1 – winter type and C2 – intermediate type)

Wheat type	Temperature (°C)	Kernel weight per spike		
		Primary Spike	*Secondary Spike*	*Average*
Winter type	**-5**	0.626	0.269	0.318
	-7	0.359	0.164	0.196
	-9	0.215	0.101	0.115
	LSD (0.05) =	*0.251*	*0.078*	*0.089*
Inter-mediate type	**-5**	0.967	0.519	0.642
	-7	0.600	0.246	0.323
	-9	0.403	0.164	0.214
	LSD (0.05) =	*0.350*	*0.157*	*0.159*

Table 6.17 Kernel weight per spike as affected by growth stage (C1 – winter type and C2 – intermediate type)

Wheat type	Flowering stage (%)	Kernel weight per spike		
		Primary Spike	*Secondary Spike*	*Average*
Winter type	**0**	0.481	0.196	0.237
	50	0.381	0.178	0.203
	100	0.338	0.160	0.189
	LSD (0.05) =	*NS*	*NS*	*NS*
Inter-mediate type	**0**	0.330	0.184	0.219
	50	0.794	0.359	0.464
	100	0.847	0.385	0.496
	LSD (0.05) =	*0.355*	*0.157*	*0.159*

Cultivar 2 showed that the growth stage had a significant influence on the kernel weight per spike (Table 6.17). At 50 and 100% flowering no significant differences occurred, but at 0% flowering the reduction in kernel weight was higher than 52% where the primary spike experienced the highest degree of reduction (61.0%). This showed that cultivar 2 was extremely sensitive to frost injury at 0% flowering and that the primary spike also showed a higher degree of sensitivity than the secondary spikes.

6.3.2.5 Mass per 100 kernels

Treatment combinations (temperature x growth stage) had no significant influence on the mass per 100 kernels in the primary and secondary spikes as well as the total (spikes combined) with the exception of the primary spikes in cultivar 2 (Appendix 6.57 – 6.62).

There were only two treatment combinations that showed severe reductions in the mass per 100 kernels produced by the primary spikes in cultivar 2 and that was at –7 and –9° C at 0% flowering. Table 6.18 clearly shows that at 0% flowering and at temperatures below –5°C the mass per 100 kernels was negatively influenced.

Temperature and growth stage had a significant effect on the mass per 100 kernels in secondary spikes as well as the total (primary and secondary spikes combined); (Table 6.19 – 6.20). In cultivar 1 the mass per 100 kernels in the secondary spikes was reduced by 17.7 and 47.3% at –7 and –9°C respectively and only the mass obtained at –9°C was significantly lower than that produced at –5°C. When the primary and secondary spikes were combined the reduction in mass per 100 kernels was 20.3 and 46.0% at –7 and –9°C respectively and only the latter was significantly lower than that at –5°C (Table 6.19).

In cultivar 2 (Table 6.19) the mass per 100 kernels at –9°C was significantly lower than that at –5°C for the secondary spikes. When the primary and secondary spikes were combined this was significantly lower at temperatures below –5°C with a reduction of 30.5 and 43.7% at –7 and –9°C, respectively.

Table 6.18 Mass per 100 kernels produced by the primary as affected by different temperatures at different growth stages for different cultivars (C1 – winter type and C2 – intermediate type)

Wheat Type	Temperature (°C)	Growth stage			
		0% flowering	*50% flowering*	*100% flowering*	*Average*
Winter type	**-5**	2.395	2.330	1.781	***2.169***
	-7	1.584	1.344	1.732	***1.553***
	-9	1.606	1.172	0.857	***1.211***
	Average	***1.862***	***1.582***	***1.457***	***1.645***
Intermediate type	**-5**	2.223	3.918	2.812	***2.985***
	-7	0.075	2.898	3.337	***2.103***
	-9	1.017	2.161	2.406	***1.861***
	Average	***1.105***	***2.99***	***2.851***	***2.316***

Winter type - T = $LSD_{0.05}$ = NS; G = $LSD_{0.05}$ = NS; T x G = $LSD_{0.05}$ = NS

Intermediate type - T = $LSD_{0.05}$ = 0.735; G = $LSD_{0.05}$ = 0.735; T x G = $LSD_{0.05}$ = 1.718

T = Temperature G = Growth stage T x G = Temperature growth stage interaction NS = Not significant

The mass per 100 kernels in the primary and secondary spikes as well as the average (primary and secondary spikes combined) showed significant differences in cultivar 2 as affected by growth stage (Table 6.20). No significant differences were observed between the 50 and 100% flowering stages, but the most sensitive stage was at 0% flowering when the highest degree of frost injury occurred. This was the case for the primary and secondary spikes separately as well as for the total. Though the mass per 100 kernels in the secondary spikes differed significantly according to the ANOVA, the Tukey test showed that these differences were not significant. This occurrence is a result of the strictness of the Tukey test.

From the results it is clear that wheat plants, especially cultivar 2, are very vulnerable at an early flowering stage (0% flowering) and that at temperatures below –5°C the plants do experience severe frost injury that reduces the mass per 100 seeds produced.

Table 6.19 Mass per 100 kernels as affected by temperature (C1 – winter type and C2 – intermediate type)

Wheat type	Temperature (°C)	Mass per 100 seeds		
		Primary Spike	*Secondary Spike*	*Average*
Winter type	**-5**	2.169	2.317	2.320
	-7	1.553	1.907	1.850
	-9	1.211	1.220	1.253
	LSD $_{(0.05)}$ **=**	*0.760*	*0.669*	*0.611*
Inter-mediate type	**-5**	2.985	3.518	3.533
	-7	2.103	2.424	2.454
	-9	1.861	1.936	1.988
	LSD $_{(0.05)}$ **=**	*0.735*	*1.266*	*0.833*

Table 6.20 Mass per 100 kernels as affected by growth stage (C1 – winter type and C2 – intermediate type)

Wheat type	Flowering stage (%)	Mass per 100 seeds		
		Primary Spike	*Secondary Spike*	*Average*
Winter type	**0**	1.862	2.007	1.954
	50	1.615	1.750	1.807
	100	1.457	1.689	1.672
	LSD $_{(0.05)}$ **=**	*NS*	*NS*	*NS*
Inter-mediate type	**0**	1.105	1.861	1.845
	50	2.992	3.033	3.018
	100	2.851	2.984	3.112
	LSD $_{(0.05)}$ **=**	*0.735*	*1.266*	*0.833*

This section dealt with the influence of temperature and different growth or developing stages and the reaction of two wheat cultivars to determine the effect of these main factors separately and in combination on the yield components of the crop during the flowering stage. All parameters showed that cultivar 1 (winter wheat) were more tolerant than cultivar 2 (intermediate wheat).

Both cultivar 1 and 2 have the ability to only withstand temperatures not lower than –5°C where significant negative effects occurred during the flowering stage. This differed from the previous growth stage (flag leaf) where cultivar 1 could withstand temperatures of up to –7°C without any significant reductions. In contrast, cultivar 2 encountered severe frost injury at temperatures lower than –5°C. This usually coincided with an early flowering stage (0% flowering). No significant differences were obtained at the different flowering stages of cultivar1, but cultivar 2 showed to be highly sensitive at 0% flowering. Cultivar 1 produced more kernels than cultivar 2, but these were lean when compared to that of cultivar 2. The secondary spikes of both cultivars experienced a higher degree of reduction in kernel number, but once more these kernels had a higher mass per 100 kernels than that of the primary spikes. This could be ascribed to the compensation ability of the plant to produce heavier ("fatter") kernels when the number of kernels was reduced.

6.4 DISCUSSION and CONCLUSION

The type of wheat used (winter, intermediate) showed that they reacted differently to frost temperatures at different growth stages (flag leaf and flowering stages). According to Single (1964) the ears of wheat may endure long periods at temperatures of –5°C and below without damage as long as crystallisation of internal moisture is not induced by contact with ice nuclei. Supercooling of floral parts within the flag leaf sheath is possible even in a plant which visually appears to be entirely frozen and this is due to the nature of the leaf cuticle and stem nodes. The freezing boundary usually fails to pass across the interior cuticle of the leaf sheath to the ear within the 'boot', and may be arrested at the nodes of the stem or rachis although it may travel rapidly in the leaf tissue (Single and Marcellos, 1974).

The developmental stage of wheat determines tiller frost avoidance. According to Hoogendoorn (1985) and Malse, Doussinault and Sun (1989) the onset of internode elongation is a critical stage, the timing is closely related with the time of heading that is determined by photoperiodic response and narrow-sense earliness, which is also called ”earliness” or “intrinsic” earliness. Once the ear has emerged from the boot a different situation occurs. Not only is the tissue susceptible to the effects of the freezing boundaries travelling upwards via the peduncle and rachis, but each floret is exposed to the danger of inoculation by ice nuclei in the atmosphere.

Both cultivars showed that severe losses occurred at temperatures below –5 (intermediate type) and –7°C (winter type) during the flag leaf stage. These losses or reductions were evident in the yield for the floral parts were killed or became sterile, dependent on the growth stage. The early flag leaf stage showed to be the most sensitive flag leaf stage and during this growth stage the floral parts usually were killed. Both the flag leaf and flowering growth stages are well within the range of when frost incidences do occur, that is during the month of September and the first week of October (Table 2.1). During the early flag leaf stage, that is the end of stem elongation, the young spike consists of expanding cells and lack intercellular spaces and can therefore not tolerate the least degree of freezing. Thus even a brief return to minimum and sub zero temperatures may become lethal to the ears (Single, 1988).

The only manner in which the ear could survive is to stay supercooled and on the capacity of developing stem nodes below the ear has to restrict the spreading of freeze boundaries from surrounding leaves and crowns. Single (1988) also stated that when active growing plants are subjected to hardening temperatures, that is temperatures in the range of 0°C, the restriction by the mentioned nodes could be able to operate to temperatures of approximately –8°C.

At temperatures closer to 0°C, when light frost do occur, the visual symptoms of frost injury becomes very difficult to recognise for the wheat ears become sterile and flaccid (Afanasiev, 1966).

Single and Marcellos (1974) showed the importance to encompass freezing of wheat ears after emergence from the leaf sheath for improving resistance in new cultivars may be considered within three categories, viz. the role of rachis and rachilla in impeding the internal movement of ice to floral parts (ear resistance), the importance of glaucousness in relation to ice nucleation in the field, and genetic variation in ear resistance. According to Single and Marcellos (1974), if glaucousness could prove to be a useful protective character in the field, few difficulties can be expected in screening for it in new cultivars because only a few genes appear to be involved, although waxless types are dominant. Screening of cultivars for rachis and rachilla resistance has revealed the existence of useful variation. This variation was not only to be seen between the cultivars used in Chapter 4, but also for two of the cultivars used to determine if there were differences in the different growth stages for the flag leaf and flowering stages.

Generally cultivars 1 and 2 (winter and intermediate types) showed, at the flowering stages, that temperatures below –5°C led to a reduction in yield. Cultivar 1 showed no difference in terms of growth stage but cultivar 2 (intermediate type) was more sensitive at 0% flowering whereas at 50 and 100% flowering the differences were insignificant. According to Single (1988) damaging temperatures for wheat in the head occurred at –1.8°C if abundant water was present and as low as –7°C if no ice formed on the ears. Furthermore, he stated that for practical purposes the range would be between –3 and –5°C as by the time the wheat crop have reached the heading stage the temperatures are usually such as to preclude hardening, and therefore water is always present (Single, 1985).

To conclude, both cultivars showed severe reductions with regard to the parameters used to evaluate the effect of temperature and/or growth stage during the flag leaf (early flag leaf, flag leaf and emergence of the awns) and flowering (0, 50 and 100%) stages. Once more cultivar 1 (winter type) proved to be more tolerant than cultivar 2 (intermediate type). Both cultivars were more sensitive to frost injury during the early flag leaf stage and cultivar 2 showed to be sensitive during the flowering stage at 0% flowering while cultivar 1 showed no difference with regard to growth stage. The difference in the cultivar's reaction to frost injury co-ordinated with reports by Single and Marcellos (1974), Fowler and Carles (1979),

Marcellos and Single (1984), Brule-Babel and Fowler (1988) and Roberts (1990). Various researchers have reported that after emergence from the flag leaf sheath, the wheat ear is highly susceptible to frost injury (Single, 1964; Single and Marcellos, 1974; Marcellos and Single, 1984). This is true, but it was shown in Chapter 4 that the flag leaf stage was even more sensitive than the flowering stage. This chapter confirmed that the flag leaf stage was more sensitive for a total loss in yield was the result for the intermediate wheat type (cultivar 2) as a result of frost injury. Fowler and Carles (1979), Brule-Babel and Fowler (1988) and Roberts (1990) also confirmed that earliness in heading time is generally seen in spring wheat cultivars, which are less hardy than winter wheat cultivars.

CHAPTER 7

GENERAL CONCLUSION AND RECOMMENDATIONS

7.1 CONCLUSIONS

Frost injury to wheat has been recognised as a major cause of economic loss, and still it has received little attention from research workers or plant breeders in South Africa. The successful cultivation of wheat depends on the continuity of the vegetative, reproductive and grain filling phases during which the crop initiates and grows its organs and eventually completes its life cycle. Each of these growth phases has to be completed within a limited period and is influenced and determined through genetic and environmental interaction. The interaction of the cultivar (genotype) and the degree of frost occurrence (environment) ultimately determine the quantity and quality of the yield. The recent occurrence of extreme climatic conditions, and therefore the occasional appearance of late frost, had a major effect on cultivar responsiveness and ultimately the yield and quality of wheat produced in this country. The latter prompted this study.

