THE GROWTH, YIELD AND PHYSIOLOGICAL RESPONSE OF RAIN FED MAIZE TO FOLIAR APPLIED FERTILIZER

by

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DECLARATION

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CHAPTER 1

Introduction and Rationale

Increasing yield for an ever growing world population has currently become a topic of great concern with regard to food security. Especially in Africa, agricultural productivity has not been able to cope with population growth, leading to increased annual imports and food insecurity (Mugo *et al*., 2005). Food insecurity has been exacerbated by consecutive years of below normal seasons and poor harvests since 2002 in Southern Africa (SADC & RVAC, 2006). Although there have been maize surpluses in South Africa for the 2005/06 and 2009/2010 seasons, maize imports for most of the SADC countries have increased. Although speculative, this is most probably the result of reduced production. Mugo *et al*. (2005) maintained that approximately 12 million people are facing food insecurity in Southern Africa. This situation is chronic, contributes to increased vulnerability at household and community level and clearly shows that the expected maize green revolution in Africa did not take off. According to Bradfield (1970), as cited by Jugenheimer (1976), a country can only increase its food production by applying the following three principles: i) expanding the area planted with food crops, (ii) increasing crop yields per unit area and (iii) increasing the number of crops grown on a specific land per annum.

In the light of the preceding, taking into account that most of the arable land in the world is already under cultivation and secondly that the cultivation of a crop such as maize is seasonal, increasing crop yield per unit area by means of better crop management practices such as foliar nutrition might have the potential, not individually but as part of an agricultural system, to contribute towards our survival on planet earth (Fageria, 2009). According to the author increasing crop yield in the 21st century has become an essential component of modern society with food demands projected to increase by a staggering 50% in the next 30 years.

Maize (*Zea mays* L.) is the most widely cultivated grain crop in the world, representing both a major component in the diets of many people from developing countries as well as one of the crops with the highest potential for bio-fuel production (McLaren, 2005). The ability of agricultural systems to sustain crop production at rates needed to feed a growing world population, that are predicted to increase from 6 billion (estimation in 1999) to 9 billion by the year 2042, is concerning (Cassman, 1999; MacKenzie, 2008). The worldwide maize crop forecast for the 2010/2011 growing season is

809 million tons, while the world consumption is around 842 million tons, resulting in a shortage of 33 million tons for the current season (International Grains Council, 2011).

With this predicted shortage in mind, it is highly likely that both food and bio-fuel production will be more dependent on the manipulation of staple C_4 crops such as maize in order to increase yields in future. Moreover, it is predicted that climate change in the 21st century will further impact many aspects of nature, and will also have a negative effect on agricultural crop production (IPCC, 2007). The latter tendency was already noticeable in 2006, when approximately 825 million people over the world were undernourished (FAO, 2006). By 2020, global demand for maize as a major food source is projected to exceed that for wheat and rice, which will make it the world's most important grain crop (Pingali, 2001). However, there are substantial regional and country-to-country differences in terms of how the importance of crops, belonging to the C_4 plant group, for food production is perceived. Be it as it may, the countries that will probably be most affected by a possible negative response of C_4 crops to predicted climate change, and subsequent yield losses, are countries in Africa and Central America (Leakey, 2009).

In this study, and in light of this brief introduction, it was considered important to undertake a project focusing on a rather affordable agricultural practice that might contribute towards counteracting anticipated yield losses by manipulating maize plants via foliar applied fertilizers with the principle aim to increase yields. In particular, three different salts were foliar applied at different growth stages and at different concentrations while the morphological, physiological and yield responses of maize plants were followed under rain fed conditions.

Research towards foliar fertilization of crops possibly started in the late 1940's or early 1950's (Dion, 1949). Unlike many technologies, both the development and application pace of foliar fertilization as a new technique over the span of six decades was rather slow in terms of a general acceptance by farmers and scientists alike. It is probably not necessary to speculate on possible reasons, but one of the obvious is that agricultural scientists most likely did not lead the way due to either a lack of conviction or the fact that the concept did not sound appealing and worthy of investigating. For example, already more than five decades ago Thorne (1958) saw the necessity to do trials in order to understand the mechanism of foliar nutrient uptake. In labeling experiments he demonstrated that the uptake rate of ^{32}P from a labeled NaH_2PO_4 solution sprayed onto one leaf of swedes (*Brassica napus*) or French beans (*Phaseolus vulgaris*) was rapid during the first few hours

after application. Further, radioactive phosphorous was detected in the roots of the above plants three hours after application.

However, it was not until the early 1980's, when studies on foliar application of fertilizers were investigated with more interest on selected crops. Early research was, however, limited to micro nutrients that were applied to high value horticultural crops such as vegetables and fruit crops (Fritz, 1978). In recent years more studies (Kaya *et al.*, 2001) were conducted, especially investigating the effect of macro nutrients such as phosphorous (P) applied foliar to crops like tomatoes (*Lycopersicon esculentum* L.). If the level of, for example, available P in soil is not adequate for optimum crop growth, P fertilizers can be foliar applied with more accuracy in order to ensure that there are adequate amounts of this nutrient available to the plant (Chen and Barber, 1990).

Unfortunately, regardless of past research, great uncertainty still exists amongst scientists, farmers, fertilizer agents as well as extension officers with regard to the desirability of the foliar inorganic fertilizer application practice ([1]personal communication; S.J. Van der Schyff, 2011). According to the latter, foliar fertilizers are marketed and used in South Africa on maize without knowing exactly what the optimum application stage is, what the optimum concentration to apply is, what the best inorganic fertilizer salt to be used is and which moiety within a specific salt molecule is actually responsible for the crop response. Further, possible synergism between the different moieties contained in a specific salt in inducing a crop response, is not excluded. This list of uncertainties is rather disturbing and emphasizes the responsibility of scientists to contribute, through research, towards finding the answers that farmers and fertilizer agents lack.

In light of the uncertainty surrounding the foliar application of inorganic fertilizers to agricultural crops, the main objectives of this study were to quantify the vegetative growth (chapter 3), yield (chapter 3) and physiological (chapter 4) responses of maize to foliar treatment with selected inorganic nutrients under rain fed conditions. The study was undertaken over three consecutive seasons on the same land. During season one treatments were designed to identify the optimum

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application growth stage of maize for potassium nitrate (KNO_3) and mono potassium phosphate (MKP). During season two both these salts were applied at one growth stage, but at different concentrations in an attempt to identify the optimum concentration rate for each. During the third season a third salt mono ammonium phosphate (MAP) was applied, due to the absence of potassium (K) in its structure, in an attempt to distinguish between K and P regarding its potential to evoke a response in maize plants following foliar application.

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CHAPTER 2

Literature Review

2.1   Introduction

It is believed that maize was first discovered by two Spaniards accompanying Columbus on an expedition to Cuba (Jugenheimer, 1976). On return they reported the discovery of a good tasting grain which they called "maiz". Further, according to Sauer (1993), *Zea mexicana* L. commonly known as teosinte (*Euchlaena*) belonging to the family *Gramineae*, gave rise to the single species of maize today known as *Zea mays* L. Genetic evidence also proved that maize did originate from the Balsas race of teosinte which is found in the Balsas River basin, Michoacan-Guerrero border region of western Mexico. *Z. mays* is thought to have speciated from *Z. mexicana* into a separate gene pool many thousands of years ago after which it diversified into a number of different races as known today (Jugenheimer, 1976).

Maize has unisexual inflorescences, the tassel (male) and the ear (female). According to Hoseney (1994) many types of maize are grown around the world, but it is the dent type corn with large flattened seed weighing on average 350 mg that is by far the largest of the common cereal seeds. Maize kernels also vary in colour, with yellow and white kernels being the most common colours cultivated today. White maize is biologically and genetically very similar to yellow maize, although there is a difference in appearance due to the absence of carotin oil pigments in the kernel, which otherwise causes the yellow colour of the grain (FAO, 2011).

Maize was introduced to Africa in the 16th century and was readily accepted by African farmers, partly because it was grown and used in a similar way to grain sorghum, then their traditional crop (Sauer, 1993). Maize eventually displaced sorghum as the staple grain in all but the drier regions of the continent due to the fact that maize can be grown over a wider range of climatic conditions. Compared to other important grain crops such as wheat, maize remains a very attractive crop to South African farmers. According to statistics between two and four million hectares are planted under maize each year in the Republic of South Africa (RSA), making it the largest crop in the country (National Department of Agriculture, 2002). White maize is largely cultivated under rain fed conditions in the RSA.

As many of the important white maize producing areas are located in regions susceptible to drought, dependence on rainfall has resulted in marked annual fluctuations in terms of yield output. For example, production in the RSA decreased sharply from 3.8 million tons in 1991 to 1.3 million tons in 1992, but recovered again reaching 4.4 million tons in 1993 and advanced further to 6.1 million tons in 1994 (FAO, 2011). The amount of hectares planted currently (2010/2011 growing season) under maize has been revised downwards by 13.1% to 2.383 million hectares compared to the 2.742 million hectares planted in the 2009/2010 growing season (South African Crops Estimate Committee, 2011). The committee attributed the decrease in planting areas to a switch from maize to oil seed crops, and this was probably strongly influenced by the profitability of producing maize. Since profitability is linearly dependant on production output per hectare, it is necessary for a maize farmer to consider alternative agricultural practices, e.g. foliar fertilization, in an attempt to increase yields ([1]personal communication; A. Prins, 2011).

In light of the underlying study and a constant search for improving maize cultivation practices, related aspects are discussed in this chapter. These include growth stages of maize, important factors during planting, seedling establishment, plant development and yield, fertilizer nutrients in general, chlorophyll (Chl) content, Chl *a* fluorescence and photosynthesis, dry matter accumulation and yield, assimilate partitioning, kernel number and foliar fertilization in general.

2.2   Growth stages of maize

A rather comprehensive summary of the growth stages of maize, as mostly accepted worldwide by agronomists, including days after emergence and an explanation of each stage (features), is presented in Table 2.1.