In this study (Experiment 1) it was concluded that different cultivars, especially different genotypes, reacted differently to frost stress. It was evident that exposure to frost stress at different growth stages (tillering, flag leaf, flowering and hard dough) evoked different responses from different cultivars with regard to sensitivity to frost damage. Results obtained in this study strongly indicated that a close association exists between the development stages and the yield components during the pre-anthesis period. During the pre-anthesis growth stages (tillering and flag leaf) all test cultivars were severely influenced at temperatures below –6°C while the flowering and hard dough stages were more tolerant to frost stress in terms of dry matter and the number of spikes produced. The latter indicated plants that have completed their vegetative growth to be more tolerant to low temperature exposure.

Although all cultivars showed some level of sensitivity to frost stress the degree of damage was primarily determined by the interaction between specific growth stage and temperature.

Cultivars 1 to 3 showed that temperatures below –6°C reduced the number of spikelets, number of kernels, number of kernels per spike, kernel weight as well as mass per 100 kernels drastically in the flag leaf stage. In cultivar 4, the flowering stage was the most sensitive growth stage, as shown by reductions in terms of all of the above parameters except for the number of spikelets where a reduction was observed at –9°C.

From this it is concluded that temperatures below –6°C have a significant reducing effect on the growth and yield component parameters of wheat in the tillering and especially the flag leaf stage for cultivars 1 to 3 and to a lesser degree in the flowering stage of cultivar 4. Differences in cultivars (genotypes) were also observed with the spring type being the least tolerant to frost damage. Generally, evaluation of the quality characteristics showed that a decrease in temperature moderately reduced the quality (protein content and stirring number) of wheat grain for all cultivars at different growth stages.

Evaluation of different flag leaf stages to frost damage (Experiment 2) showed that the early flag leaf stage was the most sensitive growth stage for both cultivars. Cultivars also differed in their ability to tolerate frost stress with the winter type being more tolerant than the intermediate type. This was illustrated by the fact that the winter type sustained moderate injury in terms of dry matter production, number of spikes and spikelets per spike at –7°C for the different flag leaf growth stages. Moreover, the intermediate type experienced severe injury at temperatures below –5°C and this was especially evident at the early flag leaf stage. The number of kernels, kernel weight and 100 kernel mass showed that the primary spike of cultivar 1 was mainly negatively influenced by temperatures irrespective of the growth stage. In cultivar 2, the mentioned parameters were negatively influenced at –5°C.

Evaluation of different flowering stages to frost damage (Experiment 3) showed that no differences were obtained in kernel count, total kernel weight and 100 kernel mass at the different flowering stages of cultivar 1 (winter type). Conversely, cultivar 2 (intermediate type) was severely affected at 0% flowering in terms of all test parameters. Data obtained for kernel count, kernel weight and 100 kernel mass showed that both cultivars were severely affected when exposed to temperatures below –5°C. This differed from the recorded

responses of different cultivars in Experiment 2 but confirmed the tolerance differences in growth stages to frost damage.

Generally, it can be concluded that South African wheat cultivars (genotypes) react differently to frost stress/damage under controlled conditions as shown by quantitative reduction in virtually all test parameters below –5°C. A moderate reduction in kernel quality was obtained at temperatures below 0°C. Finally, a guide that illustrates frost injury on wheat under South African conditions was compiled that will surely be an asset to wheat growers, agronomists and agricultural insurance companies alike. All illustrations were collected from frost injured wheat under field and controlled conditions. The guide has been compiled to assist all interested participants in the wheat industry and is presented in Chapter 8.

7.2 RECOMMENDATIONS

i) Yields of especially irrigated wheat can significantly be influenced by the time of planting. By delaying the date of planting the growth period is reduced as well as the grain filling period and ultimately yield losses is obtained. Conversely, planting before the recommended date extends the growing period, but this also increases the risk of frost damage. Therefore, the producer should not alter recommended planting dates in order to avoid risk.

ii) Cultivar differences should be recognised and used to the advantage of the producer. The selection of tolerant cultivars, especially in areas prone to the occurrence of frost, should be a high priority in future breeding programmes. There is a need for the selection and breeding of frost tolerant cultivars. In this regard the selection of spring type cultivars that are used under irrigation should receive special attention.

iv) The influence of frost damage on the qualitative characteristics of wheat should be investigated more comprehensively in future.

v) Currently, much confusion exists in terms of ascertaining the cause of alleged frost damage to wheat kernels after delivery to silo's in the event of a dispute between producers, grain graders and insurance companies. In order to settle this dispute either an analysis procedure or a simple test specific for frost damage needs to be developed. Additionally, this test should distinguish between frost damage and damaged caused by other stress factors and/or diseases. Whether such a test procedure is at all possible is questionable, but a solution might be found on the biochemical level and this is a challenge.

CHAPTER 8

A GUIDE TO FROST DAMAGE TO SOUTH AFRICAN WHEAT

No South African or southern African literature was, until now, available on the visual symptoms of frost damage on wheat. The importance of this chapter, therefore, goes without saying.

A guide has been compiled as outcome of this study by making use of simulated glasshouse as well as field frost injured plants and/or plant parts with a discussion and/or explanation (Shroyer *et al*., 1995) on each of the visual symptoms observed during different growth stages. It is virtually impossible to capture each and every symptom in the form of a photograph and therefore only the most obvious and recognisable symptoms were included because of its value (educational) to the producer (farmer) and other role players in the wheat producing industry, especially insurance companies. The latter was responsible for the funding of this project by compiling visible symptoms under South African conditions and to verify this with literature from the United States of America where freezing seems to be a major constraint and that of Australia where a great amount of research has been done on frost damage. The majority of symptoms do correlate, but the degree as well as the time for these symptoms to develop does differ slightly due to cultivar differences as well as day temperatures.

The visual symptoms were compiled and confined to specific growth stages according to the classification of growth stages explained in Chapter 2 by the ARC-Small Grain Institute (Joubert system). The wheat producing industry of South Africa is familiar with this system and it has therefore been used. In addition to the frost damage symptoms other symptoms that might be confused with that of frost damage have been included to clear, where possible, the confusion. The guide had to be reader friendly to wheat producers and bearing this in mind, it was written in a popular fashion to be easily understandable and not confusing to the reader accessing the information.

FROST DAMAGE

TO SOUTH AFRICAN **WHEAT**

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INTRODUCTION

Frost damage has reached alarming proportions the previous decade, specifically in the central wheat producing areas of South Africa. The occurrence of frost damage is more dominant in certain regions due to the planting techniques and management practices that are followed.

Generally, it is impossible to avert the occurrence of frost but through a knowledgeable choice of cultivars, by abiding to recommended planting dates and by managing water and nitrogen applications (especially under irrigation) with sound principles, this risk factor could either be minimised and/or averted.

Wheat is subjected to adverse weather conditions in all of its growth stages during the full growth period. Low temperatures (< 0°C), particularly during early spring, can be destructive.

Damage usually occurs when low temperatures coincide with sensitive growth stages. Frost tolerance of wheat decreases with an increase in temperature and accelerated growth at the end of winter.

Wheat plants are most sensitive to frost damage during the initiation of anthesis when acceleration in the development of the plants occurs.

WHEN AND WHERE DOES FROST DAMAGE OCCUR?

Frost damage occurs when low temperatures coincide with sensitive growth stages and this damage can occur over large areas, in fields and/or parts of fields.

The intensity of frost damage is usually more severe along rivers, valleys and depressions where cold air settles. The occurrence of frost damage is not confined to a specific area in South Africa.

The risk of spring frost damage increases when wheat progresses faster through its growth stages due to above average temperatures and stress conditions (water stress, nutrient deficiencies, etc.). If frost does occur under these conditions, wheat would have a greater chance of sustaining permanent damage due to its advanced development (reproductive stages, that is jointing, ear emergence, anthesis and early grain filling) and therefore will be less tolerant than during vegetative growth stages.

Various factors (plant growth stage, plant water content, duration of exposure, wind and rainfall) as well as the interaction between these factors influence frost damage to wheat and complicates the process of predicting the degree of damage. This process is further complicated through differences in elevation and topography in and between wheat fields as well as cultivar differences.

TEMPERATURES THAT CAUSE FROST STRESS

Wheat proceeds through complex processes of cold hardening during late fall and at the beginning of winter that increases its tolerance to frost damage. The hardening process takes place over a longer period in the cooler areas of the country.

In association with this, it has also been found that the degree of frost tolerance is also higher when spring temperatures do not rise drastically. To the contrary it has been found that if winter temperatures are exceptionally low in the warmer regions of the country followed by a rapid rise in the temperature at the beginning of spring, the hardening process of the plants is interrupted and frost tolerance is decreased.

The frost tolerance of wheat is decreased with an increase in temperature during spring, due to accelerated cell growth and development.

Although wheat is subjected to frost conditions throughout its growing period, the most sensitive period or phase is the reproductive growth phase (that is during jointing, ear emergence, anthesis and early grain filling, but especially during the flag leaf stage and during pollination).

Temperatures slightly below freezing can severely damage wheat during these reproductive stages and greatly reduce yields.

The duration of the exposure to these low temperatures, as well as the level of low temperature that is reached, have an influence on the degree of damage to wheat due to frost.

Therefore, a prolonged exposure to frost causes more damage than a brief exposure at the same temperature.

SYMPTOMS OF FROST DAMAGE

Frost damage to wheat can be reported to insurance companies only if the symptoms are known. This guide will enable early assessment of frost damage as will the extent thereof and subsequently provide the producer with more options to deal with the damaged crop if severe damage has occurred. Waiting until the commencement of harvesting only to learn that the wheat has been severely damaged by frost, decreases the value of the damaged crop and limits management options. Assessment of frost damage is aided by several characteristic symptoms that develop at each growth stage. Damage to vital plant parts can usually be detected by careful examination although cold temperatures delay the development of these symptoms after spring frost.

It is important to know the plant parts that are most vulnerable at each growth stage, where they are located on the plant and their appearance when they are normal as well as when they have been injured. Early maturing wheat (reaching physiological maturity) is more sensitive to frost than late maturing wheat. The reason for this being that early maturing wheat is at a more sensitive stage (flag leaf, anthesis and milk dough) of development at the time of frost occurrence.

Sensitivity to temperatures below the freezing point gradually increases as wheat's development accelerates during the spring. Although cultivars differ in the degree of frost tolerance, the cause of this tolerance is mainly based on the difference in growth stages when frost occurs. Generally, there is a small difference in the sensitivity of different cultivars at the same growth period (spring, intermediate and winter cultivars) at the same growth stage and therefore a limited opportunity to enhance frost tolerance in available cultivars exits. What could be beneficial to enhance frost tolerance, is the growth habit of different cultivars, for example the rate at which the growth point is pushed upwards during jointing.

Drought stress tends to accelerates the early developmental stages (vegetative stadium) and this increases the susceptibility of plants to frost damage.

THE OCCURRENCE OF FROST DURING DIFFERENT GROWTH STAGES

TILLERING STAGE (Growth point stage 5)

The system used to refer to the different growth stages is that being used by the Small Grain Institute (Joubert system).

During the tillering stage the growth point is located below the soil surface and it is possibly protected by the leaves and leaf sheaths.

Figure1

Leaves are twisted and light green to yellow in colour with necrotic (burned) marks. The leaves dry quickly and disintegrate when handled.

Differences with regard to tolerance during this stage could be obtained as a result of the ability of different cultivars to retain the growth point close to the soil surface.

Most damage during this stage occurs to the leaves that become twisted and light green to yellow in colour. Leaves are also necrotic (burned) at the tip and even on the bend of older leaves one to two days after frost **(Figure 1)**.

Damage during this stage slows growth and may reduce tiller numbers, but growth of new leaves and tillers recommence with warmer temperatures during spring. Wheat damaged by frost during this stage usually recovers completely. Winter type wheat has a better re-growth and recuperation ability than spring type wheat.

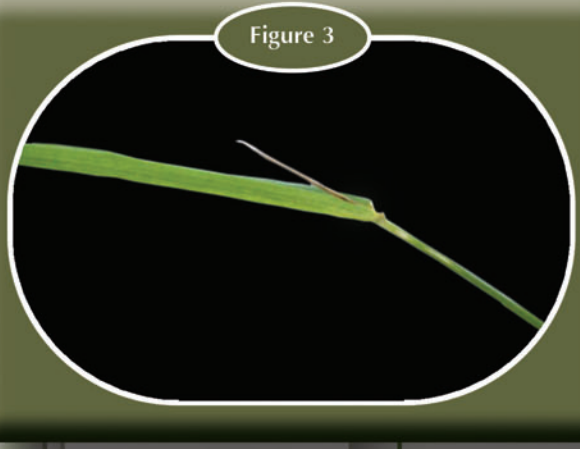
JOINTING STAGE (Growth point stage 10)

Leaves of frost-damaged plants show the same symptoms as at the tillering stage, but the most serious damage occurs to the growing points. The growth point, which is just above the uppermost node, can be located by splitting stems longitudinally. A normal, undamaged growth point is bright white to yellow green in colour and turgid, but frost damage causes it to become off-white or brown in colour and water soaked in appearance **(Figure 2)**. This injury can even occur in plants that otherwise appear normal because the growth point is more sensitive to frost than other plant parts. The growth of damaged tillers is terminated and the tillers remain green. These tillers remain visible for a long period as a light brown or pale tiller stem.

Figure 2

Eventually the growth point will become off-white or brown.

Figure 3



A dead leaf appears in the whorl of the last leaf sheath.

Damage does not commonly occur during this stage except in the case of especially low temperatures (approximately –8°C and lower).

If the growth point is damaged the tiller remains green for a period of time after growth has ceased. In certain instances a chlorotic or dead leaf may appear in the whorl, indicating that the growth point is dead **(Figure 3)**.

Stems subjected to frost during this and later growth stages may show symptoms of discoloration, roughness, lesions and enlargement of nodes **(Figure 4)**. Stem damage can also occur on higher parts of the stem in the form of ice rings or split stems. Damaged plant stems usually break at these affected areas **(Figure 5)**.

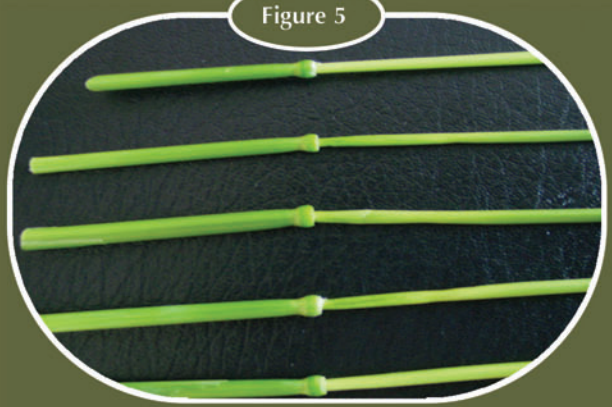
JOINTING STAGE (Growth point stage 10)

Ice rings can also occur during the jointing stage and is clearly visible just below the attachment of the growth point (**Figure 6**). It does not seem as if the growth point was damaged immediately after the occurrence of frost, but it dries and turns off-white to brown in colour if the stem has been damaged.

Figure 4

Discoloration, roughening, lesions and enlargement of the lower stem and nodes are symptoms of frost damage.

Figure 5

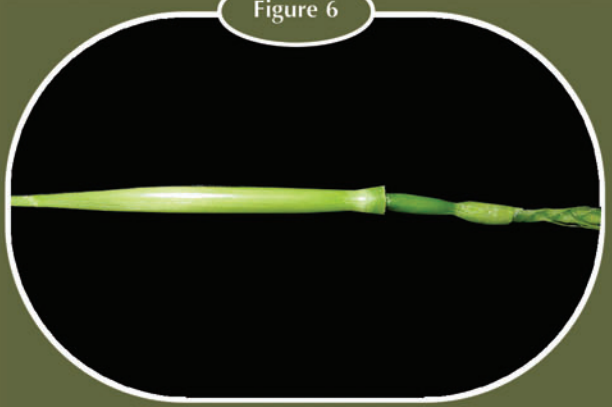


The top and bottom stems show no signs of damage. The stem, second from the top, shows an ice ring just above the node and the third stem shows an indent due to tissue damage caused by frost. The fourth stem from the top shows severe stem damage (splitting of the stem) caused by frost and the stem usually breaks easily at this point of damage.

The growth point will not move upward. The loss of these early tillers releases the later tillers that would not normally develop because of too much competition from the primary tiller and early secondary tillers.

Frost damage that occurs during this stage cannot have a direct negative influence on the quality of the grain, considering that neither fertilisation nor grain formation has taken place. This is also valid for the flag leaf stage until anthesis.

Figure 6



An evident ice ring has formed just below the attachment of the growth point. This will interrupt the translocation of nutrients to the growth point and it will eventually die.

FLAG LEAF STAGE (Growth point stage 15)

A number of visual symptoms, such as ears that are enclosed and/or pinched in the leaf sheath of the flag leaf, can be observed during the flag leaf stage when frost damage occurs.

During the flag leaf stage, the flag leaf blade can twirl around the ear and therefore the ear becomes pinched by the flag leaf. The term used for this phenomenon is that the flag leaf makes a "whip lash" **(Figure 7)**.

When this happens, the ears will remain in the boots, split out of the sides of the boots **(Figure 8)** in which event the awns of the ear will also be damaged or emerge base-first from the boots **(Figure 9)**.

This symptom can also be confused with that of aphid and/or chemical damage, especially when herbicides were applied at sensitive growth stages of the crop. Frost can cause wheat ears to be pinched in the leaf sheaths and in this event the ears will not emerge normally.

Figure 7

Ear pinched by the flag leaf blade (whip lash) after frost damage.

Figure 8

Ear emerges at the side of the flag leaf sheath after frost damage and the damaged awns is also visible.

Figure 9

Complete sterility and a ear that emerge base-first due to the awns being pinched in the flag leaf sheath.

FLAG LEAF STAGE (Growth point stage 15)

Sometimes ears emerge normally from the boot after frost damage, but remain yellow or even white instead of their usual green colour **(Figures 10 and 11)**. When this happens the, the ears die off. During severe frost conditions all flower parts will be damaged and only a rachis or empty ear will emerge from the boot **(Figure 12)**. Temperatures below freezing that are low enough to damage leaves or stems, are fatal to male plant parts, but less severe frost can also lead to male sterility without any visible symptoms of damage on the vegetative parts (leaves and stems). This form of damage can occur at temperatures as high as –2°C and causes sterility of the developing flowers through either damaging the ovum or the anthers.

Figure 10

A white ear emerges from the flag leaf sheath and the stem is twisted in the flag leaf sheath. This is also the point where the ice ring forms.

Figure 11

The ear emerges normally, but is yellow to off-white in colour and does not appear crisp green, as a normal ear should.

Figure 12

No grain filling will occur due to complete sterility.

EAR EMERGENCE

Figure 13

Awns become bleached (chlorosis) and the male flowering parts are most probably killed.

Figure 14

The tip of the ear may be damaged by frost during the beginning of ear emergence. The rest of the plant may be undamaged.

The symptoms at this stage is similar to that of the previous growth stage and include sterility, leaf abscission, leaf desiccation or drying and lesions on lower stems.

Due to ear emergence during this stage the most apparent symptom, however, is usually chlorosis or bleaching of the awns so they are white instead of the normal green colour **(Figure 13)**. The awns are also typically shrivelled and folded back.

Under certain conditions only the tip of the ear will be damaged. The symptomatic result of this is white coloured ear tips that is sterile and does not contribute to the yield **(Figure 14)**, but this symptom also occurs during certain drought stress conditions and may be confusing. During the latter the tip of the ear is being ablactated and the remainder of the seeds will be lean and shrivelled due to water stress while the older ones will die and become brownish.

During this scenario the vegetative growth of the plant will also show signs of water deficiencies. Temperatures that damage the awns may also kill the male flower parts.

Yellowish chlorotic tissue at the juncture of the stem and the flag leaf at the time of frost occurrence.

A light green or yellow "ice or frost ring" may encircle and appear the stems several days after exposure to frost temperatures. This area of yellowed chlorotic tissue marks the juncture of the stem and the flag leaf at the time when frost occurred **(Figure 15)**.

This ring may be present on damaged as well as on plants that show no other symptoms. It does not seem that this ring interfere with the movement of nutrients from the plant to the developing grain. As the plants mature, however, the stems may bend or break at the frost ring. This usually occurs more often with above average grain filling and especially during windy conditions where wheat is irrigated.

ANTHESIS (Growth point stage 20)

The flowering stage is the most sensitive growth stage of wheat and only slight changes in temperature, the duration of exposure and other conditions (wind, irrigation and the physiological well being of plants) can cause major differences in the degree of damage caused.

At this stage it seems that the critical temperature range is from –3.5°C to 0.6°C and the symptoms of the flowering and heading stages are nearly similar. Frequently, only the male parts (anthers) of the flowers in the ears die because they are more sensitive to low temperatures than the female parts.

Since wheat is self-pollinating, sterility caused by frost damage could lead to poor kernel set with consequential yield losses.

Wheat anthers are tri-lobed, light green to green in colour and turgid preceding pollination.

Possible sterility could be detected soon after the occurrence of frost by examining the anthers inside each floret. When the anthers appear from the florets during normal conditions they are yellow in colour.

During the preceding period the anthers are tri-lobed, normally light green in colour and turgid in appearance **(Figure 16)**.

During frost damage the anthers become twisted and shrivelled within 48 hours and they quickly turn white to whitish-brown in colour and may not protrude from the florets **(Figure 17)**.

ANTHESIS (Growth point stage 20)

Figure 17

Frost damaged anthers become discoloured within two days, depending on conditions, and turn from light yellow to off-white.

Figure 18

A healthy, undamaged stigma has a white feathery appearance.

Female flowering parts can also be damaged by frost although the male flowering parts are more sensitive to frost damage than the female flowering parts. If the female flowering parts are damaged, then the anthers will definitely be damaged. The stigma normally has a white, feathery appearance **(Figure 18)**, but a damaged stigma becomes off-white to brown in colour **(Figure17)**. In view of the fact that the flowering process in wheat proceeds from florets one third from the base of the ear to florets at the top and the bottom of the ear over a two to four day period, explains why slight differences during the stage of flowering can cause the appearance of void or partially filled ears. The centre or one or both ends of the ear might be void of grain because those florets were at a sensitive stage when they were frozen during the occurrence of frost.

ANTHESIS (Growth point stage 20) and MILK DOUGH STAGE (Growth point stage 21)


Figure 19

Frost damage during anthesis lead to void or partially filled ears. The interaction between the stage of flowering and the intensity and occurrence of frost determines the degree of damage.

Grain might develop in other parts of the ear, however, because flowering had not started or was already completed in those florets when frost occurred **(Figure 19)**. During the milk dough stage the young developing kernels are plump and to reach this it will grow within two weeks after anthesis to its full size (volume), but the kernel reaches its maximum weight only four weeks after anthesis. Under normal circumstances the endosperm contains a large amount of water and if pinched the contents of these young kernels have a milky appearance.

Frost damage during this stage is more severe than during the soft and hard dough stages as a result of the higher water content.

Frost damaged kernels during this stage might be white or grey and have a rough shrivelled appearance instead of their normal light green, plump appearance. A slight coloration ("a blister") on the kernel during the milk dough stage might be an indication of frost damage **(Figure 21)**.

MILK DOUGH STAGE (Growth point stage 21)

Figure 20

Both the kernels are in the milk dough stage. The left-hand kernel has not been exposed to frost, but the right-hand kernel has been exposed to frost and the contents already have a grey appearance.

The occurrence of mild frost during the milk dough stage could cause kernels to grow their normal size, but at harvest these kernels could be light and shrivelled.

Examining these kernels during the early soft dough stage, may show that their contents are grey and liquid instead of white and viscous compared to that of undamaged kernels at this stage (**Figure 20**). Mature wheat grains that were damaged during the milk dough stage are often shrivelled and has a low hectolitre mass.

Figure 21

Slight coloration ("a blister") on the kernel during the milk dough stage indicates frost damage.

Frost damage may also seriously reduce the germination of grain where seed are produced.

DOUGH STAGE (Growth stages 22–24)

A normally developed kernel has a sound green colour, is round and turgid.

The dough stage consists of a soft and a hard dough stage. The endosperm becomes firm/starched and has a doughy appearance **(Figure 22)**.

The colour of the kernel also changes from dark green to yellow. After the hard dough stage no nutrients/photosynthate are translocated or deposited to the kernel and it becomes physiologically mature.

During this stage the final development of the kernel has already occurred.

The kernel water content has already decreased and wheat is usually more resistant to freezing temperatures during this stage than during most early spring growth stages.

Visual signs of frost stress during the dough stage may be green shrivelled kernels with "blisters" on the testa and the testa has the tendency to become loose. During the soft dough stage, after the kernels started to change colour, severe frost could cause "blisters" on the surface of the kernels as a whole.

The most serious consequence of frost damage during the dough stage is reduction in the germination of seed. The embryo or germ usually has a higher water content than that of other kernel parts, and its complex of cellular contents and structures makes it more vulnerable to frost damage.

FULLY MATURED (Growth point stage 25)

During this stage the kernels has reached maturity and therefore no frost damage can occur.

Of importance are the visual symptoms after harvesting, as it has been summarised by the Department of Agriculture (Act No.119 of 1990).

The term heavily frost-damaged wheat is used and includes the following:

i) Wheat kernels which have been damaged by severe frost during the milk to soft dough stage are characterised by the kernels being fairly plump but covered entirely with small blisters extending into the crease, excluding:

a) kernels in which blistering is confined to the back of the kernel; and

b) immature wrinkled kernels in which wrinkling has been caused by frost while the kernels were still immature; and

ii) wheat kernels (flaked) which have a slightly flaked-off bran coat due to frost: Provided that evidence of frost damage is present and that the bran coat had not been rubbed off due to handling.

POSSIBLE CONFUSIONS

POSSIBLE CONFUSIONS

Considering that stress factors such as heat, drought, cold (frost), insect damage, pathogen infections, mechanical and chemical damage could all occur in wheat, the symptoms can be confusing due to similarities.

Therefore some of these symptoms, observed and positively identified in practice, are briefly presented as figures (photo's) with accompanying captions to support and explain the actual situation to clear away uncertainty and confusion where possible **(Figures 23 – 39)**.

POSSIBLE CONFUSIONS

Figure 23

Drought also causes white ear tips and during this condition the lower leaves on the plant also die/desiccate and the stem length is shorter than normal.

Figure 24

Ears may also be pinched by the flag leaf due to aphid damage and this could lead to ears that split out of the side of the leaf sheath of typically damaged awns.

Figure 25

Mechanical damage caused by a sprayer mounted on a downpipe on the overhang of a centre pivot.

Figure 26

Hail damage causes the tip of awns to be pinched in the leaf sheath after the flag leaf has been damaged.

POSSIBLE CONFUSIONS

Figure 27

Bole worm damage to wheat ears.

Figure 28

A bole worm on a wheat ear.

Figure 29

Stink-beetle damage to florets.

Figure 30

A stink-beetle on a wheat ear.

POSSIBLE CONFUSIONS

Figure 31

An ear becomee white in colour due to Chilo borer damage. Damage is usually confined to a single plant in a field.

Figure 32

A stem that has been damaged by a Chilo borer.

Figure 33

A Chilo borer.

Figure 34

Take-all infection usually occurs in patches but can cover the whole field. The appearance of white or void wheat ears are typical of take-all (Gaeumannomyces graminis var. tritici)

Figure 35

A black coloration on the bottom leaf sheaths and adjacent stems as well as the crowns of the plants is a certain sign of take-all (Gaeumannomyces graminis var. tritici).