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Table 2.1: International standardized growth stages for maize (CIMMYT, 2010)

Stage	DAS*	
VE	5	The coleoptile emerge from the soil surface.
V1	9	The collar of the first leaf is visible.
V2	12	The collar of the second leaf is visible.
Vn		The collar of the leaf number 'n' is visible. The maximum value of 'n' represents the final number of leaves, which is usually 16-23, but by flowering, the lower 4-7 leaves have disappeared.
VT	55	The last branch of the tassel is completely visible.
R 0	57	Anthesis or male flowering. Pollen shed begins.
R 1	59	Silks are visible.
R 2	71	Blister stage. Kernels are filled with clear fluid and the embryo can be seen.
R 3	80	Milk stage. Kernels are filled with a white, milky fluid.
R 4	90	Dough stage. Kernels are filled with a white paste. The embryo is about half the width of the kernel. The top part of the kernels is filled with solid starch.
R 5	102	Dent stage. If the genotype is a dent type, the grains are dented. The 'milk line' is close to the base when the kernel is viewed from the side in both flint and dent types.
R 6	112	Physiological maturity. The black layer is visible at the base of the grain. Grain moisture is usually about 35%.

* DAS: approximate number of days after sowing in lowland tropics, where maximum and minimum temperatures may be 33 and 22℃, respectively. In cooler environments, these periods are extended.

2.3   Important aspects during planting, seedling establishment, plant development and yield

Eik and Hanway (1965) reported that planting date had a small effect on the rate of maize seedling emergence. The authors observed that nutrition rather than planting date, especially a higher plant N content early in the growing season, increased the number of leaves, plant size, rate of leaf emergence and leaf area expansion. Alessi and Power (1971) concluded that, at planting, maize seeds normally need 4 to 24 days to achieve an 80% germination rate depending on temperature and planting depth. A study conducted by Pioneer (2008) in the RSA, indicated that planting depth, although it influenced germination time, also has a significant effect on the development of maize seedlings after germination, when measured seven days after planting (Figure 2.1). Maize seeds planted at depths of 2, 4, 6 and 8 cm realized grain yields of 8.4, 8.5, 9.6 and 10.1 ton ha^{-1}

respectively, indicating the 8 cm depth to be optimum. As a result maize seeds were planted at a depth of 8 cm in this study.

Figure 2.1: Maize seedling development seven days after planting for seeds planted 8, 6, 4 and 2 cm (from left to right) below the soil surface, during the 2008/2009 maize production season in the RSA (Pioneer, 2008).

The findings of Eik and Hanway (1965) in terms of the importance of plant nutrition at planting as well as the interesting observation of Pioneer (2008) regarding maize seedling growth and planting depth, demands a further investigation. According to the FSSA (2003), a maize plant absorbs maximum nitrogen (N) and potassium (K) from the soil eight weeks after planting, while phosphorous (P) is taken up at a maximum rate ten weeks after planting (flowering). In order to meet the soil nutrient requirements of maize under rain fed conditions, in terms of N, P and K fertilization for a 5 ton ha^{-1} grain yield, 95 kg N ha^{-1}, 42 kg P ha^{-1} (soil P 8-14 mg kg^{-1}) and 0 kg K ha^{-1} (soil K > 120 mg kg^{-1}) is recommended for the average South African soil (FSSA, 2003). Recommended fertilizer rates must be adapted according to analyses for different soils. As a standard practise, one third of the N requirement is normally applied as a top dressing four to five weeks after planting.

The application of fertilizer to soils is not the only cultivation aspect that is considered by the international research community, but also the degree to which plants are capable of utilizing the available nutrients. In this regard insight concerning maize root distribution in soils is equally important, especially in terms of the optimal positioning of fertilizer during planting in order to obtain optimum root interception. The effect of root distribution in compacted soils (very common

in the RSA) and nutrient uptake was investigated by Kaspar *et al.* (1991). The authors reported that fertilizer placed in inter row spaces between tractor wheel tracks contributed to a significant increase in root length while root growth was restricted in seedlings from seeds placed directly on wheel tracks. It seems that fertilizer-use efficiency by seedlings growing in wheel tracks was restricted and, together with the influence of soil compaction on growth, it was recommended by the authors to avoid placing fertilizer in or near wheel tracks.

Besides fertilizer placement, climatic conditions also have a profound effect on fertilizer uptake from soil by crops. For example Richner *et al.*, 1996 reported that temperature has an effect on the uptake of certain nutrients by the maize plant during critical periods. Maize is sensitive to chilling and root growth of maize seedlings is often impeded by low temperatures. Mild chilling stress often occurs during the early stages of maize growth that might affect the metabolism, architecture and morphology of the roots hampering the uptake of nutrients. Ching and Barbers (1979) on the other hand, determined the K influx characteristics of maize roots as affected by root temperature in solution culture experiments. They reported that K influx at 15ºC was one-half of that at 29ºC. Similar results were obtained for the shoot K content where 8.1% was measured for the 29ºC treatment compared to 3.7% K for the 15ºC treatment. Root growth, measured in length, was also more than eight times greater at 29ºC than at 15ºC.

Further, additional to the influence temperature has on plant development, studies by Mackay and Barber (1985) accentuated the important effect of soil moisture on plant development. According to the authors, one of the most critical aspects of soil moisture content is that it determines the uptake of nutrients. They tested the effect of three volumetric soil moisture levels, 0.22 (M_0) [–7.5 kPa], 0.27 (M_1) [–33 kPa], and 0.32 (M_2) [–170 kPa] on the uptake of phosphorous (P) by maize roots. Total plant mass increased from 13 to 43%, while total P uptake increased from 55 to 70% and root length increased from 41 to 52% within the same period of time at the highest water potential level.

Another climate factor that has a greater potential to affect crop development and yield is atmospheric CO_2. Leakey *et al.* (2006) conducted several free-air CO_2 enrichment studies on maize to investigate its affect as a climatic factor and found that if the crop is not experiencing any drought stress, there was no CO_2-concentration effect on photosynthesis. In addition, when there was no drought stress at any stage of the season, there were also no *in vivo* or *in vitro* differences on photosynthetic enzyme capacity, leaf carbohydrates, leaf N content, bio-mass production or yield on maize. Parry *et al.* (2004) analyzed as well as predicted future global consequences of elevated CO_2

concentrations to affect crop yields of cereals, (*a*) with and (*b*) without the direct effects of elevated CO_2 concentrations, as summarized in Figure 2.2.
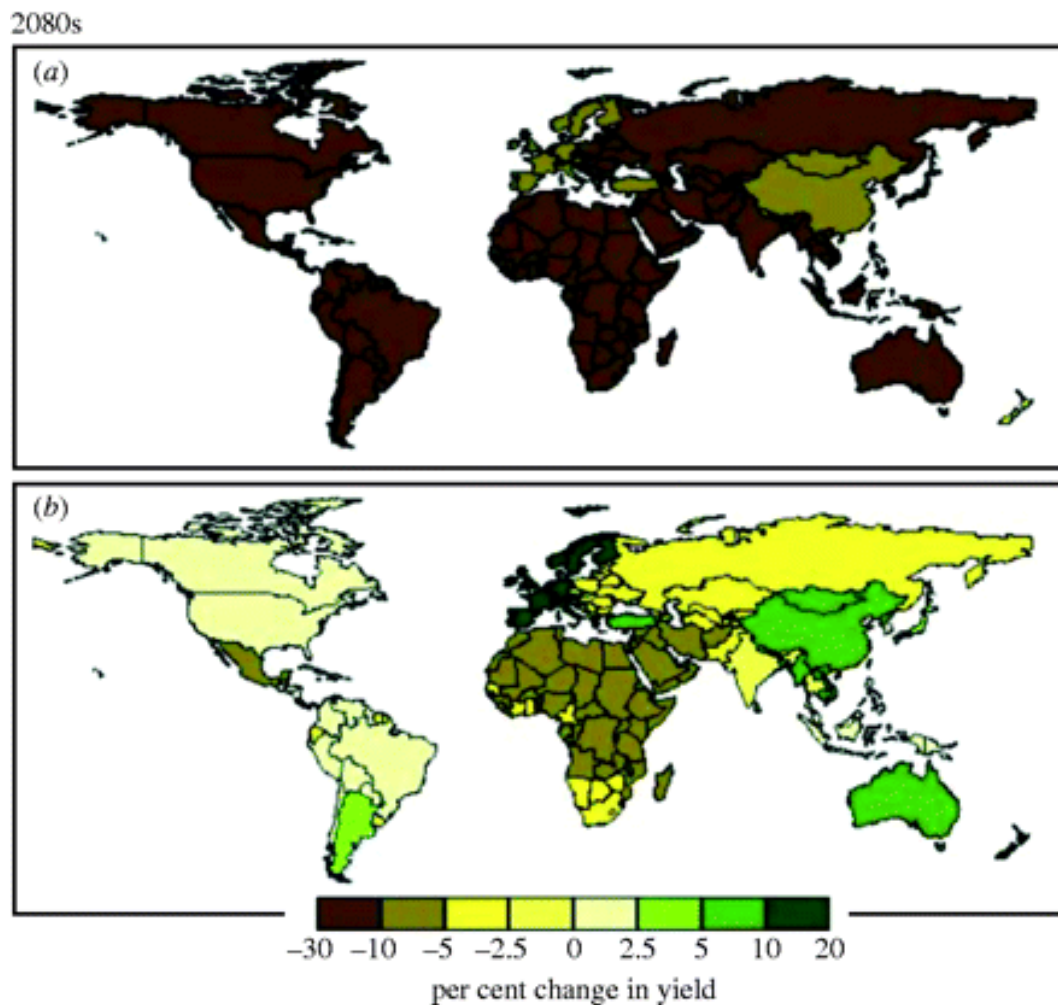
Figure 2.2: Predicted percentage changes in cereal yields for the 2080's, compared to 1990, a) with and b) without the direct effects of elevated CO_2 concentrations (Parry *et al.*, 2004).

The predictions by Parry *et al.* (2004), namely a potential reduction in cereal grain yield of 30% over the next 70 years is rather disturbing, but serves as a rationale for identifying alternative ways and means to improve crop yields on the available arable land.

2.4   Fertilizer nutrients: An overview

2.4.1   Nitrogen (N)

As a nitrate containing salt (KNO_3) was foliar applied to maize plants in this study, despite the assumed availability of NO_3^- in soil at relative large amounts, the possibility that it might influence the outcome of this study set the basis for revisiting current literature on this macro element. N is

essential for plant growth and development and has a meaningful influence on crop yield (FSSA, 2003). It is absorbed by plants from the soil mainly in the form of NO_3^-, but can also be absorbed in the form of NH_4^+, especially under anaerobic conditions. Once taken up, it is incorporated into essential organic compounds such as amino acids, proteins, adenosine triphosphate (ATP), adenosine diphosphate (ADP), dioxiribose nucleic acid (DNA), phospholipids and Chl (FSSA, 2003). From this it is clear that N plays a major role in life processes such as photosynthesis, growth and reproduction in plants. The most notable effect of this mobile element is promotion of the green colour (Chl) in leaves as well as vegetative growth in plants (Poorter and Evans, 1998 as cited by Fageria, 2009).

More than two decades ago Sanchez and Blackmer (1988) attempted to determine the fate and recovery of applied N to the soil in a maize production system. Time course studies on the recovery of N, using an anhydrous ammonia-derived ^{15}N isotope, showed that 13% to 33% of the labelled N was removed from the soil at harvest, by the first season crop. Moreover, only a small percentage (0.3-1.5%) of the labelled N was recovered from the soil by the second season crop. Soil analysis one year after application showed that 19 to 23% of the labelled N remained in the soil. According to the findings of Sanchez and Blackmer (1988), a substantial portion (49-64%) of the labelled N was lost from the surface during the first year by processes other than plant uptake. Further, only a small portion of the soil N existed as exchangeable ammonium and nitrate, while most was in the KMI-N (Kjeldahl minus inorganic-N) fraction, which includes N from both organic matter and non-exchangeable ammonium. Large portions (47-94%) of the labelled N found in the KMI-N fraction one year after application were still present three years later.

Similar to Sanchez and Blackmer (1988), Torbert *et al.* (1992) also conducted labelling experiments on fine textured soils where maize was cultivated. The authors reported that, within four weeks after planting, the maize plants absorbed 30-55% of the labelled N while about 15-30% was retained in the soil and mainly in organic form. Seo *et al*. (2006) studied the N use efficiency by using a different labelled N-source namely $^{15}NH_4SO_4$ (ammonium sulphate). The isotope was applied at planting and at the six leaf stage as a side-dressing to the soil. The authors reported only 32% absorption of labelled N by the plants when applied at planting and 46% absorption of the labelled N applied as a side dressing, in the first year following application. The fact that relatively large amounts of N is normally reserved by the soil in non-absorbable forms, might be the reason for N deficiencies so often experienced under field conditions, causing yellowing of older maize leaves

along the midrib, regardless of the fact that large amounts of N were applied to the soil (FSSA, 2003).

Moreover, N mineralization is a microbiologically mediated process consisting of the transformation of organic N to NH_4^+ and is a major contributor to the amount of soil N available for plant uptake (Sanchez and Blackmer, 1988). According to the authors, maize may derive up to 70% of its required N from the mineralization of soil N. Further, plant roots have a significant effect on the soil microbial population because conditions for microbial growth are especially favourable in the rhizosphere (Foster, 1988). For example, soil microflora is heavily influenced by carbon sources derived from plant root rhizosphere deposits (Curl and Harper, 1990). Rhizosphere deposits are easily decomposable substrates that are translocated from the above soil parts of the plant to the roots where it is exuded into the surrounding soil as root mucilage (Qian et al., 1997). However, the role of rhizosphere deposits in the turnover of soil organic matter (releasing nutrients) has been controversial for many years. Although a number of studies have reported that root presence increases the decomposition rate of soil organic matter (Cheng and Coleman, 1990), other factors are also involved. Olness (1999) emphasized the influence of pH (H^+ and/or OH^- ions) on the rate of microbial mineralization of organic matter to form nitrate-N (NO_3^--N). Olness (1999) further showed that the conversion to nitrate-N was more negatively affected by [OH^-] ions than [H^+] ions.

From research conducted almost four decades ago (Warncke and Barber, 1973) it is known that maize roots can absorb both NH_4^+ and NO_3^- forms of N. Maximum dry matter accumulation in the maize plant resulted at a total N concentration of 67 µM, while maximum N uptake occurred at 303 µM and an NH_4^+/NO_3^- ratio of 2.46. The shoot:root ratio increased significantly with an increase in N concentration, but was unaffected by the NH_4^+/NO_3^- ratio. Primary root numbers per plant also increased with increasing N levels, but the elongation rate of an individual root did not respond strongly to elevated N concentrations. From this data it is clear that the correct amount of N supplied to plants is critical for influencing specific metabolic events in below and aerial plant parts.

N supply has an extensive effect on the growth of above soil plant parts (Muchow, 1988). Plénet and Lemaire (2000) used the N nutrition index (NNI) of the maize crop to distinguish non-limiting and limiting N conditions from each other. The NNI was determined by dividing the N concentration of the shoot biomass by the critical N concentration. The critical N concentration (N_c), defined as the minimum N concentration required to achieve maximum shoot growth, was formulated as $N_c = 34.0 \times DM^{-0.37}$, where dry mass (DM) is the shoot biomass in ton ha^{-1}. This was confirmed by Ziadi et al.

in 2007, in maize. They reported non-significant grain yields with a NNI $\geq$ 0.90, versus significant yields with a NNI < 0.80.

N not only has an effect on vegetative growth, but also plays an important role towards the manipulation of physiological processes (FSSA, 2003). Depending on the amount of available soil N, leaf N content was quantified by Zhao *et al.* (2003) to fall in the range of 11 to 48 g kg^{-1} on a DM basis. Wolfe *et al.* (1988a) found that leaves of low N supplied plants had a reduced capacity for solute accumulation. They also investigated the influence of N deficiency on leaf reflectance and found an increased leaf reflectance in two waveband ranges of green (550-580 nm) and far red (700-720 nm) light, which are not only closely related to leaf N content, but also to Chl content. The strong relationship between leaf N, leaf reflectance and leaf Chl content confirmed the influence of N on a physiological level.

The culmination of morphological and physiological responses by crops to N-fertilization can eventually be observed in yield data. Sainz Rozas *et al*. (2004) evaluated the effect of different urea rates (0, 70, 140 and 210 kg N ha^{-1}) applied at planting as well as at the V6 growth stage, and measured the fate of N in the course of NH_3 volatilization, de-nitrification, soil residual nitrate, N uptake, grain yield and unaccounted N. The yield obtained was 10.5 and 11.2 ton ha^{-1}, while N uptake at physiological maturity was 168 and 192 kg N ha^{-1} (average of N rates) for applications at planting and V6, respectively. For urea applied at planting, unaccounted N was 55, 69, 86 and 103 kg N ha^{-1} for the 0, 70, 140 and 210 kg N ha^{-1} application rates respectively, versus N applied at the V6 growth stage where it was 55, 46, 49 and 34 kg N ha^{-1} for the 0, 70, 140, and 210 kg N ha^{-1} application rates respectively. N losses were mostly attributed to NO_3^- leaching. These results showed that nutrient use efficacy, concomitant with economically viable grain yields can be obtained when N is applied at the V6 growth stage rather than at planting. However, this is dependent on residual soil N at planting. Split application may be a better alternative (Sainz Rozas *et al*., 2004).

In this regard the work of Sainz Rozas *et al*. (2004) showed that, although large quantities of N was applied to the soil, relatively large quantities have been lost, confirming the importance to find methods (possibly by means of foliar fertilization) whereby N can be supplied later in the season when N demands are generally high in crops. It is equally important to apply N at the correct growth stage, when contributions to yield can still be realized, which further makes foliar application a possibility to consider. Subedi and Ma (2005) indicated that there was no yield reduction when N

was restricted at silking or three weeks after silking up to physiological maturity. N supply was, therefore, more critical before silking than after silking, probably because of a N supply limitation that lead to a reduced ear size, kernel yield and N uptake (Subedi and Ma, 2005). The authors also indicated that restriction in N supply from planting to V8 caused an irreparable reduction in ear size and kernel yield of about 30%. Withholding N supply between the V8 growth stage and maturity reduced grain yield by 22% and N uptake by 53%. In practice, N containing fertilizer is generally applied to maize shortly before planting and partially side dressed at about the V6 to V8 growth stages (Ritchie *et al.*, 1993). Delayed side dressing can be critical and may lead to irreversible yield loss (Binder *et al.,* 2000). The authors reported that delaying N application till the V6 growth stage resulted in a near 12% reduction in kernel yield. Although the data supplied here is applicable to conditions in the USA, the principles also apply for South African conditions.

Equally important for maize cultivation is the management of P fertilizer application.

2.4.2   Phosphorus (P)

Two phosphate containing salts were applied in this study, i.e. mono potassium phosphate (MKP) and mono ammonium phosphate technical (MAP). P deficiency is a major abiotic stress condition that limits crop productivity on 30–40% of the world's arable land (Von Uexküll and Mutert, 1995) and it is the second most limiting mineral nutrient in crop production after N (Vance *et al.*, 2003). Low inorganic phosphorous (Pi) availability in soil, therefore, is one of the major constraints for maize production worldwide (Leakey *et al.,* 2006). It is mainly absorbed from the soil by plant roots in the form of soluble orthophosphate ($H_2PO_4^-$) (FSSA, 2003). After it has been absorbed, P is not only incorporated into essential organic compounds such as ATP, ADP and phospholipids but also into plant structures. As a result, P plays an important role in physiological processes such as photosynthesis, growth, respiration and reproduction and is primarily associated with cell division, root growth, flowering and ripening of crops (FSSA, 2003). P has been shown to enhance the number, length and diameter of roots when available in sufficient quantities (Chassot and Richner, 2002). According to the authors, and different from N, P is relatively immobile in plants that can result in deficiencies, especially in younger tissue, as well as impediment of ear formation, specifically in maize.

P deficiency in plants initiates a myriad of transcriptional, biochemical and physiological responses that serve either to enhance the plant's ability to acquire P from the soil or improve the efficiency

with which plants utilize P internally. P deficiency in crops is usually ascribed to the uptake of Pi, or lack thereof, from the soil as well as the translocation, or lack thereof, within the plant (Flügge *et al.*, 2003). Concerning uptake of P from the soil, and since maize roots occupy less than 1% of the soil volume in the plough layer (FSSA, 2003), it is not only diffusion that determines P uptake, but also antagonism or synergism between elements. Kovaevic *et al.* (2004) demonstrated a 25, 20 and 22% increase in the ear leaf nutrient content for P, calcium (Ca) and magnesium (Mg), respectively, when additional P was applied to the soil. Conversely, the authors showed that additional K application resulted in a 7, 13 and 30% decrease in the ear leaf content for P, Ca and Mg, respectively, while leaf K content increased by 16%. When both P and K was applied additionally and simultaneously while nutrient content was analyzed in the ear leaf afterwards, Kovaevic *et al.* (2004) reported an increase of 14% and 15% for P and K respectively, while leaf calcium was reduced by 5% and leaf magnesium by 30%. The latter indicates an antagonistic effect between K and P in the soil or an overriding effect as the latter results resembled that when K was additionally applied on its own.