POSSIBLE CONFUSIONS

Figure 36

The top part of the ear infected by Fusarium graminearum.

Figure 37

The brown coloration of the stem is a typical sign of Fusarium infection. The flag leaf dies suddenly and white ears appear either in patches or covers the entire field during severe infection.

Figure 38

Stem cavities at the base of stems are infested with mycelium growth and during moist conditions a reddish pink fungal growth may occur on the basal leaf sheaths.

Figure 39

The influence of Fusarium infection, drought, heat, and frost damage on grain filling. Kernels are shrivelled and light in mass.

Kernels that have been damaged by heat show a dark coloration.

The degree and stage of grain filling will also determine the degree of shrivelled kernels and furthermore it has been established that the deep groove found on the kernels was not due to frost damage only.

From the above it is evident that it is extremely difficult to establish the cause of shrivelled kernels after harvesting and that this can not be used as the norm to qualify frost damage.

SUMMARY OF SYMPTOMS

SUMMARY OF SYMPTOMS

TILLERING STAGE

- Growth points – Below the soil surface and protected by leaves.
- Leaf damage – Within one to two days after the occurrence of frost the leaves will be twisted, discolour from light green to yellow with necrotic marks to leaf tips and even on the bend of older leaves.
- Kernel damage – None.

JOINTING STAGE

- Leaf damage – Similar to that of the tillering stage.
- Growth point damage – Growth points become off-white or turn brown and water soaked in appearance.
- Stem damage – Initially stems remain green, but growth ceases immediately and die later on.
- Uneven growth due to a mixture of normal and late tillers. Lower parts of stems show discolouration, roughness, lesions and enlargement of nodes; stems easily bent and are also easily pulled by hand (break at the juncture of the ice/frost ring).
- Kernel damage – None.

FLAG LEAF STAGE

- Leaf damage – Dead leaf tips and brown in colour.
- Ears – Pinched in flag leaf sheaths. Ears split out to the side or the ear base appears first.
- Ear may also emerge sterile from the flag leaf sheath.
- Kernel damage – None.

SUMMARY OF SYMPTOMS

EAR EMERGENCE

- Sterility, leaf desiccation or drying and lesions on lower stems. Other symp toms are similar to that of previous growth stages.
- The most apparent symptom, however, is usually chlorosis or bleaching of the awns so they are white instead of green, and typically disordered. Temperature that damage awns may also kill the male flower parts.
- Stems – "Ice/frost ring" appear on stems; yellowish chlorotic tissue at the juncture of the stem and flag leaf. Stems easily bend at this point.
- Kernel damage – None.

ANTHESIS

- Symptoms virtually similar to the above.
- Flower parts – Anthers twisted and shrivelled, white to white-brown in colour and does not protrude from the floret. Anthers die and turn off-white or brown and appear to be dehydrated.
- Ears – Void or partially filled.
- Kernel damage – None.

MILK DOUGH STAGE

- Kernels – Frost damaged kernels may be white or grey in colour and have a rough, shrivelled appearance instead of their normal light green, plump appearance. The contents could be grey and liquid instead of white and viscous as it should be during this stage.

DOUGH STAGE

- Kernels – kernels are unsightly, shrivelled and with "blisters" on the seed and a reduction in hectolitre mass.
- A reduction in the ability of seed to germinate.

NOTES

CHAPTER 9

SUMMARY

In South Africa loss of income as a result of frost damage to wheat has had far reaching consequences for the wheat industry in recent times. This is the result of early maturing wheat as well as the occurrence of late frost in early spring when wheat is most susceptible to frost damage, therefore intensifying the risk of frost stress.

The objectives of this study were to: a) evaluate the quantitative and qualitative characteristics of different growth types (winter, intermediate and spring wheat) for tolerance to frost (freezing) during the tillering, flag leaf, flowering and hard dough growth stages at different sub-zero temperatures; b) evaluate different growth types for frost tolerance at different flag leaf growth stages; c) evaluate different growth types for frost tolerance at different flowering stages; and d) compile a guide with illustrations of frost injury symptoms that could be used by wheat growers and other participants in the wheat industry.

a) Artificial freezing was used to evaluate the reaction response of different growth types at different temperatures (0 to –12°C with 3°C increments) and at different growth stages (tillering, flag leaf, flowering and hard dough). A quantitative evaluation showed that all cultivars were to some degree sensitive to frost damage. Cultivars 1 to 3 showed the highest degree of sensitivity at the flag leaf stage, while cultivar 4 proved to be more sensitive at the flowering stage. Though the growth stages differed in terms of sensitivity to frost stress, it was evident that temperatures below –6°C led to a reduction in growth and development, and subsequently a reduction in the parameters measured. Finally, the different genotypes had a profound influence on the reaction of wheat to frost injury, with the winter type being more tolerant than the spring type.

b) A qualitative evaluation of different growth types, in terms of protein content and stirring number, was conducted at different temperatures and different growth stages. Results obtained at the different growth stages, before grain filling commenced, showed no

differences. Only the hard dough stage seemed to be negatively influenced by the cumulative effect of a decrease in temperature. Generally a decrease in temperature led to a decrease in grain quality at different growth stages.

c) Artificial freezing was also used to evaluate the reaction of two growth types at different temperatures (–5° to –9°C with 2°C increments) and at different flag leaf stages (early flag leaf, flag leaf and emerging of awns). The quantitative evaluation showed cultivar 1 (winter type) to be more tolerant to frost injury than cultivar 2 (intermediate type). However, both cultivars were highly sensitive to frost injury at the early flag leaf than at the flag leaf and emergence of the awns stages. Furthermore, the primary spikes were shown to sustain the highest degree of frost injury during this trial.

d) During this trial the reaction response of two growth types at different temperatures (–5° to –9°C with 2°C increments) and at different flowering stages (0, 50 and 100% flowering), was evaluated. The quantitative evaluation showed cultivar 1 (winter type) to be more frost tolerant than cultivar 2 (intermediate type). At the different flowering stages both cultivars proved to be highly sensitive to frost injury at temperatures lower than –5°C. No significant differences were obtained for cultivar 1 at the different flowering stages, but cultivar 2 was more sensitive at 0% flowering than at other growth stages.

e) A guide was finally compiled to assist the producer, agronomist, insurance companies and other role players in the wheat industry. This guide consists of short discussions supported by photographs to illustrate frost damage to South African wheat.

Key words: wheat, frost, freezing, temperature, growth stage, flag leaf, flowering, yield components, protein, symptoms

OPSOMMING

In Suid-Afrika het die verlies aan inkomste as gevolg van rypskade op koring onlangs verrykende gevolge op die koringbedryf gehad. Hierdie is die gevolg van koring wat voortydig wasdom bereik sowel as die voorkoms van laat ryp vroeg in die lente wanneer koring die gevoeligste vir rypskade is, wat gevolglik die risiko vir rypskade verhoog.

Die doelstellings van die studie was om: a) die kwantitatiewe en kwalitatiewe eienskappe van verskillende koringtipes (winter, intermediêre en lente tipes) vir ryptoleransie (vriestoleransie) gedurende die stoel-, vlagblaar-, blom- en hardedeegstaduims by verskillende temperature benede vriespunt te evalueer; b) verskillende koring tipes vir ryptoleransie by verskillende vlagblaarstadiums te evalueer; c) verskillense koringtipes vir ryptoleransie by verskillende blomstadiums te evalueer; en d) ’n handleiding wat rypskade simptome illustreer saam te stel wat deur produsente en ander belanghebbendes in die koringbedryf gebruik kan word.

a) ’n Kunsmatige vriesmetode is gebruik om die reaksie van verskillende koringtipes by verskillende temperature (0 tot –12°C met 3°C inkremente) by verskillende groeistadia (stoel-, vlagblaar-, blom- en hardedeegstadium) te evalueer. Al die cultivars het tydens die kwantitatiewe evaluering ’n mate van sensitiwiteit teenoor rypskade getoon. Cultivars 1 tot 3 het die hoogste graad van sensitiwiteit tydens die vlagblaarstadium getoon, terwyl cultivar 4 meer sensitief tydens die blomstadium was. Alhoewel die groeistadiums sensitiwiteitsverskille getoon het, was daar ’n duidelike aanduiding dat temperature benede –6°C tot ’n verlaging in die groei en ontwikkeling van koring gelei het en gevolglik ’n verlaging in die gemete parameters. Laastens het die verskillende koringtipes (genotipes) ’n duidelike invloed op die die reaksie van koring op rypskade uitgeoefen waar die wintertipe ’n groter mate van toleransie as die lentetipes getoon het.

b) ’n Kwalitatiewe evaluering van verskillende koringtipes, in terme van proteïn-inhoud en roergetal, is by verskillende temperature en verskillende groeistadiums uitgevoer. Resultate wat vir die groeistadiums voor graanvulling het geen verskille getoon nie. Dit

blyk dat slegs die hardedeegstadium negatief beïnvloed is deur die kumulatiewe effek van dalende temperature. In die algemeen het dalende temperature to die verlaging in graankwaliteit by verskillende groeistadiums gelei.

c) ’n Kunsmatige vriesmetode is gebruik om die reaksie van twee koring tipes by verskillende temperature (–5 tot –9°C met 2°C inkremente) en by verskillende vlagblaarstadiums (vroeë vragblaar, vlagblaar en verskeining van angels). Tydens die kwantitatiewe evaluering het cultivar 1 (wintertipe) ’n groter mate van ryptoleransie as cultivar 2 (intermediêre tipe) getoon. Beide cultivars het ’n groot mate van sensitiwiteit tydens die vroeë vlagblaarstadium as die vlagblaar en/of aarverskyning van angels getoon. Verder het die primêre are die grootste mate van rypskade tydens die proef getoon.

d) Tydens die proef is die reaksie van twee verskillende koringtipes by verskillende temperature (–5 tot –9°C met 2°C inkremente) en by verskillende blomstadiums (0, 50 en 100% blom), geëvalueer. Die kwantitatiewe evaluering het getoon dat cultivar 1 (wintertipe) meer ryptolerant as cultivar 2 (intermediêre tipe) was. By al die blomstadiums het albei cultivars ’n hoër mate van rypsensitiwiteit by temperature laer as –5°C getoon. Geen betekenisvolle verskille is vir cultivar 1 by die verskillende blomstadiums gevind nie, maar cultivar 2 het ’n hoër mate van sensitiwiteit by 0% blom as die ander blomstadiums getoon.

e) ’n Handleiding is saamgestel as hulpmiddel wat deur produsente, agronome, versekerings-instansies en ander rolspelers in die koringindustrie gebruik kan word. Die handleiding bestaan uit bondige besprekings wat deur foto’s ondersteun word om rypskade op Suid-Afrikaanse koring te illustreer.

Sleutelwoorde: koring, ryp, temperatuur, vriesing, groeistadiums, vlagblaar, blom, opbrenskomponente, proteïen, simptome

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APPENDICES

APPENDIX 4

Appendix 4.1: Analysis of variance of dry matter as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	170.7893	42.69733	9.61	0.000002*	0.997660
B: Grst	3	41.1193	13.70643	3.08	0.031945*	0.616373
AB	12	181.299	15.10825	3.40	0.000481*	0.988280
S	80	355.5563	4.444454			
Total (Adjusted)	99	748.7639				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 4.887 (Temp x Grst)

Appendix 4.2: Analysis of variance of dry matter as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	150.5437	37.63591	13.19	0.000000*	0.999911
B: Grst	3	41.24797	13.74932	4.82	0.003906*	0.824999
AB	12	92.8832	7.740267	2.71	0.004020*	0.956754
S	80	228.318	2.853975			
Total (Adjusted)	99	512.9929				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 3.916 (Temp x Grst)

Appendix 4.3: Analysis of variance of dry matter as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	288.2986	72.07465	27.35	0.000000*	1.000000
B: Grst	3	40.88633	13.62878	5.17	0.002561*	0.853444
AB	12	122.3825	10.19855	3.87	0.000114*	0.995565
S	80	210.7916	2.634895			
Total (Adjusted)	99	662.3591				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 3.763 (Temp x Grst)

Appendix 4.4: Analysis of variance of dry matter as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	84.16638	21.0416	17.55	0.000000*	0.999999
B: Grst	3	11.9013	3.9671	3.31	0.024270*	0.650436
AB	12	39.84035	3.320029	2.77	0.003376*	0.960945
S	80	95.93051	1.199131			
Total (Adjusted)	99	231.8385				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 2.538 (Temp x Grst)

Appendix 4.5: Analysis of variance of spikes per plant as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	61.665	15.41625	13.28	0.000000*	0.999918
B: Grst	3	26.4875	8.829166	7.60	0.000155*	0.961399
AB	12	61.675	5.139583	4.43	0.000021*	0.998682
S	80	92.9	1.16125			
Total (Adjusted)	99	242.7275				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 2.498 (Temp x Grst)

Appendix 4.6: Analysis of variance of spikes per plant as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	40.885	10.22125	22.97	0.000000*	1.000000
B: Grst	3	20.1475	6.715833	15.09	0.000000*	0.999731
AB	12	29.415	2.45125	5.51	0.000001*	0.999896
S	80	35.6	0.445			
Total (Adjusted)	99	126.0475				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 1.546 (Temp x Grst)

Appendix 4.7: Analysis of variance of spikes per plant as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	42.265	10.56625	23.29	0.000000*	1.000000
B: Grst	3	3.6475	1.215833	2.68	0.052469	0.549732
AB	12	15.315	1.27625	2.81	0.002948*	0.963945
S	80	36.3	0.45375			
Total (Adjusted)	99	97.5275				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 1.562 (Temp x Grst)