Importantly, the influence of synergism and antagonism between elements as far as foliar application is concerned, is not fully understood and needs further investigation according to Kovaevic *et al.* (2004).

In recent years, the transport of P over plant membranes were investigated extensively and progress was made in our understanding of this aspect in terms of membrane and gene involvement. Although this falls outside the scope of this investigation, a short discussion seemed necessary in light of the uncertainties pertaining to P uptake by roots and leaves. In addition to the plastid Pi translocator family known in membranes, three subfamilies of Pi transporters have been identified namely Pht1, Pht2 and Pht3 (Mudge *et al.*, 2002; Rausch and Bucher, 2002). The Pht1 subfamily of Pi transporters is principally responsible for the uptake of Pi from the soil solution by the roots and gives a new understanding of the regulation of plant P uptake from the soil. According to Rausch and Bucher (2002), P uptake by roots is actually regulated by genes via the synthesis of specific transporter proteins, and is not simply a passive system. Hammond and White (2008) concluded that plant P deficiency has a direct effect on the expression of specific genes coding for the synthesis of plant growth regulators involved in the initiation of root meristem activity, anthocyanin accumulation and root hair development, probably in an attempt to counteract P deficiencies.

On a physiological level, keeping in mind that physiological events regulate morphological or growth events, plants respond to nutrient deficiencies in very specific ways (Müller *et al.*, 2007).

The authors implicated the involvement of shoot-derived carbohydrate signals, in particular sucrose, to be involved in the prevention or circumvention of low plant P levels. They speculated that sucrose not only functions as the primary compound for the delivery of carbon to sink tissues (roots) from the source (leaves), but also acts as a signal that alters the expression of genes involved in the response to nutrient deficiencies. Jain *et al.* (2007a) suggested that the availability of both P and sucrose has a strong influence on the development of root hairs following their observation that P-starved roots supplied with sucrose produced three fold more root hairs (of substantially longer length) than control P-starved roots not supplied with sucrose. This is in concert with the findings of Wissuwa *et al.* (2005) namely that plants alter their root system morphology in response to their P status by allocating more resources, such as sucrose and triacylglycerol (Dieuaide *et al.*, 1992), to the roots, subsequently increasing their root:shoot ratio. In this regard, Svistoonoff *et al.* (2007) reported that contact of the root cap with P-deficient media is thought to be necessary in order to initiate the latter response.

Further, Halsted and Lynch (1996) reported that P-induced reduction in plant biomass accumulation may result from the disturbance of several interacting physiological processes. However, at the canopy level, this reduction may be ascribed to either a limited amount of absorbed photosynthetically active radiation (PAR) or a less efficient conversion of the intercepted radiation into dry matter. Colomb *et al.* (1995) have previously shown that light interception by crops is generally the most sensitive component of biomass accumulation under varying P supply levels. Plants grown under low soil P conditions developed a smaller leaf area index (LAI), but how it is reduced is not well understood (Colomb *et al.*, 1995). In this regard Pellerin *et al.* (2000) reported that reduced LAI was the consequence of a delayed appearance of leaves in P-deficient plants. In the same year Plénet *et al.* (2000a) confirmed that soil P deficiency had a definite effect on the leaf area index (LAI) as well as the leaf expansion rate of maize, and this was in concert with the findings of Colomb *et al.* (2000).

As all morphological and physiological events in crops collectively determine the final yield of cultivated crops, and final yield is that part of organised agriculture that interests the farmer most, the essential role of P in this regard has been established to a large extent. During the 70's and 80's much attention was given to optimizing phosphate application to crops in terms of the final yield outcome, without contributing much to its physiological role in plants. In this regard Greenwood *et al.* (1980) showed that agricultural crops generally absorb only 5-10% of the applied P in the year of application while Barber (1985) demonstrated that most of the P is absorbed from the upper soil

surface. Johnston *et al*. (1986) contributed much to our understanding of P uptake by plants by showing that this is mainly accomplished from residual P in the soil and that freshly applied P cannot compensate for a low soil P status. It, therefore, seems that a given pool of plant available P has to be present for optimal, economic and sustainable crop production. However, in the event of P being limited plants respond on both a developmental and biochemical level in an attempt to maintain optimal P levels. Sibbesen and Sharpley (1997) succeeded in quantifying an optimum P level for cereal crops. The authors calculated that P content in soil must be between 21-35 mg P kg^{-1} soil (Olsen method), 50-90 mg P kg^{-1} soil (Bray method) or 9-15 mg P kg^{-1} soil (water extraction method) in order to obtain acceptable yields. The latter P levels in soil are seldom obtained. Further, the upper 2 mm of the soil A-horizon is usually dry during the period of maximum P uptake by plants cultivated under rain fed conditions, and this might be an important underlying, and possibly overlooked, factor influencing final maize yields. Again the question arose whether methods, such as foliar nutrition, that are 8 to 10 times more efficient than soil applications (Akanbi *et al.*, 2007), might contribute towards overcoming problems experienced with P uptake by roots during critical development stages in terms of crop development and yield.

2.4.3   Potassium (K)

Two K-containing salts, KNO$_3$ and MKP, were investigated in this study. K is the fourth most abundant mineral constituting about 2.5% of the lithosphere. However, actual soil concentrations of this mineral vary widely, ranging from 0.04 to 3% (Sparks and Huang, 1985). In accordance with its availability to plants, soil K is ascribed to four different pools: (i) soil solution, (ii) exchangeable K, (iii) fixed K and (iv) lattice K (Syers, 1998).

K is also one of the major nutrients essential for plant growth and development and has been recognized in the eighteen forties. Plants accumulate large quantities of this element, which constitutes between 2% and 10% of plant DM (Tisdale *et al.*, 1993). The exchange of K between different pools in the soil is strongly dependent upon the concentration of other macronutrients in the soil solution, e.g. nitrate (Yanai *et al.*, 1996). Further, the release of exchangeable K is often slower than the rate of K acquisition by plants (Johnston, 2005).

Unlike N and P, K is not found in organic compounds and structures inside plants, but is present in the cell sap (FSSA, 2003). However, it is involved in a list of divergent activities accentuating its

macro nutrient status. These include its role in i) the transport of N and starch within the plant, ii) the uptake of other nutrients by plants (Blevins *et al*., 1978b), iii) photosynthesis, iv) the strengthening of plant fibres, v) the regulation of stomatum opening and closing (Talbott *et al.*, 1998), vi) plant growth (Jordan-Meille and Pellerin, 2004), vii) maintenance of turgor pressure within cells (Mengel, 1998) and viii) the synthesis of proteins (Blevins *et al*., 1978b), carbohydrates and lipids (FSSA, 2003). K is rather mobile in plants and K deficiency symptoms show up first in older leaves as yellowing (necrosis) of leaf margins (FSSA, 2003).

Similarly to N and P, the uptake of K by plants is also affected by antagonistic and synergistic events. The K-status of a plant may deteriorate in the presence of high levels of other mono valent cations such as Na^+ and NH_4^+ that interfere with K uptake (Rus *et al.,* 2004). Diffusion is the dominant mechanism of K delivery to the root surface and constitutes up to 96% of the total soil K transported to the roots (Oliveira *et al.*, 2004). On the other hand, when plants are deficient in K (K starvation), it is known that K itself initiates uptake by the roots (Shin and Schachtman, 2004). This activation has been conventionally associated with the induction and expression of high affinity transporters and is considered as a major mechanism of plant adaptation to K starvation. The Shaker-type K channels KAT1 (Anderson *et al.*, 1992) and AKT1 (Sentenac *et al.*, 1992) were the first K transporting proteins cloned from plants. Both AKT1 and KAT1 are activated by a more negative membrane potential and are highly selective for K. Of these two channels, only AKT1 is expressed in roots and is involved directly in mineral nutrient uptake (Hirsch *et al*., 1998). KAT1 is a guard cell-specific channel and is likely to mediate K fluxes for turgor-dependent regulation of the stomatal aperture (Nakamura *et al.*, 1995). Another Shaker-type channel in *Arabidopsis*, SKOR, is expressed in the root pericycle and stelar parenchyma cells, and is likely to be involved in xylem loading (Gaymard *et al.*, 1998). The molecules that signal low K status in plants have been identified and include reactive oxygen species and phytohormones such as auxin, ethylene and jasmonic acid (Ashley *et al.*, 2006).

Our knowledge of how K distribution in the root zone influences K uptake by the plant is important in developing efficient practices for K fertilization. More than three decades ago Claassen and Barber (1977) showed that bathing roots in the presence or absence of K in a solution did not affect root growth. Comparisons of K influx into K supplied-roots attached to the shoot, as opposed to roots bathed in a solution that did not contain K and that was also attached to the shoot from the same plants, indicated that the K level in the shoot had a greater effect on K influx than the K level

of the root. Although Claassen and Barber (1977) did not speculate on the possible mechanism involved, probably because of a lack of knowledge at the time, the recent work of Kronzucker and Britto (2008) supplies some insight. K uptake in plants is biphasic, involving both low (passive K^+ influx down a electrochemical gradient through specific K channels) and high (active energy dependant K^+ pump against a electrochemical gradient) affinity K uptake mechanisms (Britto, 2008). In maize, the majority of K accumulation occurs before silking, according to studies conducted by Karlen *et al*. (1988).

In terms of yield, contrasting results have been documented. For instance, Heckman and Kamprath (1992) observed that K fertilization had a positive effect on ear size and stover dry matter accumulation. Later Ebelhar and Varsa (2000) as well as Haq and Mallarino (2005) reported yield increases in response to K fertilization while Bruns and Ebelhar (2006) observed no effect of K nutrition on grain yield in maize. However, maybe the views of Sweeney *et al.* (2000) should be accepted as the rule of thumb. The authors maintained that yield improvement in maize due to K fertilization is the rule rather than the exception and is the result of either increases in kernel weight or occasionally due to elevated number of kernels per ear.

The fact that N, P and K are involved in photosynthesis (Fageria and Gheyi, 1999, as cited by Fageria, 2009) necessitated a literature investigation in this regard.

2.5   Photosynthesis: Chlorophyll *a* fluorescence

Photosynthesis and the pathway of energy transduction (conversion of radiant energy to chemically stable forms) is complex in plants. Light is absorbed by antenna molecules within the photosynthetic membrane and energy is transferred as excitation energy that is either trapped by a reaction centre or dissipated as heat and fluorescence (Strasser *et al.*, 1999). A typical fluorescence transient is exhibited upon illumination of a dark-adapted photosynthetic sample by saturating light and under *in vivo* and *in situ* conditions the fluorescence behaviour of the photosynthetic system changes following a continuous changing environment (Srivastava *et al.*, 1995). Variations in fluorescence intensity are, therefore, solely due to changes in the redox state of the reaction centre complex of photosystem II (PSII) (Strasser *et al.*, 1999). The PSII unit catalyses the light-induced electron transport from water to the plastoquinone (PQ) pool molecule (Hankamer *et al*., 1997). The first part is the core complex, a well-defined structure responsible for all electron transfer reactions in PSII

and organized as a dimer in the stacked regions of the thylakoid membrane. The second part is the peripheral antenna, which in plants consists of a collection of light-harvesting complex II proteins which absorb most of the light for PSII (Dekker and Grondelle, 2000).

Interpretation of data captured by means of the fluorescence measuring instrument are based on the fast fluorescence rise that starts at an initial low value F_o and reaches a maximal value F_m. Out of these values several expression are calculated (Figure 2.3), with the basis of all of these expressions being F_o/F_m and the difference $F_v = F_m - F_o$ (Strasser $et\ al.$, 1999).
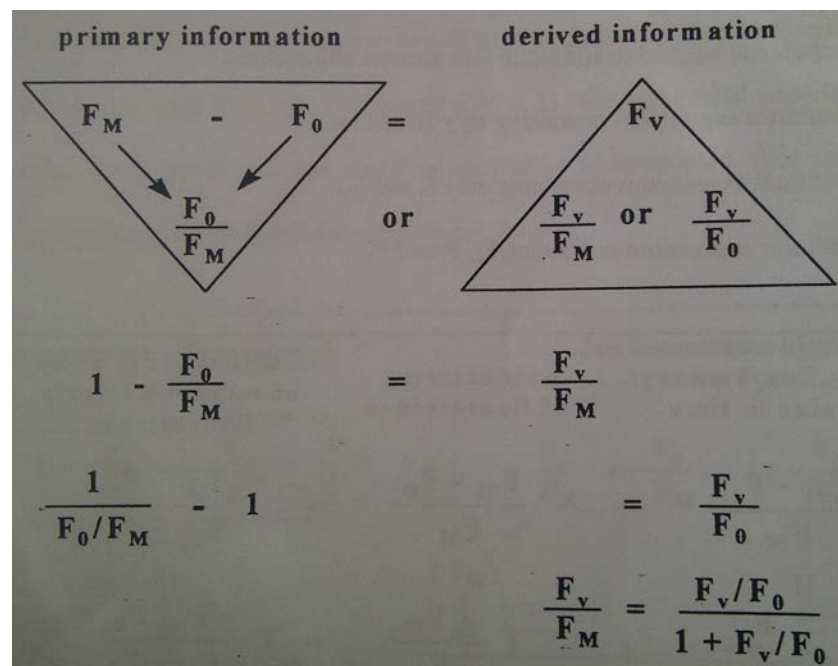
Figure 2.3: A demonstration of how several expressions combining the extrema F_o and F_m are converted into one another (Strasser $et\ al.$, 1999).

Chl a fluorescence is a handy tool to measure both physical (temperature, ozone) and chemical (fertilizer) effects on the vitality, sensitivity, productivity and resistance responses of plants towards these effects (Ouzounidou $et\ al.$, 1997; Clark $et\ al.$, 1998).

Based on the analysis of how fluorescence data can be processed, a test namely the JIP-test was developed for the translation of original fluorescence measurements into biophysical expressions (Table 2.2, Strasser $et\ al.$, 1999) quantifying the stepwise flow of energy through PSII (Strasser and Strasser, 1995).

Table 2.2: Summary of the JIP-test formulae, using data extracted from the fast fluorescence transient (Strasser *et al.*, 1999). These parameters are applied in order to quantify Chl a fluorescence using mathematical equations

Extracted and technical fluorescence parameters		
F_0	=	$F_{50\mu s}$, fluorescence intensity at 50μs
F_{150}	=	fluorescence intensity at 150μs
F_{300}	=	fluorescence intensity at 300μs
F_J	=	fluorescence intensity at the J-step (at 2ms)
F_M	=	maximal fluorescence intensity
^tF_M	=	time to reach F_M, in ms
V_J	=	$(F_{2ms} - F_0) / (F_M - F_0)$
Area	=	area between fluorescence curve and F_M
$(dV/dt)_0$ or M_0	=	$4 . (F_{300} - F_0) / (F_M - F_0)$
S_m	=	Area $/ (F_M - F_0)$
N	=	$S_m . M_0 . (1 / V_J)$ turn over number of Q_A
Quantum efficiencies or flux ratios		
ϕ_{Po} or TR_0/ ABS	=	$(1 - F_0) / F_M$ or F_v/F_M
ϕ_{Eo} or ET_0/ ABS	=	$(1 - F_0) / F_M . \Psi_0$
Ψ_0 or ET_0/ TR_0	=	$1 - V_J$
Specific fluxes or specific activities		
ABS/RC	=	$M_0 . (1/V_J) . (1/\phi_{Po})$
TR_0/RC	=	$M_0 . (1/V_J)$
ET_0/RC	=	$M_0 . (1/V_J) . \Psi_0$
DI_0/RC	=	$(ABS/RC) - (TR_0/RC)$
Phenomenological fluxes or phenomenological activities		
ABS/CS_0	=	F_0 or other useful expression*
TR_0/CS_0	=	$\phi_{Po} (ABS/CS_0)$
ET_0/CS_0	=	$\phi_{Po} . \Psi_0 . (ABS/CS_0)$
DI_0/CS_0	=	$(ABS/CS_0) - (TR_0/CS_0)$
RC/CS_0	=	$\phi_{Po} (V_J/M_0) . F_0$ *

* When expressed per CS_M, F_0 is replaced by F_M

In this study the photosynthetic response of maize to foliar applications of N, P and K containing salts were, *inter alia*, followed by measuring Chl levels and Chl *a* fluorescence. A short discussion on the process as it pertains to maize follows.

Currently, there is no doubt that the production of dry matter by crops is limited by the amount of Chl in leaves due to the pivotal role this pigment plays in photosynthesis (Dawson *et al.*, 2003). Further, virtually all of the known abiotic stress conditions, including drought, heat, cold, waterlogging and mineral deficiencies, have a direct effect on the process. Particularly the availability of nutrients, as it relates to the underlying study, is important. Wolfe *et al.* (1988b) reported that leaf photosynthetic capacity strongly correlated with N content during senescence. The maize plant follows the C_4-photosynthetic pathway while photorespiration is absent. The first steps of C_4-photosynthesis occurs in the mesophyll cells and involves the hydration of CO_2 into bicarbonate, which reacts with phosphoenolpyruvate (PEP) under the control of PEP carboxylase (PEPC), an enzyme with a higher affinity for CO_2 than Ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), to produce oxaloacetate (Hatch, 1987, as cited by Ghannoum, 2009). Oxaloacetate is converted into other C_4 acids (malate, aspartate or alanine) which diffuse into the bundle sheath cells where they are de-carboxylated, releasing CO_2 for fixation by RUBISCO and the rest of the C_3 cycle. The C_3 product, following the de-carboxylation reaction, returns to the mesophyll cells completing the C_3 cycle (Hatch, 1987 as cited by Ghannoum, 2009).

Ghannoum (2009) further explained that C_4 photosynthesis is equally or even more sensitive to water stress compared to its C_3 counterpart, in spite of the greater capacity and water use efficiency of the C_4 photosynthetic pathway. According to the author, declining leaf water status resulted in a lower CO_2 assimilation rate and stomatal conductance with photosynthesis going through several stomatal and non-stomatal phases. The work of Ghannoum (2009) revealed that the main non-stomatal factor, namely water, reduced the activity of photosynthetic enzymes, inhibited nitrate assimilation, induced early senescence and changed the leaf anatomy and ultra structure. The author also suggested that elevated CO_2 concentrations alleviate the effect of water stress on plant productivity indirectly.

In light of the worldwide focus on climate change, the importance of elevated CO_2 levels and its impact on photosynthesis is under scrutiny amongst scientists. Leakey *et al.* (2006) observed that elevated CO_2 concentrations, at normal soil water levels, did not stimulate photosynthesis, biomass

or yield of C$_4$-plants, such as maize. The authors also found that under elevated CO$_2$ levels, stomatal conductance was lower (–34%) with a higher soil moisture content, which resulted in a reduction in water use by the C$_4$-crop. The results obtained by Leakey *et al.* (2006) provided field evidence that photosynthesis as well as the production of maize may be unaffected by rising CO$_2$ concentrations, when sufficient water is available and drought conditions do not prevail.

With the development of photo-electric devices in the 1930's, fluorometry of Chl *a*, *in vivo*, emerged as a major method in the science of photosynthesis (Papageorgiou and Govindjee, 2011). Previous measurements of leaf photosynthetic rates by using gas exchange techniques have proven to be laborious and not practical under field conditions (Earl and Tollenaar, 1999). Alternatively, Chl *a* fluorescence techniques may serve as a more practical means for indirectly assessing leaf photosynthetic rates with a single instrument used under field conditions (Earl and Davis, 2003). Papageorgiou and Govindjee (2004) highlighted Chl *a* fluorescence as a convenient, non-invasive, highly sensitive, rapid and quantitative probe of oxygenic photosynthesis.