Appendix 4.8: Analysis of variance of spikes per plant as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	33.875	8.46875	13.63	0.000000*	0.999942
B: Grst	3	4.5475	1.515833	2.44	0.070403	0.507288
AB	12	14.065	1.172083	1.89	0.048382*	0.832892
S	80	49.7	0.62125			
Total (Adjusted)	99	102.1875				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 1.827 (Temp x Grst)

Appendix 4.9: Analysis of variance of spikelets per primary spike as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	483.815	120.9538	15.56	0.000000*	0.999991
B: Grst	3	526.2875	175.4292	22.56	0.000000*	0.999999
AB	12	859.925	71.66042	9.22	0.000000*	1.000000
S	80	622	7.775			
Total (Adjusted)	99	2492.028				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 6.464 (Temp x Grst)

Appendix 4.10: Analysis of variance of spikelets per primary spike as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	402.56	100.64	12.36	0.000000*	0.999806
B: Grst	3	466.64	155.5467	19.11	0.000000*	0.999986
AB	12	728.06	60.67167	7.45	0.000000*	0.999999
S	80	651.3	8.14125			
Total (Adjusted)	99	2248.56				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 6.614 (Temp x Grst)

Appendix 4.11: Analysis of variance of spikelets per secondary spike as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	930.985	232.7462	56.24	0.000000*	1.000000
B: Grst	3	531.6075	177.2025	42.82	0.000000*	1.000000
AB	12	1058.855	88.23792	21.32	0.000000*	1.000000
S	80	331.1	4.13875			
Total (Adjusted)	99	2852.548				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 4.761 (Temp x Grst)

Appendix 4.12: Analysis of variance of spikelets per primary spike as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	548.235	137.0587	35.20	0.000000*	1.000000
B: Grst	3	139.58	46.52667	11.95	0.000002*	0.997562
AB	12	394.045	32.83708	8.43	0.000000*	1.000000
S	80	311.5	3.89375			
Total (Adjusted)	99	1393.36				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 4.574 (Temp x Grst)

Appendix 4.13: Analysis of variance of spikelets per secondary spike as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	217.8319	54.45798	2.01	0.100674	0.502133
B: Grst	3	449.4535	149.8178	5.53	0.001669*	0.878352
AB	12	842.4536	70.20447	2.59	0.005789*	0.946658
S	80	2165.439	27.06799			
Total (Adjusted)	99	3675.178				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 12.060 (Temp x Grst)

Appendix 4.14: Analysis of variance of spikelets per secondary spike as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	362.2636	90.56591	5.73	0.000415*	0.947642
B: Grst	3	242.3033	80.76778	5.11	0.002748*	0.848969
AB	12	665.5018	55.45848	3.51	0.000342*	0.990638
S	80	1263.734	15.79668			
Total (Adjusted)	99	2533.803				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 9.213 (Temp x Grst)

Appendix 4.15: Analysis of variance of spikelets per secondary spike as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	843.8563	210.9641	18.72	0.000000*	1.000000
B: Grst	3	263.5766	87.85886	7.79	0.000125*	0.965505
AB	12	475.9826	39.66522	3.52	0.000333*	0.990790
S	80	901.7773	11.27222			
Total (Adjusted)	99	2485.193				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 11.686 (Temp x Grst)

Appendix 4.16: Analysis of variance of spikelets per secondary spike as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	613.8611	153.4653	10.68	0.000001*	0.999089
B: Grst	3	39.01662	13.00554	0.90	0.442676	0.206130
AB	12	356.172	29.681	2.06	0.028725*	0.872225
S	80	1150.042	14.37553			
Total (Adjusted)	99	2159.092				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 8.789 (Temp x Grst)

Appendix 4.17: Analysis of variance of the average number of spikelets per spike as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	5770.45	1442.613	7.30	0.000046*	0.984034
B: Grst	3	2701.74	900.58	4.56	0.005342*	0.801214
AB	12	9420.51	785.0425	3.97	0.000084*	0.996424
S	80	15813.3	197.6662			
Total (Adjusted)	99	33706				
Total	100					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 7.957 (Temp x Grst)

Appendix 4.18: Analysis of variance of the average number of spikelets per spike as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	195.894	48.9735	6.12	0.000239*	0.960510
B: Grst	3	224.2957	74.76525	9.34	0.000023*	0.986571
AB	12	623.2057	51.93381	6.49	0.000000*	0.999991
S	80	640.3616	8.00452			
Total (Adjusted)	99	1683.757				
Total	100					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 6.558 (Temp x Grst)

Appendix 4.19: Analysis of variance of the average number of spikelets per spike as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	604.3707	151.0927	24.34	0.000000*	1.000000
B: Grst	3	430.4479	143.4826	23.11	0.000000*	0.999999
AB	12	960.3168	80.0264	12.89	0.000000*	1.000000
S	80	496.6216	6.207769			
Total (Adjusted)	99	2491.757				
Total	100					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 5.776 (Temp x Grst)

Appendix 4.20: Analysis of variance of the average number of spikelets per spike as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	326.8067	81.70168	25.09	0.000000*	1.000000
B: Grst	3	110.8729	36.95763	11.35	0.000003*	0.996352
AB	12	492.2918	41.02431	12.60	0.000000*	1.000000
S	80	260.4939	3.256174			
Total (Adjusted)	99	1190.465				
Total	100					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 4.183 (Temp x Grst)

Appendix 4.21: Analysis of variance of the number of kernels produced by the primary spike as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	1873.5	468.375	7.09	0.000061*	0.981193
B: Grst	3	1459.807	486.6025	7.37	0.000202*	0.955684
AB	12	3674.68	306.2233	4.64	0.000012*	0.999180
S	80	5284.7	66.05875			
Total (Adjusted)	99	12292.69				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(\text{Tukey} =0.05)}$ = 18.841 (Temp x Grst)

Appendix 4.22: Analysis of variance of the number of kernels produced by the primary spike as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	3589.985	897.4963	11.62	0.000000*	0.999613
B: Grst	3	2136.028	712.0092	9.22	0.000026*	0.985494
AB	12	4580.235	381.6862	4.94	0.000005*	0.999596
S	80	6179.8	77.2475			
Total (Adjusted)	99	16486.05				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(\text{Tukey} =0.05)}$ = 20.347 (Temp x Grst)

Appendix 4.23: Analysis of variance of the number of kernels produced by the primary spike as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	7831.925	1957.981	23.64	0.000000*	1.000000
B: Grst	3	7035.567	2345.189	28.31	0.000000*	1.000000
AB	12	8969.895	747.4913	9.02	0.000000*	1.000000
S	80	6626.3	82.82875			
Total (Adjusted)	99	30463.69				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(\text{Tukey} =0.05)}$ = 21.175 (Temp x Grst)

Appendix 4.24: Analysis of variance of the number of kernels produced by the primary spike as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	5910.465	1477.616	25.10	0.000000*	1.000000
B: Grst	3	889.9675	296.6558	5.04	0.003002*	0.843205
AB	12	2697.795	224.8163	3.82	0.000133*	0.995047
S	80	4710.2	58.8775			
Total (Adjusted)	99	14208.43				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(\text{Tukey} =0.05)}$ = 17.787 (Temp x Grst)

Appendix 4.25: Analysis of variance of the number of kernels produced by secondary spikes as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	5399.06	1349.765	2.54	0.046460*	0.612508
B: Grst	3	2906.188	968.7292	1.82	0.150252	0.389052
AB	12	12189.5	1015.792	1.91	0.045489*	0.838052
S	80	42592	532.4			
Total (Adjusted)	99	63086.75				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 53.488 (Temp x Grst)

Appendix 4.26: Analysis of variance of the number of kernels produced by secondary spikes as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	17290.62	4322.654	14.53	0.000000*	0.999976
B: Grst	3	5495.49	1831.83	6.16	0.000806*	0.912670
AB	12	7791.085	649.2571	2.18	0.020210*	0.893831
S	80	23797.3	297.4662			
Total (Adjusted)	99	54374.49				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 39.981 (Temp x Grst)

Appendix 4.27: Analysis of variance of the number of kernels produced by secondary spikes as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	10234.74	2558.684	11.49	0.000000*	0.999565
B: Grst	3	1116.54	372.18	1.67	0.179767	0.359553
AB	12	1677.085	139.7571	0.63	0.812730	0.306329
S	80	17813.9	222.6738			
Total (Adjusted)	99	30842.26				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 13.170 (Temp)

Appendix 4.28: Analysis of variance of the number of kernels produced by secondary spikes as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	5481.925	1370.481	5.95	0.000303*	0.955403
B: Grst	3	1056.76	352.2533	1.53	0.213064	0.331191
AB	12	4405.215	367.1013	1.59	0.109867	0.748576
S	80	18416.1	230.2012			
Total (Adjusted)	99	29360				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 17.586 (Temp)

Appendix 4.29: Analysis of variance of the total number of kernels produced by primary and secondary spikes as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	13349.58	3337.396	4.30	0.003347*	0.860252
B: Grst	3	8419.05	2806.35	3.62	0.016682*	0.693560
AB	12	24637.97	2053.165	2.64	0.004944*	0.951264
S	80	62100.4	776.255			
Total (Adjusted)	99	108507				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 64.586 (Temp x Grst)

Appendix 4.30: Analysis of variance of the total number of kernels produced by primary and secondary spikes as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	36626.41	9156.604	19.74	0.000000*	1.000000
B: Grst	3	13824.75	4608.249	9.94	0.000012*	0.990794
AB	12	21550.96	1795.914	3.87	0.000113*	0.995578
S	80	37105.5	463.8188			
Total (Adjusted)	99	109107.6				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 49.924 (Temp x Grst)

Appendix 4.31: Analysis of variance of the total number of kernels produced by primary and secondary spikes as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	35669.66	8917.415	32.87	0.000000*	1.000000
B: Grst	3	11327.61	3775.869	13.92	0.000000*	0.999378
AB	12	16924.98	1410.415	5.20	0.000002*	0.999781
S	80	21701.1	271.2638			
Total (Adjusted)	99	85623.34				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 31.180 (Temp x Grst)

Appendix 4.32: Analysis of variance of the total number of kernels produced by primary and secondary spikes as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	22418.54	5604.635	16.68	0.000000*	0.999997
B: Grst	3	1486.108	495.3692	1.47	0.227806	0.319930
AB	12	8874.78	739.565	2.20	0.019120*	0.896920
S	80	26879.9	335.9987			
Total (Adjusted)	99	59659.33				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 42.492 (Temp x Grst)

Appendix 4.33: Analysis of variance of the number of kernels per secondary spike as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	169.6204	42.4051	0.58	0.681064	0.160798
B: Grst	3	505.1846	168.3949	2.29	0.085018	0.478982
AB	12	1219.726	101.6438	1.38	0.192929	0.669593
S	80	5892.757	73.65946			
Total (Adjusted)	99	7787.288				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = NS (Temp, Grst and Temp x Grst)

Appendix 4.34: Analysis of variance of the number of kernels per secondary spike as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	1798.052	449.513	5.33	0.000744*	0.930041
B: Grst	3	685.0436	228.3479	2.71	0.050794	0.554284
AB	12	1947.251	162.271	1.92	0.043552*	0.841605
S	80	6750.989	84.38736			
Total (Adjusted)	99	11181.34				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 21.295 (Temp x Grst)

Appendix 4.35: Analysis of variance of the number of kernels per secondary spike as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	5027.72	1256.93	8.52	0.000009*	0.994075
B: Grst	3	1227.277	409.0923	2.77	0.046854*	0.565499
AB	12	1719.314	143.2762	0.97	0.483483	0.482731
S	80	11807.57	147.5946			
Total (Adjusted)	99	19781.88				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 10.722 (Temp) LSD $_{(Tukey =0.05)}$ = 9.016 (Grst)

Appendix 4.36: Analysis of variance of the number of kernels per secondary spike as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	2944.546	736.1365	11.88	0.000000*	0.999697
B: Grst	3	221.5696	73.85652	1.19	0.318131	0.263081
AB	12	1127.067	93.92223	1.52	0.135693	0.721317
S	80	4956.366	61.95457			
Total (Adjusted)	99	9249.548				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 6.947 (Temp)

Appendix 4.37: Analysis of variance of the average number of kernels per spike as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	305.1915	76.29788	1.42	0.233309	0.363512
B: Grst	3	868.4082	289.4694	5.41	0.001942*	0.869959
AB	12	1558.353	129.8628	2.43	0.009694*	0.928744
S	80	4283.55	53.54437			
Total (Adjusted)	99	7015.502				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 16.963 (Temp x Grst)

Appendix 4.38: Analysis of variance of the average number of kernels per spike as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	1065.956	266.4889	4.07	0.004709*	0.838226
B: Grst	3	644.2496	214.7499	3.28	0.025159*	0.646091
AB	12	2749.112	229.0927	3.50	0.000356*	0.990390
S	80	5239.571	65.49464			
Total (Adjusted)	99	9698.889				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 18.760 (Temp x Grst)

Appendix 4.39: Analysis of variance of the average number of kernels per spike as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	3586.9	896.725	10.82	0.000000*	0.999197
B: Grst	3	3809.315	1269.772	15.32	0.000000*	0.999771
AB	12	6221.63	518.4692	6.25	0.000000*	0.999984
S	80	6632.482	82.90604			
Total (Adjusted)	99	20250.33				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 21.107 (Temp x Grst)

Appendix 4.40: Analysis of variance of the average number of kernels per spike as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	4586.372	1146.593	31.65	0.000000*	1.000000
B: Grst	3	567.2325	189.0775	5.22	0.002420*	0.856938
AB	12	1882.05	156.8375	4.33	0.000029*	0.998366
S	80	2897.785	36.22231			
Total (Adjusted)	99	9933.439				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 13.952 (Temp x Grst)

Appendix 4.41: Analysis of variance of the primary kernel weight as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	2.831771	0.7079427	12.22	0.000000*	0.999779
B: Grst	3	0.5346355	0.1782119	3.08	0.032244*	0.615180
AB	12	3.284776	0.2737314	4.73	0.000009*	0.999333
S	80	4.6344	0.05793			
Total (Adjusted)	99	11.28558				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.558 (Temp x Grst)

Appendix 4.42: Analysis of variance of the primary kernel weight as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	3.835633	0.9589081	12.64	0.000000*	0.999850
B: Grst	3	1.06235	0.3541166	4.67	0.004679*	0.811569
AB	12	2.89463	0.2412191	3.18	0.000950*	0.981904
S	80	6.070678	7.588347E-02			
Total (Adjusted)	99	13.86329				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.639 (Temp x Grst)

Appendix 4.43: Analysis of variance of the primary kernel weight as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	11.17039	2.792598	27.61	0.000000*	1.000000
B: Grst	3	6.336303	2.112101	20.89	0.000000*	0.999997
AB	12	8.217367	0.6847805	6.77	0.000000*	0.999996
S	80	8.09022	0.1011278			
Total (Adjusted)	99	33.81428				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.737 (Temp x Grst)

Appendix 4.44: Analysis of variance of the primary kernel weight as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	11.65066	2.912665	31.02	0.000000*	1.000000
B: Grst	3	2.360595	0.7868649	8.38	0.000065*	0.975700
AB	12	4.143689	0.3453074	3.68	0.000205*	0.993347
S	80	7.512252	9.390315E-02			
Total (Adjusted)	99	25.6672				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.710 (Temp x Grst)

Appendix 4.45: Analysis of variance of the secondary kernel weight as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	7.618859	1.904715	6.03	0.000272*	0.957820
B: Grst	3	1.800521	0.6001738	1.90	0.136295	0.404876
AB	12	5.343203	0.4452669	1.41	0.179023	0.681304
S	80	25.27346	0.3159183			
Total (Adjusted)	99	40.03605				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.496 (Temp)

Appendix 4.46: Analysis of variance of the secondary kernel weight as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	16.26974	4.067436	18.76	0.000000*	1.000000
B: Grst	3	2.398827	0.7996089	3.69	0.015270*	0.703173
AB	12	4.061668	0.3384723	1.56	0.120313	0.737167
S	80	17.34576	0.216822			
Total (Adjusted)	99	40.076				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.411 (Temp) LSD $_{(Tukey\ =0.05)}$ = 0.346 (Grst)

Appendix 4.47: Analysis of variance of the secondary kernel weight as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	13.84185	3.460463	17.60	0.000000*	0.999999
B: Grst	3	0.4938742	0.1646247	0.84	0.477308	0.193077
AB	12	1.582032	0.131836	0.67	0.774371	0.328363
S	80	15.72751	0.1965939			
Total (Adjusted)	99	31.64527				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.391 (Temp)

Appendix 4.48: Analysis of variance of the secondary kernel weight as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	5.294424	1.323606	6.53	0.000133*	0.971120
B: Grst	3	0.6366759	0.2122253	1.05	0.376127	0.234279
AB	12	4.313186	0.3594322	1.77	0.066687	0.803570
S	80	16.20389	0.2025486			
Total (Adjusted)	99	26.44817				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.397 (Temp)

Appendix 4.49: Analysis of variance of the total kernel weight as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	19.49488	4.873721	8.96	0.000005*	0.995935
B: Grst	3	4.259613	1.419871	2.61	0.057049	0.537866
AB	12	14.59035	1.215862	2.24	0.017206*	0.902575
S	80	43.49839	0.5437299			
Total (Adjusted)	99	81.84324				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.709 (Temp x Grst)

Appendix 4.50: Analysis of variance of the total kernel weight as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	35.76281	8.940701	22.13	0.000000*	1.000000
B: Grst	3	6.219919	2.073306	5.13	0.002690*	0.850318
AB	12	12.37771	1.031476	2.55	0.006568*	0.942697
S	80	32.32769	0.4040962			
Total (Adjusted)	99	86.68813				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.474 (Temp x Grst)

Appendix 4.51: Analysis of variance of the total kernel weight as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	48.21563	12.05391	42.21	0.000000*	1.000000
B: Grst	3	6.194176	2.064725	7.23	0.000236*	0.952047
AB	12	14.5973	1.216441	4.26	0.000035*	0.998089
S	80	22.84744	0.285593			
Total (Adjusted)	99	91.85455				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.239 (Temp x Grst)

Appendix 4.52: Analysis of variance of the total kernel weight as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	31.68902	7.922255	28.55	0.000000*	1.000000
B: Grst	3	1.871971	0.6239904	2.25	0.089003	0.471987
AB	12	10.4227	0.868558	3.13	0.001105*	0.980119
S	80	22.19889	0.2774861			
Total (Adjusted)	99	66.18258				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.221 (Temp x Grst)

Appendix 4.53: Analysis of variance of the kernel weight per secondary spike as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	0.446628	0.111657	2.12	0.086053	0.525924
B: Grst	3	0.3310266	0.1103422	2.09	0.107557	0.442607
AB	12	0.5499144	0.0458262	0.87	0.580269	0.431317
S	80	4.215643	5.269555E-02			
Total (Adjusted)	99	5.543212				
Total	100					

* Term significant at alpha = 0.05

$LSD_{(Tukey=0.05)}$ = NS (Temp, Grst and Temp x Grst)

Appendix 4.54: Analysis of variance of the kernel weight per secondary spike as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	2.227487	0.5568717	13.41	0.000000*	0.999928
B: Grst	3	0.4862428	0.1620809	3.90	0.011765*	0.730259
AB	12	1.012627	0.0843856	2.03	0.031669*	0.865568
S	80	3.322775	4.153468E-02			
Total (Adjusted)	99	7.049131				
Total	100					

* Term significant at alpha = 0.05

$LSD_{(Tukey=0.05)}$ = 0.472 (Temp x Grst)

Appendix 4.55: Analysis of variance of the kernel weight per secondary spike as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	5.902367	1.475592	11.16	0.000000*	0.999411
B: Grst	3	0.3188728	0.1062909	0.80	0.495447	0.186604
AB	12	1.518787	0.1265656	0.96	0.496219	0.475835
S	80	10.57988	0.1322485			
Total (Adjusted)	99	18.3199				
Total	100					

* Term significant at alpha = 0.05

$LSD_{(Tukey=0.05)}$ = 0.321 (Temp)

Appendix 4.56: Analysis of variance of the kernel weight per secondary spike as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	2.953061	0.7382652	10.10	0.000001*	0.998486
B: Grst	3	0.1224577	4.081924E-02	0.56	0.643856	0.140841
AB	12	1.412975	0.1177479	1.61	0.104952	0.754149
S	80	5.845294	7.306618E-02			
Total (Adjusted)	99	10.33379				
Total	100					

* Term significant at alpha = 0.05

$LSD_{(Tukey=0.05)}$ = 0.239 (Temp)

Appendix 4.57: Analysis of variance of the average kernel weight per spike as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	0.7080637	0.1770159	4.28	0.003438*	0.858614
B: Grst	3	0.4400382	0.1466794	3.55	0.018119*	0.684388
AB	12	0.9164851	7.637376E-02	1.85	0.054217	0.822973
S	80	3.307782	4.134728E-02			
Total (Adjusted)	99	5.372369				
Total	100					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.179 (Temp) $LSD_{(Tukey=0.05)}$ = 0.151 (Grst)

Appendix 4.58: Analysis of variance of the average kernel weight per spike as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	1.764482	0.4411204	9.74	0.000002*	0.997916
B: Grst	3	0.4231144	0.1410381	3.11	0.030783*	0.621084
AB	12	1.619373	0.1349478	2.98	0.001759*	0.973537
S	80	3.623144	0.0452893			
Total (Adjusted)	99	7.430113				
Total	100					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.493 (Temp x Grst)

Appendix 4.59: Analysis of variance of the average kernel weight per spike as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	4.519116	1.129779	11.31	0.000000*	0.999489
B: Grst	3	2.731982	0.9106608	9.12	0.000029*	0.984587
AB	12	5.478983	0.4565819	4.57	0.000014*	0.999054
S	80	7.988107	9.985133E-02			
Total (Adjusted)	99	20.71819				
Total	100					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.733 (Temp x Grst)

Appendix 4.60: Analysis of variance of the average kernel weight per spike as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	7.737723	1.934431	30.73	0.000000*	1.000000
B: Grst	3	1.434464	0.4781547	7.60	0.000156*	0.961234
AB	12	3.147816	0.262318	4.17	0.000046*	0.997660
S	80	5.035928	0.0629491			
Total (Adjusted)	99	17.35593				
Total	100					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.582 (Temp x Grst)

Appendix 4.61: Analysis of variance of the mass per 100 kernels produced by the primary spike as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	33.41702	8.354255	16.30	0.000000*	0.999996
B: Grst	3	7.988067	2.662689	5.20	0.002492*	0.855141
AB	12	29.04846	2.420705	4.72	0.000009*	0.999330
S	80	41.00093	0.5125116			
Total (Adjusted)	99	111.4545				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.660 (Temp x Grst)

Appendix 4.62: Analysis of variance of the mass per 100 kernels produced by the primary spike as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	16.55034	4.137585	13.03	0.000000*	0.999897
B: Grst	3	5.349297	1.783099	5.62	0.001518*	0.883368
AB	12	15.79906	1.316588	4.15	0.000049*	0.997551
S	80	25.40253	0.3175316			
Total (Adjusted)	99	63.10122				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.306 (Temp x Grst)

Appendix 4.63: Analysis of variance of the mass per 100 kernels produced by the primary spike as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	52.76989	13.19247	50.50	0.000000*	1.000000
B: Grst	3	10.99252	3.664175	14.03	0.000000*	0.999423
AB	12	36.12717	3.010597	11.52	0.000000*	1.000000
S	80	20.89985	0.2612482			
Total (Adjusted)	99	120.7894				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.185 (Temp x Grst)

Appendix 4.64: Analysis of variance of the mass per 100 kernels produced by the primary spike as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	162.4477	40.61192	34.50	0.000000*	1.000000
B: Grst	3	41.39235	13.79745	11.72	0.000002*	0.997158
AB	12	55.44726	4.620605	3.93	0.000096*	0.996055
S	80	94.16337	1.177042			
Total (Adjusted)	99	353.4507				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 2.515 (Temp x Grst)

Appendix 4.65: Analysis of variance of the mass per 100 kernels produced by the secondary spikes as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	50.55813	12.63953	7.65	0.000029*	0.987918
B: Grst	3	6.477988	2.159329	1.31	0.278107	0.286080
AB	12	19.63395	1.636163	0.99	0.465905	0.492338
S	80	132.223	1.652787			
Total (Adjusted)	99	208.893				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.135 (Temp)

Appendix 4.66: Analysis of variance of the mass per 100 kernels produced by the secondary spikes as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	32.6255	8.156376	22.14	0.000000*	1.000000
B: Grst	3	3.982325	1.327442	3.60	0.016924*	0.691980
AB	12	12.00531	1.000443	2.72	0.003975*	0.957040
S	80	29.47029	0.3683786			
Total (Adjusted)	99	78.08344				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.407 (Temp x Grst)

Appendix 4.67: Analysis of variance of the mass per 100 kernels produced by the secondary spikes as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	103.4744	25.86861	52.66	0.000000*	1.000000
B: Grst	3	1.070673	0.3568909	0.73	0.539143	0.171907
AB	12	10.30323	0.8586022	1.75	0.071926	0.796016
S	80	39.30027	0.4912534			
Total (Adjusted)	99	154.1486				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.619 (Temp)

Appendix 4.68: Analysis of variance of the mass per 100 kernels produced by the secondary spikes as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	91.05298	22.76324	11.74	0.000000*	0.999655
B: Grst	3	8.415521	2.805173	1.45	0.235289	0.314472
AB	12	41.33658	3.444715	1.78	0.066186	0.804309
S	80	155.0616	1.938269			
Total (Adjusted)	99	295.8666				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.383 (Temp)

Appendix 4.69: Analysis of variance of the mass per 100 kernels as affected by temperature and growth stage for cultivar 1

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	40.25537	10.06384	13.57	0.000000*	0.999938
B: Grst	3	7.568142	2.522714	3.40	0.021673*	0.663863
AB	12	20.72424	1.72702	2.33	0.013027*	0.916184
S	80	59.34301	0.7417876			
Total (Adjusted)	99	127.8908				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 2.000 (Temp x Grst)

Appendix 4.70: Analysis of variance of the mass per 100 kernels as affected by temperature and growth stage for cultivar 2

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	20.63125	5.157813	18.60	0.000000*	1.000000
B: Grst	3	4.029039	1.343013	4.84	0.003789*	0.827185
AB	12	13.60445	1.133704	4.09	0.000059*	0.997221
S	80	22.18439	0.2773049			
Total (Adjusted)	99	60.44913				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.221 (Temp x Grst)

Appendix 4.71: Analysis of variance of the mass per 100 kernels as affected by temperature and growth stage for cultivar 3

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	55.33713	13.83428	53.98	0.000000*	1.000000
B: Grst	3	8.118832	2.706277	10.56	0.000006*	0.993853
AB	12	32.86861	2.73905	10.69	0.000000*	1.000000
S	80	20.50272	0.2562841			
Total (Adjusted)	99	116.8273				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 1.174 (Temp x Grst)

Appendix 4.72: Analysis of variance of the mass per 100 kernels as affected by temperature and growth stage for cultivar 4

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	4	132.428	33.10699	32.17	0.000000*	1.000000
B: Grst	3	31.79882	10.59961	10.30	0.000008*	0.992727
AB	12	51.05143	4.254285	4.13	0.000051*	0.997487
S	80	82.31771	1.028971			
Total (Adjusted)	99	297.5959				
Total	100					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 2.351 (Temp x Grst)