O'Neill *et al.* (2006) demonstrated that Chl *a* fluorescence can be used to detect plant stress in maize. They observed, under water stress conditions, that PSII quantum efficiency and electron transport rates were 25% lower for stressed plants versus non-stressed plants. Using a PAM-2000 fluorometer leaf temperature, PSII quantum efficiency and electron transport rates were measured for two drought tolerant and two susceptible maize hybrids grown under deficit and adequate water levels on three post-flowering dates, in order to determine whether these measurements could be used to differentiate hybrid photosynthetic responses to post-flowering stress (O'Neill *et al.*, 2006). Responses of the photosynthetic indicators, namely Chl content, PSII quantum efficiency and electron transport rates to water levels, were in general similar to leaf temperature responses.

Leaf temperature is considered to be a proven indicator of plant water stress (Raskin and Ladyman, 1988), and is based on the principle that increasing plant water deficits lead to stomatal closure, decreased leaf transpirational cooling and consequently increased leaf temperature relative to well-watered plants. O'Neill *et al.* (2006) observed distinct effects on measured variables including an increase in the average leaf temperature by 2.5°C resulting in PSII quantum efficiency and electron transport rates to decrease by 25% for deficit *vs.* adequate water level treated maize, during the critical R3 reproductive growth stage. This indicated that when water stress was intensified, photosynthesis decreased under water deficit conditions (O'Neill *et al.*, 2006). Under water deficit conditions the authors also reported that leaf temperature was 2.8°C cooler while PSII quantum

efficiency and electron transport rate values increased by 50% in tolerant vs. susceptible hybrids. Interestingly, similar leaf temperatures, PSII quantum efficiency and electron transport rate values were measured in both tolerant and susceptible hybrids under normal non-stressed conditions.

Baker *et al*. (1988) showed that not only water stress, but also chilling stress can reduce the quantum yield of photosynthesis (PHI, a fluorescence parameter) measured in maize and may take up to several days before it recovers. In particular, it was shown that PSII functioning and its regulation is not qualitatively changed during leaf desiccation and that the variations in PSII photochemistry can simply be understood by changes in substrate availability (Cornic and Fresneau, 2002). In this condition, the CO_2 molar fraction in the chloroplast declines when stomata close, while ribulose-1,5-bisphosphate oxygenation increases and becomes the main sink for photosynthetic electrons.

Studies conducted a decade ago have shown that N deficient plants had a significantly decreased CO_2 assimilation capacity compared to control plants having been supplied with sufficient amounts of N (Lu and Zhang, 2000). The authors concluded that N deficiency had little effect on PSII photochemistry, even under natural illumination, but that modification in the PSII photochemistry, under steady state conditions of photosynthesis, was induced. The latter indicated a decreased PHI for PSII electron transport as well as a lower efficiency of excitation energy captured by PSII in plants that were experiencing an N deficiency (Lu and Zhang, 2000). In concert Ju and Yu (2006) showed that N applied on wheat (jointing stage) improved the maximum photochemical efficiency (F_v/F_m), coefficient of photochemical fluorescence quenching (qP) and PHI of PSII.

Yu *et al.* (2010) compared five different N sources to evaluate their performance in maize via Chl fluorescence parameters (F_v/F_m) and found no difference in the relative Chl fluorescence capacity between treatments. From this it seems that Chl *a* florescence can be used to distinguish between the efficacies of different types of fertilizers, while Lu *et al.* (2001) found Chl *a* fluorescence as a useful indicator of leaf senescence. More recently, Chl *a* fluorescence was shown to be a useful technique for measuring the effect of heavy metals, such as nickel (Ni), on the photosynthetic capacity of crops (Drazkiewicz and Baszyński, 2010). They found that maize seedlings treated with Ni (200 µM) for 13 days showed a marked reduction in Fv/Fm versus control plants. Application of chemicals may negatively affect the environment. Abiotic stress conditions, such as unconformable nutrient supply, may affect the operation of photosynthetic mechanisms. Measurements of Chl *a* fluorescence parameters make it possible to evaluate the plant's photosynthetic performance and the extent of its tolerance towards abiotic stresses (Veres *et al.,* 2009).

2.6 Dry matter accumulation and yield

From a crop production perspective, accumulation of dry matter, as it relates to yield, is of particular importance. In this regard, Borrás *et al.* (2004) maintained that yield is mostly limited by sink capacity and seldom by the source (i.e. the leaf via photosynthesis). In terms of fertilization, a direct relationship exists between the amount applied and the expected yield outcome (Karlen *et al.,* 1987). For maize produced on a sandy loam soil under irrigation, the authors measured peak dry matter (V12-V18/600 growing degree units) accumulation in maize concurrent with N, P and K accumulation rates and calculated it to be 650, 10, 1.6 and 28 kg ha^{-1} day^{-1} respectively for a 10.9-13.4 ton ha^{-1} grain yield potential.

Maize grain yield appears to be more closely associated with kernel number than the phytomass of individual kernels (Andrade *et al.*, 1999). In this regard Subedi and Ma (2005) compared a leafy hybrid cultivar to a conventional cultivar. The authors reported that, despite a greater number of leaves and significantly larger leaf area, higher leaf DM and total DM in the leafy hybrid at silking, no difference in kernel DM and total DM between the two cultivars types were observed at physiological maturity. This strongly indicated that the leafy hybrid was possibly sink-limited or that the large number of leaves and leaf area was not efficient for dry matter production. Subedi and Ma (2005) also observed that the leafy hybrid had set significantly greater number of kernels, but their mass was lower indicating poor grain filling in the leafy hybrid despite the larger amount of DM in the leaves and stalk. Andrews *et al.* (2000) conducted a similar study five years prior to Subedi and Ma (2005) and observed that, despite twofold higher carbohydrate content in the leafy hybrid compared to a conventional control hybrid, grain yield was not significantly increased.

In the case of maize, dry matter accumulation (kernel set) is closely correlated with starch formation in kernels before physiological maturity. Maize leaves (source) export large quantities of carbon, in the form of sucrose, to storage organs (sinks) where sucrose is largely converted to starch (Kalt-Torres *et al.*, 1987). This is believed to occur on the basis of a concentration gradient between the sources and sinks. Phloem loading, on the other hand occurs against a concentration gradient (Sonnewald and Willmitzer, 1992). K, for example, plays an important role in the active process of phloem loading (Giaquinta, 1983). However, a previous report by Crafts-Brandner (1992) indicated that P does not have a major influence on sucrose accumulation. This apparent contradiction is of

interest in the current investigation and special attention will be given in chapter 4 dealing with this aspect.

In their study of K-stressed maize plants, Pretorius *et al.* (1999) showed by means of [U-^{14}C]-glucose labelling experiments that 40% less radioactivity was transported from the leaves to the stem and roots compared grown in soil containing sufficient K. However, K-stressed plants tended to translocate more radioactivity to the roots while much less was transported to the storage tissue in the stem. The authors concluded that K shortages might have inhibited sucrose translocation. Widstrom *et al.* (1988) previously investigated the distribution of sugars (soluble solids) in the stalks of maize plants. They observed that for older maize varieties, soluble solids increased from the bottom nodes of the stalk to the top, while newer varieties had the highest concentrations in nodes above the ear with a decrease in soluble solids from soil level to the ear node.

From the observations of Widstrom *et al.* (1988) it becomes apparent that phenotype and vegetative growth patterns have to be taken into account when carbon partitioning in plants is considered.

2.7   Assimilate partitioning

2.7.1   Assimilates, phenotype and vegetative growth stage

More than four decades ago, as far as phenotype and assimilate distribution is concerned, Pendleton and Hammond (1969) reported that positioning of the ear on the plant (a phenotypical trait) relative to the site of assimilate production affects ear growth. Similarly, the position of a leaf relative to the ear strongly influence the rate and direction of assimilate translocation (Palmer *et al.*, 1973). Upper leaves export carbon principally to the ear during the post-silking period, while lower leaves export relatively less to the ear and more to the lower internodes and roots (Tollenaar, 1977). In this regard Wilhelm *et al.* (1995) investigated the effect of tassel and leaf removal on final yield. The authors confirmed that grain and stover yield declined linearly with the number of leaves removed together with the tassel. For each leaf that was removed, together with the tassel, grain yield was reduced by 0.36 ton ha^{-1} and this was the result of decreased kernel size (poor grain fill).

Moreover, kernel set in cereals such as maize and wheat (*Triticum aestivum* L.) has been associated with intercepted radiation around anthesis (Lizaso *et al.*, 2003a). According to Lizaso *et al.* (2003b) post-silking assimilates are the most important source for kernel set and ear growth.

2.7.2   Assimilates and kernel set

Kernel set as well as kernel abortion has been linked to a shortage of assimilate supply to developing maize kernels (Boyle *et al.*, 1991). More recently, Subedi and Ma (2005) reported that grain filling is affected when all leaves above the ear leaf were removed, indicating that assimilate reserved in the stalk before silking was not remobilized to meet the requirement for grain filling. When there were no top leaves above the ear leaf, kernel was reduced by 50% and when there was no ear leaf 15 to 25% kernels were reduced in the conventional hybrids. Rajcan *et al*. (1999) also found that the effect of defoliation was greater for the ear leaf and ear leaf plus three leaves above the ear than for the ear leaf minus three leaves below the ear. This is concomitant with earlier findings (Pendleton and Hammond, 1969) that the relative photosynthetic potential of maize leaves in the top one-third of the canopy was twice as high as the middle leaves and five times as high as the bottom third. In contrast, Subedi and Ma (2005) observed that for conventional grain-yield hybrids the ear leaf and all leaves below the ear leaf significantly contributed to ear development and final grain yield.

On the other hand, in their study of maize under rain fed conditions, Schussler and Westgate (1995) noted that a lack of currently produced as well as reserved photosynthate accounted for much of the kernel loss in maize, while partitioning to ovaries at low ovary water potential may also have been limited by lack of assimilate demand. The concentration of sucrose and glucose increased in ovaries of water-deficient plants while ovary turgor remained at or above control levels. According to the authors inhibition of ovary growth at a low water potential was not related to a loss of turgor, nor was it caused by a depletion of ovary sugars, indicating that sugar accumulation at a low water potential could have been the result of impaired metabolism. Coupled with a low level of reserves, failure to utilize available sugars at a low water potential would severely inhibit assimilate flux to the ear and render kernel set highly vulnerable to water deficits during pollination (Schussler and Westgate, 1995). Eventually normal kernel set is measured in terms of starch formation in the endosperm.