APPENDIX 6

Appendix 6.1: Analysis of variance of dry matter as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	26.67856	13.33928	1.74	0.190567	0.299460
B: Grst	2	27.98961	13.99481	1.82	0.176328	0.312513
AB	4	0.9920267	0.2480067	0.03	0.997902	0.055155
S	36	276.5435	7.681763			
Total (Adjusted)	44	332.2037				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = NS (Temp, Grst and Temp x Grst)

Appendix 6.2: Analysis of variance of dry matter as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	334.3746	167.1873	13.38	0.000045*	0.989659
B: Grst	2	18.80114	9.400569	0.75	0.478648	0.150737
AB	4	28.4641	7.116025	0.57	0.686548	0.159435
S	36	449.9812	12.49948			
Total (Adjusted)	44	831.621				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 3.156 (Temp)

Appendix 6.3: Analysis of variance of spikes per plant as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	96.13333	48.06667	14.01	0.000032*	0.992238
B: Grst	2	11.63333	5.816667	1.70	0.197825	0.293190
AB	4	18.03333	4.508333	1.31	0.283269	0.336250
S	36	123.5	3.430556			
Total (Adjusted)	44	249.3				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 1.653 (Temp)

Appendix 6.4: Analysis of variance of spikes per plant as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	154.5333	77.26667	31.94	0.000000*	0.999999
B: Grst	2	17.5	8.75	3.62	0.037047*	0.565924
AB	4	3.166667	0.7916667	0.33	0.857848	0.108585
S	36	87.1	2.419445			
Total (Adjusted)	44	262.3				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 1.388 (Temp) LSD $_{(Tukey =0.05)}$ = 1.388 (Grst)

Appendix 6.5: Analysis of variance of spikelets per primary spike as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	233.7333	116.8667	7.88	0.001446*	0.897597
B: Grst	2	76.8	38.4	2.59	0.088884	0.427376
AB	4	161.0667	40.26667	2.72	0.044843*	0.646091
S	36	533.6	14.82222			
Total (Adjusted)	44	1005.2				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 8.028 (Temp x Grst)

Appendix 6.6: Analysis of variance of spikelets per primary spike as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	215.5111	107.7556	17.54	0.000005*	0.998511
B: Grst	2	650.7111	325.3556	52.95	0.000000*	1.000000
AB	4	337.6889	84.42223	13.74	0.000001*	0.999943
S	36	221.2	6.144444			
Total (Adjusted)	44	1425.111				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 5.169 (Temp x Grst)

Appendix 6.7: Analysis of variance of spikelets per secondary spike as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	9.800373	4.900187	0.50	0.609931	0.115112
B: Grst	2	121.4181	60.70905	6.21	0.004819*	0.810987
AB	4	171.0252	42.7563	4.37	0.005519*	0.865732
S	36	351.9247	9.775686			
Total (Adjusted)	44	654.1684				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 6.520 (Temp x Grst)

Appendix 6.8: Analysis of variance of spikelets per secondary spikes as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	1495.401	747.7003	87.99	0.000000*	1.000000
B: Grst	2	0.9500844	0.4750422	0.06	0.945711	0.056758
AB	4	7.275862	1.818966	0.21	0.928914	0.086826
S	36	305.9061	8.497393			
Total (Adjusted)	44	1809.532				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 2.602 (Temp)

Appendix 6.9: Analysis of variance of the average number of spikelets per spike as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	2.041693	1.020847	0.15	0.858421	0.068867
B: Grst	2	64.59525	32.29763	4.85	0.013640*	0.701638
AB	4	136.903	34.22574	5.14	0.002217*	0.919379
S	36	239.7145	6.658736			
Total (Adjusted)	44	443.2544				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 5.381 (Temp x Grst)

Appendix 6.10: Analysis of variance of the average number of spikelets per spike as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	146.7401	73.37006	8.48	0.000961*	0.918580
B: Grst	2	319.0108	159.5054	18.43	0.000003*	0.999036
AB	4	345.7421	86.43553	9.99	0.000015*	0.998243
S	36	311.4936	8.652599			
Total (Adjusted)	44	1122.987				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 6.134 (Temp x Grst)

Appendix 6.11: Analysis of variance of the number of kernels produced by primary spike as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	3525.033	1762.517	10.75	0.000218*	0.967651
B: Grst	2	889.2333	444.6167	2.71	0.079916	0.444900
AB	4	348.3333	87.08334	0.53	0.713475	0.151122
S	36	5900.4	163.9			
Total (Adjusted)	44	10663				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 11.427 (Temp)

Appendix 6.12: Analysis of variance of the number of kernels produced by primary spike as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	6814.044	3407.022	77.26	0.000000*	1.000000
B: Grst	2	116.8111	58.40556	1.32	0.278591	0.236353
AB	4	639.9556	159.9889	3.63	0.013889*	0.787048
S	36	1587.5	44.09722			
Total (Adjusted)	44	9158.312				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 13.847 (Temp x Grst)

Appendix 6.13: Analysis of variance of the number of kernels produced by secondary spikes as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	8593.9	4296.95	3.23	0.051116	0.516659
B: Grst	2	4277.433	2138.717	1.61	0.214075	0.279977
AB	4	2434.367	608.5917	0.46	0.765982	0.135395
S	36	47841.5	1328.931			
Total (Adjusted)	44	63147.2				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey=0.05)}$ = NS (Temp, Grst and Temp x Grst)

Appendix 6.14: Analysis of variance of the number of kernels produced by secondary spikes as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	31916.34	15958.17	29.80	0.000000*	0.999997
B: Grst	2	1456.544	728.2722	1.36	0.269501	0.241797
AB	4	1415.322	353.8306	0.66	0.623250	0.179893
S	36	19276.1	535.4472			
Total (Adjusted)	44	54064.31				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey=0.05)}$ = 20.653 (Temp)

Appendix 6.15: Analysis of variance of the total number of kernels produced by primary and secondary spikes as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	22928.63	11464.32	5.75	0.006817*	0.778251
B: Grst	2	9062.8	4531.4	2.27	0.117701	0.380553
AB	4	4559.167	1139.792	0.57	0.685022	0.159913
S	36	71801.6	1994.489			
Total (Adjusted)	44	108352.2				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey=0.05)}$ = 39.860 Temp)

Appendix 6.16: Analysis of variance of the number of kernels produced by primary and secondary spikes as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	64926.48	32463.24	43.14	0.000000*	1.000000
B: Grst	2	2376.578	1188.289	1.58	0.220126	0.275324
AB	4	3570.489	892.6222	1.19	0.333431	0.304930
S	36	27091.9	752.5528			
Total (Adjusted)	44	97965.45				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey=0.05)}$ = 24.485 (Temp)

Appendix 6.17: Analysis of variance of the number of kernels per secondary spike as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	24.93185	12.46593	0.24	0.788950	0.079798
B: Grst	2	252.9654	126.4827	2.42	0.103152	0.402637
AB	4	137.0229	34.25573	0.66	0.626702	0.178739
S	36	1880.699	52.24165			
Total (Adjusted)	44	2295.619				
Total	45					

* Term significant at alpha = 0.05

$LSD_{(Tukey=0.05)}$ = NS (Temp, Grst and Temp x Grst)

Appendix 6.18: Analysis of variance of the number of kernels per secondary spike as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	2370.35	1185.175	18.77	0.000003*	0.999180
B: Grst	2	49.57096	24.78548	0.39	0.678247	0.100202
AB	4	102.539	25.63475	0.41	0.803131	0.124525
S	36	2273.547	63.15409			
Total (Adjusted)	44	4796.007				
Total	45					

* Term significant at alpha = 0.05

$LSD_{(Tukey=0.05)}$ = 7.093 (Temp)

Appendix 6.19: Analysis of variance of the average number of kernels produced by the primary and secondary spikes as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	165.9245	82.96223	1.62	0.212636	0.281104
B: Grst	2	281.3214	140.6607	2.74	0.077972	0.448937
AB	4	160.091	40.02274	0.78	0.545635	0.207307
S	36	1847.363	51.31565			
Total (Adjusted)	44	2454.7				
Total	45					

* Term significant at alpha = 0.05

$LSD_{(Tukey=0.05)}$ = NS (Temp, Grst and Temp x Grst)

Appendix 6.20: Analysis of variance of the average number of kernels produced by the primary and secondary spikes as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	2732.763	1366.381	26.32	0.000000*	0.999983
B: Grst	2	50.87508	25.43754	0.49	0.616693	0.113542
AB	4	126.1693	31.54232	0.61	0.659805	0.167906
S	36	1869.137	51.92048			
Total (Adjusted)	44	4778.944				
Total	45					

* Term significant at alpha = 0.05

$LSD_{(Tukey=0.05)}$ = 6.432 (Temp)

Appendix 6.21: Analysis of variance of the primary kernel weight as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	1.41227	0.7061352	9.39	0.000524*	0.943193
B: Grst	2	0.4129537	0.2064769	2.74	0.077721	0.449464
AB	4	0.1436875	3.592187E-02	0.48	0.751952	0.139546
S	36	2.708095	7.522487E-02			
Total (Adjusted)	44	4.677007				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.245 (Temp)

Appendix 6.22: Analysis of variance of the primary kernel weight as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	8.145191	4.072596	83.40	0.000000*	1.000000
B: Grst	2	0.4763529	0.2381765	4.88	0.013351*	0.704230
AB	4	1.127869	0.2819671	5.77	0.001072*	0.948414
S	36	1.757887	4.883019E-02			
Total (Adjusted)	44	11.5073				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.461 (Temp x Grst)

Appendix 6.23: Analysis of variance of the secondary kernel weight as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	3.087562	1.543781	1.85	0.171635	0.317052
B: Grst	2	3.6757	1.83785	2.20	0.125009	0.370437
AB	4	1.067158	0.2667896	0.32	0.862725	0.107151
S	36	30.01606	0.8337795			
Total (Adjusted)	44	37.84649				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = NS (Temp, Grst and Temp x Grst)

Appendix 6.24: Analysis of variance of the secondary kernel weight as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	28.93417	14.46709	32.63	0.000000*	0.999999
B: Grst	2	2.30971	1.154855	2.60	0.087788	0.429427
AB	4	2.302867	0.5757167	1.30	0.288983	0.332439
S	36	15.96023	0.4433396			
Total (Adjusted)	44	49.50698				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.594 (Temp)

Appendix 6.25: Analysis of variance of the total kernel weight as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	8.589819	4.294909	3.53	0.039709*	0.555505
B: Grst	2	6.531663	3.265831	2.69	0.081741	0.441191
AB	4	1.837077	0.4592694	0.38	0.822932	0.118768
S	36	43.75899	1.215527			
Total (Adjusted)	44	60.71755				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.984 (Temp)

Appendix 6.26: Analysis of variance of the total kernel weight as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	65.41146	32.70573	47.96	0.000000*	1.000000
B: Grst	2	4.773789	2.386894	3.50	0.040832*	0.551284
AB	4	6.378636	1.594659	2.34	0.073740	0.572109
S	36	24.55069	0.6819637			
Total (Adjusted)	44	101.1146				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.737 (Temp) LSD $_{(Tukey\ =0.05)}$ = 0.737 (Grst)

Appendix 6.27: Analysis of variance of the kernel weight per secondary spike as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	2.530964E-02	1.265482E-02	0.46	0.635635	0.109260
B: Grst	2	0.2218882	0.1109441	4.02	0.026493*	0.614578
AB	4	3.784275E-02	9.460689E-03	0.34	0.847055	0.111744
S	36	0.9927884	2.757746E-02			
Total (Adjusted)	44	1.277829				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.148 (Grst)

Appendix 6.28: Analysis of variance of the kernel weight per secondary spike as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	28.93417	14.46709	32.63	0.000000*	0.999999
B: Grst	2	2.30971	1.154855	2.60	0.087788	0.429427
AB	4	2.302867	0.5757167	1.30	0.288983	0.332439
S	36	15.96023	0.4433396			
Total (Adjusted)	44	49.50698				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.233 (Temp)

Appendix 6.29: Analysis of variance of the kernel weight per spike as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	9.563418E-02	4.781709E-02	1.85	0.171408	0.317275
B: Grst	2	0.2135472	0.1067736	4.14	0.024128*	0.627610
AB	4	4.279582E-02	1.069896E-02	0.41	0.796954	0.126324
S	36	0.9289852	2.580514E-02			
Total (Adjusted)	44	1.280962				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.143 (Grst)

Appendix 6.30: Analysis of variance of the kernel weight per spike as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	2.671216	1.335608	24.37	0.000000*	0.999952
B: Grst	2	0.1923843	9.619216E-02	1.76	0.187296	0.302368
AB	4	0.2464246	6.160615E-02	1.12	0.360378	0.289834
S	36	1.972631	0.0547953			
Total (Adjusted)	44	5.082656				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.209 (Temp)

Appendix 6.31: Analysis of variance of the mass per 100 kernels produced by the primary spikes as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	20.78931	10.39465	14.98	0.000018*	0.995027
B: Grst	2	0.9739955	0.4869978	0.70	0.502270	0.143496
AB	4	2.378116	0.5945291	0.86	0.498961	0.225418
S	36	24.97572	0.6937699			
Total (Adjusted)	44	49.11713				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.743 (Temp)

Appendix 6.32: Analysis of variance of the mass per 100 kernels produced by the primary spikes as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	91.75404	45.87702	34.95	0.000000*	1.000000
B: Grst	2	18.42918	9.214588	7.02	0.002666*	0.858557
AB	4	15.59892	3.89973	2.97	0.032205*	0.690718
S	36	47.25747	1.312707			
Total (Adjusted)	44	173.0396				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 2.389 (Temp x Grst)