2.8   Starch content and fertilization

Singh *et al.* (2004) observed that the starch and extractable starch content in kernels decreased significantly as the application of N to the soil increased. The extractable starch ranged from 63.4% to 72.1%, with the highest extractable starch content (72.1%) found in the plots that received no N application, and the lowest extractable starch content occurred in the plots that received 202 kg N

ha[-1]. According to the authors the total starch content ranged from 72 to 76.1%. Interestingly, despite the fact that the starch content was higher in kernels of plants receiving no N, these plots yielded 10.5 tons ha^{-1} compared to the plots receiving 202 kg N ha^{-1} that yielded on average 11.5 tons ha^{-1}. This indicates that the accumulation of starch in sinks is not the only aspect influencing kernel yield in maize.

Porter *et al.* (2001) applied different combinations and volumes of N and P fertilizer to the soil with the aim of observing its influence on starch content. They found that for maize grain to have more uniform starch content (69.9 to 75.6%), N and P fertilization rates must be adequate in order to also realize high yields. However, in maize, yield is not only dependant on kernel starch content or phytomass, but also on the number of kernels produced per ear.

2.9 Kernel number

Maize grain yield is closely associated with kernel number at harvest and this yield component is a function of the physiological condition of the crop during the period bracketing flowering (Otegui and Andrade, 2000). The authors found no significant differences between the effects of sowing dates on the number of spikelets per ear at flowering, indicating that kernel abortion was the main factor determining the differences in final kernel number per ear. This coincided with earlier findings of Cirilo and Andrade (1994) namely that reductions in crop growth rate after silking rather determined decreases in the number of kernels per ear than growth rate reduction during the pre-silking period in late sowings of maize. The authors based their postulate on the assumption that the contribution of assimilates to structural vegetative growth and maintenance of respiration in late sowings, would be associated with high ear infertility.

Andrade *et al.* (2002) investigated the effect of plant growth rate during the critical period of kernel set and found the latter to be a good predictor of the capacity of the maize plant to set kernels under a wide range of environmental conditions and management practices. The latter authors illustrated a positive correlation ($R^2 = 0.88$, $n = 34$) between plant growth and kernel number in the uppermost ear, during the silking period of maize (cv. Dekalb hybrid 636).

Otegui and Bonhomme (1998) reported that kernel number per ear was related closely to intercepted photosynthetically active radiation, accumulated during ear growth from 227 growing degree days (°Cd) before silking to 100°Cd after silking. Moreover, shading experiments based on the amount of radiation intercepted by the crop at flowering not only enabled prediction of the effect of altered

plant density on kernel number but also showed that suboptimal densities produced a strong negative effect on the efficiency with which the crop or plant converts intercepted radiation into grain sink capacity (Andrade *et al.,* 1993). This is in concert with previous findings of Edmeades and Daynard (1979) namely that, under most circumstances, kernel set in maize is predominately a source-limited process.

With regards to a delay in the onset of silk emergence relative to pollen shed, Bassetti and Westgate (1994) concluded that it has a direct effect and often decreases kernel set in maize. According to the authors the lack of pollen, failure of silks to emerge and loss of silk receptivity are all probable causes for poor kernel set. They went on to demonstrate that pollen shed followed a normal distribution pattern with time, peaking three days after anthesis and ended 13 days after anthesis. On ears with silks exposed to pollen for only one day, nearly all florets set kernels when pollen was shed at intensities greater than 100 grains cm^{-2} d^{-1}. These results indicated that synchrony between silk emergence and pollen shed is very important. Where a decreased kernel set was observed, silks emerged after the amount of pollen became limiting. Further, silks emerged prior to anthesis remained receptive to pollen for at least five days. Collectively, these findings of Bassetti and Westgate (1994) suggest that selection for silk emergence prior to pollen shed (progeny) may improve kernel set in maize under conditions known to delay silk emergence and might have an effect on final kernel number per ear.

The time between ovary fertilization and kernel set at the tip of the ear versus at the base of the ear also has an effect on the number of kernels produced per ear (Cárcova and Otegui, 2007). The authors reported that the longer the time gap between base and tip fertilization, the larger the difference in the development rate between kernels found at the base of the ear (dominant sinks) as opposed to those at the tip (recessive sinks), indicating a reduced opportunity for kernel set at the ear tip. In other words, the larger the tip-to-base ratio in ovary growth rate, the larger the number of kernels set per ear (r^2= 0.94; P = 0.03).

Total nutrient content in plants was also found to have an effect on ear kernel number. Barry and Miller (1989) found, for example, that a high P use efficiency in maize plants (optimum P fertilization versus uptake) from seeding resulted in a greater number of kernels produced per ear and hence also yield. The authors also observed that to obtain maximum yield, a shoot P concentration of at least 5.0 g kg^{-1} at the six leaf growth stage (V6) of maize is required.

Especially in light of the underlying study, the relationship between all of the aspects covered thus far in this review and its relation to foliar fertilization is of particular importance.

2.10   Foliar fertilization

2.10.1 Solubility of foliar fertilizers and mechanism of uptake by the leaf

Nutrients applied to plant foliage gain access to the plant by diffusion through the leaf surface or stomatal openings (Li *et al*., 2009). The epicuticular layer is the most hydrophobic part of the leaf surface and therefore the uptake of foliar fertilizers that do not enter the plant via the stomatal complex is dependent on the penetration and diffusion of atoms through the waxy layers, making the use of non-ionic compatible surfactants important (Wojcik, 2004, as cited by Keller, 2011). The rate at which a foliar applied fertilizer salt penetrates the leaf is greatly affected by humidity over cuticles as well as the hygroscopicity of salts and the size of ions (Tagliavini *et al.,* 2002). Penetration of the leaf requires firstly, that the salt is dissolved before uptake can take place. This is determined by the point of deliquescence (POD) of a salt, defined as the humidity over a saturated solution containing a solid fertilizer salt, with values for some salts indicated in Table 2.3. According to Tagliavini *et al.* (2002), when the humidity is above POD the salt residue on the cuticle dissolves, while if the humidity is below the POD value, a solid residue is formed and penetration ceases.

Table 2.3: Physical properties of selected fertilizer salts for foliar nutrition ([b]Lide, 1991, as cited by [a]Tagliavini *et al.,* 2002)

Salt	POD[a] (%)	Solubility[b] (g kg^{-1} H$_2$O)	Molecular weight (g mol^{-1})
CaCl$_2$ x 6H$_2$O	33	2790	219
MgCl$_2$ x 6H$_2$O	33	1670	203
K$_2$CO$_3$ x 2H$_2$O	44	1469	174
Ca(NO$_3$)$_2$ x 4H$_2$O	56	6600	236
Mg(NO$_3$)$_2$ x 6H$_2$O	56	1250	256
NH$_4$NO$_3$	63	1183	80
KCl	86	244	75
K$_2$HPO$_4$	92	167	174
KH$_2$PO$_4$	95a	33	136
KNO$_3$	95	133	101
Ca-propionate x H$_2$O)	95a	490	204
Ca-acetate	100a	374	308

Once nutrients passed through the waxy layer and the negative charges contained there, it must pass through water containing gaps of 10 to 100 Å (angstrom) in size [1Å = 1 x 10^{-8} cm] in the cell wall, called ectodesmata, and finally it must penetrate the plasmalemma (4 Å in size) to reach the first cell cytoplasm. Molecules or atoms (a typical atom is about 1 Å) reaching this site (plasmalemma) then requires active transport in the form of molecular carrier transport by means of pinocytosis (Figure 2.4) for entry into the plant cell (Jeppson, 2008, as cited by Keller, 2011). In cellular biology, pinocytosis is a form of endocytosis in which small particles are brought into the cell suspended within small vesicles that subsequently fuse with lysosomes to hydrolyze, or to break down, the particles. This process requires a lot of energy in the form of ATP (Jacek, 2007).
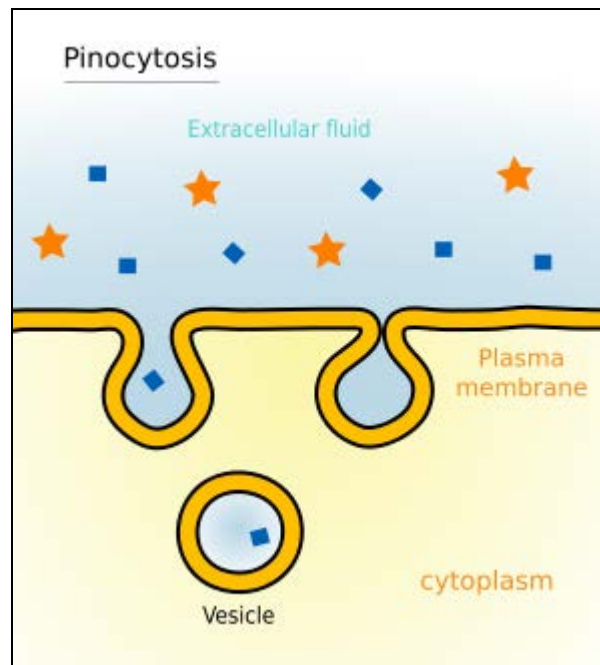
Figure 2.4: Pinocytosis, a form of endocytosis, which contributes to the active transport of particles over membranes (Jacek, 2007).

Nutrients that accumulate on the leaf surface and are not absorbed, may become toxic to the plant, and is generally referred to as "burnout".

Lewis and Kettlewell (1993) reported that in maize and other cereals the foliar application of macro nutrients, for example P, had not contributed largely towards plant growth and development and for this reason the authors did not consider the technique as one with possibilities. However, Eddy (2000) considered foliar fertilization as a viable practice and an economic means of supplementing

crop plants with specific nutrients. In light of this controversy surrounding foliar fertilization and its application potential a more comprehensive literature review follows.

2.10.2 Application potential of foliar fertilization as a viable practice

Despite the possible controversy still surrounding the foliar fertilization technique, it is currently used rather widely as an acknowledged practice with the objective of correcting nutritional deficiencies in plants caused by improper supply of nutrients to roots (Girma *et al.*, 2011). Almost three decades ago Papanicolaou *et al.* (1985) showed by means of field experiments on a heavy to medium calcareous soil that foliar application of urea was nearly as effective as a soil application (side dressing) in increasing maize grain yields, while the percentage N applied by the two methods were the same. Four years later Suwanarit and Sestapukdee (1989) reported that a single foliar application of KNO_3 on maize on any given day between 50% tasselling and 10 days after tasselling increased grain yield, although the uptake of the nutrients was dependant on the extent of leaf coverage. The authors observed that the yield response to additional KNO_3 application was less marked when the leaf area covered declined. More specifically, coverage of all aerial parts contributed to a significantly higher increase in yield compared to where KNO_3 was applied to only the ear leaf, only leaves above the ear or the soil. Interestingly, they also maintained that a balance between N and K was important for K fertilization to be optimally effective.