Appendix 6.33: Analysis of variance of the mass per 100 kernels produced by the secondary spikes as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	7.309814	3.654907	8.04	0.001294*	0.903656
B: Grst	2	3.166583	1.583292	3.48	0.041358*	0.549340
AB	4	3.487597	0.8718991	1.92	0.128389	0.480553
S	36	16.35662	0.4543505			
Total (Adjusted)	44	30.32061				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.602 (Temp) $LSD_{(Tukey=0.05)}$ = 0.602 (Grst)

Appendix 6.34: Analysis of variance of the mass per 100 kernels produced by the secondary spikes as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	75.31454	37.65727	61.87	0.000000*	1.000000
B: Grst	2	5.735017	2.867509	4.71	0.015227*	0.688107
AB	4	3.608751	0.9021878	1.48	0.227957	0.377215
S	36	21.91219	0.6086719			
Total (Adjusted)	44	106.5705				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.693 (Temp) $LSD_{(Tukey=0.05)}$ = 0.696 (Grst)

Appendix 6.35: Analysis of variance of the mass per 100 kernels as affected by temperature and growth stage for cultivar 1 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	7.46448	3.73224	9.67	0.000436*	0.949269
B: Grst	2	2.414773	1.207387	3.13	0.055950	0.502434
AB	4	3.506434	0.8766086	2.27	0.080665	0.557909
S	36	13.89987	0.3861075			
Total (Adjusted)	44	27.28556				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.555 (Temp)

Appendix 6.36: Analysis of variance of the mass per 100 kernels as affected by temperature and growth stage for cultivar 2 during the flag leaf stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	81.83446	40.91723	72.56	0.000000*	1.000000
B: Grst	2	6.327034	3.163517	5.61	0.007571*	0.767628
AB	4	3.980399	0.9950997	1.76	0.157410	0.444767
S	36	20.30044	0.5639011			
Total (Adjusted)	44	112.4423				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)}$ = 0.670 (Temp) $LSD_{(Tukey=0.05)}$ = 0.670 (Grst)

Appendix 6.37: Analysis of variance of the number of kernels produced by the primary spike as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	1408.144	704.0722	5.20	0.010357*	0.733844
B: Grst	2	79.87778	39.93889	0.30	0.746225	0.087193
AB	4	1020.889	255.2222	1.89	0.134129	0.472963
S	36	4871.9	135.3306			
Total (Adjusted)	44	7380.811				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 10.383 (Temp)

Appendix 6.38: Analysis of variance of the number of kernels produced by the primary spike as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	1343.011	671.5056	4.98	0.012291*	0.714099
B: Grst	2	2399.244	1199.622	8.90	0.000722*	0.931041
AB	4	151.7556	37.93889	0.28	0.888007	0.099628
S	36	4851.3	134.7583			
Total (Adjusted)	44	8745.312				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 10.361 (Temp) LSD $_{(Tukey =0.05)}$ = 10.361 (Grst)

Appendix 6.39: Analysis of variance of the number of kernels produced by secondary spikes as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	10262.58	5131.289	6.40	0.004201*	0.822915
B: Grst	2	1404.253	702.1266	0.88	0.425506	0.168731
AB	4	3009.524	752.381	0.94	0.453236	0.244667
S	36	28884.81	802.3558			
Total (Adjusted)	44	43561.16				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 25.282 (Temp)

Appendix 6.40: Analysis of variance of the number of kernels produced by secondary spikes as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	3309.733	1654.867	7.41	0.002023*	0.877388
B: Grst	2	1170.433	585.2167	2.62	0.086701	0.431485
AB	4	452.4333	113.1083	0.51	0.731395	0.145691
S	36	8043.7	223.4361			
Total (Adjusted)	44	12976.3				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 13.341 (Temp)

Appendix 6.41: Analysis of variance of the total number of kernels produced by primary and secondary spikes as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	18764.81	9382.405	6.92	0.002861*	0.853426
B: Grst	2	1254.129	627.0646	0.46	0.633324	0.109774
AB	4	6878.438	1719.609	1.27	0.300261	0.325113
S	36	48796.91	1355.47			
Total (Adjusted)	44	75694.29				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 32.860 (Temp)

Appendix 6.42: Analysis of variance of the total number of kernels produced by primary and secondary spikes as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	8861.812	4430.906	7.98	0.001352*	0.901304
B: Grst	2	6667.811	3333.906	6.01	0.005616*	0.797002
AB	4	1105.622	276.4055	0.50	0.737368	0.143897
S	36	19986	555.1667			
Total (Adjusted)	44	36621.25				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 21.030 (Temp) LSD $_{(Tukey =0.05)}$ = 21.030 (Grst)

Appendix 6.43: Analysis of variance of the number of kernels per secondary spike as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	294.1401	147.07	11.07	0.000179*	0.971717
B: Grst	2	1.176999	0.5884995	0.04	0.956716	0.055344
AB	4	100.175	25.04375	1.89	0.134236	0.472824
S	36	478.2098	13.28361			
Total (Adjusted)	44	873.7019				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 3.253 (Temp)

Appendix 6.44: Analysis of variance of the number of kernels per secondary spike as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	465.9335	232.9668	8.28	0.001103*	0.911908
B: Grst	2	256.0782	128.0391	4.55	0.017324*	0.671785
AB	4	67.04951	16.76238	0.60	0.668150	0.165238
S	36	1013.294	28.14707			
Total (Adjusted)	44	1802.356				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 4.735 (Temp) LSD $_{(Tukey =0.05)}$ = 4.735 (Grst)

Appendix 6.45: Analysis of variance of the average number of kernels per spike as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	403.724	201.862	11.01	0.000186*	0.970989
B: Grst	2	1.382847	0.6914233	0.04	0.963023	0.054544
AB	4	167.338	41.83451	2.28	0.079427	0.560373
S	36	659.9436	18.33177			
Total (Adjusted)	44	1232.389				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 3.821 (Temp)

Appendix 6.46: Analysis of variance of the average number of kernels per spike as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	754.6286	377.3143	12.13	0.000094*	0.982035
B: Grst	2	540.8582	270.4291	8.69	0.000832*	0.925061
AB	4	80.50868	20.12717	0.65	0.632718	0.176739
S	36	1120.133	31.11479			
Total (Adjusted)	44	2496.128				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 4.979 (Temp) LSD $_{(Tukey\ =0.05)}$ = 4.979(Grst)

Appendix 6.47: Analysis of variance of the primary kernel weight as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	1.302471	0.6512356	8.26	0.001119*	0.911192
B: Grst	2	0.1631144	8.155722E-02	1.03	0.365929	0.192353
AB	4	0.3706989	9.267472E-02	1.17	0.338205	0.302176
S	36	2.83974	7.888167E-02			
Total (Adjusted)	44	4.676024				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.251 (Temp)

Appendix 6.48: Analysis of variance of the primary kernel weight as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	2.462658	1.231329	8.00	0.001332*	0.902126
B: Grst	2	2.424567	1.212284	7.88	0.001451*	0.897392
AB	4	0.5923729	0.1480932	0.96	0.439855	0.250628
S	36	5.53884	0.1538566			
Total (Adjusted)	44	11.01844				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.350 (Temp) LSD $_{(Tukey\ =0.05)}$ = 0.350(Grst)

Appendix 6.49: Analysis of variance of the secondary kernel weight as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	7.796918	3.898459	8.36	0.001042*	0.914717
B: Grst	2	0.9927049	0.4963524	1.06	0.355524	0.196935
AB	4	1.280972	0.3202429	0.69	0.605821	0.185798
S	36	16.78682	0.4663004			
Total (Adjusted)	44	26.85741				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 0.610 (Temp)

Appendix 6.50: Analysis of variance of the secondary kernel weight as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	7.939045	3.969523	13.47	0.000043*	0.990072
B: Grst	2	2.307047	1.153523	3.91	0.028984*	0.601825
AB	4	0.4128785	0.1032196	0.35	0.842167	0.113171
S	36	10.61168	0.294769			
Total (Adjusted)	44	21.27065				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 0.485 (Temp) LSD $_{(Tukey =0.05)}$ = 0.485 (Grst)

Appendix 6.51: Analysis of variance of the total kernel weight as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	15.35694	7.67847	9.76	0.000410*	0.951192
B: Grst	2	1.65391	0.8269551	1.05	0.359982	0.194954
AB	4	2.886656	0.7216641	0.92	0.464421	0.239804
S	36	28.31894	0.7866372			
Total (Adjusted)	44	48.21645				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 0.792 (Temp)

Appendix 6.52: Analysis of variance of the total kernel weight as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	19.23404	9.617021	15.92	0.000011*	0.996791
B: Grst	2	8.849361	4.424681	7.33	0.002142*	0.873652
AB	4	1.238357	0.3095892	0.51	0.726853	0.147060
S	36	21.74364	0.6039899			
Total (Adjusted)	44	51.0654				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 0.694 (Temp) LSD $_{(Tukey =0.05)}$ = 0.694 (Grst)

Appendix 6.53: Analysis of variance of the kernel weight per secondary spike as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	0.2146115	0.1073058	14.10	0.000030*	0.992561
B: Grst	2	9.222577E-03	4.611289E-03	0.61	0.550926	0.129813
AB	4	2.908849E-02	7.272122E-03	0.96	0.443407	0.249030
S	36	0.2738764	7.607678E-03			
Total (Adjusted)	44	0.526799				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.078 (Temp)

Appendix 6.54: Analysis of variance of the kernel weight per secondary spike as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	1.032626	0.5163128	16.99	0.000006*	0.998063
B: Grst	2	0.3574612	0.1787306	5.88	0.006167*	0.788090
AB	4	0.1515284	0.0378821	1.25	0.308893	0.319673
S	36	1.094148	3.039299E-02			
Total (Adjusted)	44	2.635763				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.157 (Temp) LSD $_{(Tukey\ =0.05)}$ = 0.157 (Grst)

Appendix 6.55: Analysis of variance of the kernel weight per spike as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	0.3114648	0.1557324	15.75	0.000012*	0.996516
B: Grst	2	1.772298E-02	8.861489E-03	0.90	0.417078	0.171829
AB	4	5.469315E-02	1.367329E-02	1.38	0.259373	0.352978
S	36	0.3560136	9.889266E-03			
Total (Adjusted)	44	0.7398946				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.089 (Temp)

Appendix 6.56: Analysis of variance of the kernel weight per spike as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	1.486778	0.743389	23.35	0.000000*	0.999918
B: Grst	2	0.6916769	0.3458384	10.86	0.000203*	0.969133
AB	4	0.1072856	2.682139E-02	0.84	0.507421	0.222030
S	36	1.145906	3.183071E-02			
Total (Adjusted)	44	3.431646				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey\ =0.05)}$ = 0.159 (Temp) LSD $_{(Tukey\ =0.05)}$ = 0.159 (Grst)

Appendix 6.57: Analysis of variance of the mass per 100 kernels produced by the primary spike as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	7.057286	3.528643	4.86	0.013495*	0.702934
B: Grst	2	1.248216	0.6241078	0.86	0.431552	0.166552
AB	4	1.686969	0.4217423	0.58	0.678079	0.162093
S	36	26.11654	0.7254595			
Total (Adjusted)	44	36.10901				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 0.760 (Temp)

Appendix 6.58: Analysis of variance of the mass per 100 kernels produced by the primary spike as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	10.48467	5.242336	7.73	0.001613*	0.891312
B: Grst	2	33.15552	16.57776	24.44	0.000000*	0.999953
AB	4	11.08058	2.770146	4.08	0.007873*	0.838603
S	36	24.42225	0.6783958			
Total (Adjusted)	44	79.14303				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 1.718 (Temp x Grst)

Appendix 6.59: Analysis of variance of the mass per 100 kernels produced by the secondary spikes as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	9.195262	4.597631	8.19	0.001172*	0.908865
B: Grst	2	0.8585443	0.4292721	0.76	0.472941	0.152552
AB	4	0.4283361	0.107084	0.19	0.941690	0.082523
S	36	20.21222	0.5614504			
Total (Adjusted)	44	30.69436				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 0.669 (Temp)

Appendix 6.60: Analysis of variance of the mass per 100 kernels produced by the secondary spikes as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	19.71034	9.855169	4.90	0.013101*	0.706502
B: Grst	2	13.17577	6.587887	3.28	0.049279*	0.522376
AB	4	2.432913	0.6082281	0.30	0.874349	0.103714
S	36	72.38071	2.010575			
Total (Adjusted)	44	107.6997				
Total	45					

* Term significant at alpha = 0.05 LSD $_{(Tukey =0.05)}$ = 1.266 (Temp) LSD $_{(Tukey =0.05)}$ = 1.266 (Grst)

Appendix 6.61: Analysis of variance of the mass per 100 kernels as affected by temperature and growth stage for cultivar 1 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	8.734003	4.367002	9.33	0.000545*	0.941779
B: Grst	2	0.5982049	0.2991024	0.64	0.533832	0.134444
AB	4	0.6190906	0.1547727	0.33	0.855604	0.109243
S	36	16.85752	0.4682646			
Total (Adjusted)	44	26.80882				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)} = 0.611$ (Temp)

Appendix 6.62: Analysis of variance of the mass per 100 kernels as affected by temperature and growth stage for cultivar 2 during the flowering stage

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp	2	18.83266	9.416332	10.82	0.000209*	0.968524
B: Grst	2	14.96128	7.480642	8.59	0.000888*	0.922161
AB	4	2.466093	0.6165232	0.71	0.591555	0.190732
S	36	31.33401	0.8703893			
Total (Adjusted)	44	67.59406				
Total	45					

* Term significant at alpha = 0.05 $LSD_{(Tukey=0.05)} = 1.266$ (Temp) $LSD_{(Tukey=0.05)} = 1.266$ (Grst)