More recently, a study was conducted by Ling and Silberbush (2002) with the objective to determine the efficacy of different forms of nitrogen-phosphorus-potassium (NPK) fertilizers applied to maize, either to the soil or the leaves. All indices increased in response to all forms of foliar fertilization, but no significant difference was obtained between the different fertilizer forms.

Currently, foliar application of urea is considered an important factor affecting phenology, growth and yield components of maize (Amanullah *et al.,* 2010). A field experiment was conducted investigating the effect of foliar urea application on maize. The number of days to tasseling, silking and maturity were delayed significantly when urea was applied at a rate of 6% (v/w) at the V12 growth stage, while leaf area, number of grains per ear, 1000 kernel weight and grain and stover yields were increased significantly by the latter treatment. Yield components were higher when urea was applied at the V12 and VT growth stages, compared to foliar applications at V9 and R1. It was concluded that urea sprayed at a rate of 6% during the V12 stage was optimal in improving the grain yield of maize significantly (Amanullah *et al.* 2010).

From the latter results, it became clear that the growth stage at which fertilizer is applied foliar must be carefully considered, especially in the case of maize. Concomitant, in terms of application growth stage and concentration, Girma *et al.* (2011) conducted trials where treatments comprised of factorial combinations of three application timings and four rates of foliar P. Foliar applications were applied at growth stages V4, V8 and VT on maize, while foliar P (KH_2PO_4) rates were 0, 2, 4 and 8 kg ha^{-1}. Foliar P applied at the VT growth stage improved grain and forage P concentrations, which also reflected in enhanced grain yields obtained in some of the experiments, while the most optimum foliar P (KH_2PO_4) rate, was found to be 8 kg ha^{-1}. Girma *et al.* (2011) concluded that foliar P applied at the V8 growth stages and later, could be used as an efficient P manipulation tool in maize production.

Zhang *et al.* (2009), on the other hand, examined the effect of foliar N application on the water status and plant growth of maize under short-term moderate water stress conditions. The authors found that foliar N application increased DM content, relative water content as well as nitrate reductase activity under short-term moderate water stress conditions, compared to the control measurements.

From the above, one would expect foliar applied P to also be more efficient than when applied to the soil, but limited information is available on this postulate (Mosali *et al.*, 2006). The authors conducted trials over three seasons to determine the effect of varying concentrations of foliar applied P on winter wheat grain yields and P uptake. Treatments included P (KH_2PO_4) applied at rates of 0, 1, 2 and 4 kg ha^{-1} in 2002 and 2003 and 8, 12, 16 and 20 kg ha^{-1} in 2004. In all cases the response of wheat plants to these foliar applications in the presence or absence of pre-plant P-rates at 30 kg P ha^{-1}, were evaluated. Foliar applications of P increased grain yields and P uptake compared to the control where no additional P was applied to the leaves (Mosali *et al.*, 2006). Results from this study suggested that low rates of foliar applied P, independent of pre-plant P applied to soil when the second node is visible (Feeks 7 growth stage), might correct mid-season P deficiency in winter wheat and may result into higher P use efficiencies (Mosali *et al.*, 2006). Yield enhancements on wheat due to foliar P applications were also reported by Benbella and Paulsen (1998) who obtained, dependant on soil P status, a yield increase of up to 1 ton ha^{-1} compared to the unsprayed plots after foliar application of 5 to 10 kg KH_2PO_4 ha^{-1} after anthesis.

It has been hypothesized in the past that the application of foliar fertilizer may delay natural leaf senescence (Harder *et al.*, 1982). The authors based their statement on the estimation of leaf

photosynthesis in field-grown maize plants using a $^{14}CO_2$ uptake procedure. A small segment of the leaf material enclosed in a chamber was exposed to $^{14}CO_2$ for a short time period. Leaf samples were then analyzed using standard radiotracer techniques. $^{14}CO_2$ uptake rates were measured over a number of days between silking and plant maturity in conjunction with a larger experiment relating to the response of maize to foliar fertilizer application. Measurements indicated that the rate of leaf photosynthesis was depressed by as much as 17% the day following foliar-fertilizer application, but had nearly recovered by the second day. A similar pattern occurred in leaf stomata conductance. No difference in the seasonal trend of photosynthetic rates was detected between the control and foliar fertilizer treatments. Photosynthetic patterns after foliar fertilizer application may warrant further investigation as significant yield reductions were noted in these experiments. Further, as the technique used in the experiments of Harder *et al*. (1982) to monitor the rate of photosynthesis was on small intact leaf segments, photosynthesis and transpiration measurements on the total canopy could reveal supportive information in future.

On a different note, secondary advantages of foliar fertilizer application, in this case K-fertilization, was reported by Mann *et al*. (2004). The authors showed that foliar applied KCl reduced the incidence of septoria leaf blotch in wheat, while Beaton and Sekhon (1985) reported that K fertilization decreased stalk lodging in maize. Further, Reuveni and Reuveni (1998) showed that after just one spray of a phosphate salt at 0.1M, systemic protection against commen rust in maize, caused by *Puccinia sorghi* and northern leaf blight caused by *Exserohilum turcicum*, were induced.

However, there are several climate related factors that might have an effect on the efficacy of foliar applied fertilizers that need to be considered. One such factor is daytime of application, as was found recently by Barranco *et al*. (2010). In their study, the different responses of MKP foliar treatments (3% MKP solution applied at daylight, 3% MKP solution applied overnight) on olive trees under field conditions were evaluated, during three different application times (April, July, and November) in the northern hemisphere. All treatments, except the 3% (w/v) MKP treatment applied at daylight, improved the K nutritional status of olive trees in July (mid-summer), but not in November or April (late summer). The latter indicates that the day time of application as well as the time of the year, probably because of temperature and humidity differences, might also play a role towards the efficacy of foliar nutrition.

2.11   Summary

From the literature review it became clear that the fertilization of crops in general has been researched extensively over many decades. However, information in literature on i) the foliar application of specific macro nutrients (e.g. MKP, KNO_3 and MAP technical) and their benefits to maize, ii) the optimal growth stage (e.g. V3 and V8) for application, iii) the optimal application concentration and iv) the actual moiety contained within a specific salt that is most likely to have the most profound influence on maize growth and development as well as yield, are particularly scarce. All of these aspects have been addressed in this study. In chapter 3, data towards the vegetative growth and yield response of maize to foliar treatment with inorganic nutrients under rain fed conditions are supplied, while in chapter 4 the physiological response of maize to the above (i to iv) was followed using selected physiological parameters. Chapter 5 is a general discussion in an attempt to integrate all the acquired data and make meaningful recommendations towards the application of inorganic foliar fertilizers.

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CHAPTER 3

Vegetative growth and yield response of *Zea mays* L. to foliar applied inorganic nutrients under rain fed conditions

Abstract

Field studies were undertaken over three seasons, 2004/05, 2005/06 and 2006/07 in order to assess the effect of foliar applied mono potassium phosphate (MKP), potassium nitrate (KNO_3) and technical mono ammonium phosphate (MAP) on the vegetative growth and yield responses of *Zea mays* L., cv. DKC 78-15B, under rain fed conditions. Vegetative parameters measured in the study included plant height, number of tillers, total length of tillers expressed as a function of the number of tillers as well as plant dry and fresh mass. Yield parameters included total ear length, ear kernel length, total ear mass, kernel mass, number of kernels per ear (for the first as well as second ear) and final grain yield. During the first season 4% (w/v) MKP and 3% KNO_3 solutions were foliar applied at growth stages V3 and V8 with the objective to determine optimal application time. Different solution concentrations were used to maintain a constant K content. Results indicated V8 to be the optimum application time, while the MKP treatment proved to be the best general treatment as it contributed to increases in plant height, aerial part dry mass, total ear mass, kernel filling of the second ear as well as final yield when compared to the control and other treatments. During the second season MKP at 4% and 8% solutions and KNO_3 at 3% and 6% were applied at V8 to identify the optimum application concentration. Results showed the lower concentration rates to be best for both MKP and KNO_3 on the basis of measured vegetative and yield parameters. During the third season technical MAP was added and applied at 3.4%, to give the same P-concentration as the 4% MKP solution, in order to verify the involvement of either P or K or both in causing the measured vegetative and yield responses in maize. A reigning drought condition in the third season and the fact that MAP had no significant effect on the parameters measured eliminated the purpose of its use. Overall, it was only MKP applied at 4% and at V8 that tended to increase grain yield over the first two seasons.

Keywords: Maize, foliar fertilizer, MKP, KNO_3, MAP, growth and yield components.

3.1   Introduction

Foliar fertilization is an agricultural practise which has been studied for many years (Ling and Silberbush, 2002), but often gives contradictory results (FSSA, 2007). Foliar application of micro nutrients improves plant growth and is widely used (Fritz, 1978), while foliar application of macro nutrients is either less popular or least studied. The general consensus amongst scientists, however, is that foliar fertilization is merely a supplemental method of applying fertilizer to plants and cannot replace conventional soil application. Moreover, Ling and Silberbush (2002) identified plant size as the one critical factor influencing foliar fertilizer efficacy. They demonstrated that the larger the leaf surface area of a plant, the greater the absorption of foliar spray. This simply means that more of a foliar applied salt can be absorbed by larger plants and sends out a warning that foliar application of fertilizer has less of a chance on success when plants are in the initial vegetative growth stages when leaves are rather small.

Maize is one of the major crops produced worldwide and is, therefore, often used by researchers as a test crop because of potential financial benefits in the event that manipulation techniques can lead to yield and/or quality improvement. However, a literature review on foliar application of fertilizers to crops as an alternative method or as an additional supplement, revealed rather inconsistent results during the early years Thorne (1958). This is not surprising when the report of Li *et al.* (2009) is considered. The authors stated that several factors may influence the efficacy of foliar fertilizer, including plant type, the vegetative growth stage or time of application, the composition and characteristics of the administered fertilizer as well as environmental factors such as temperature, water supply and even illumination. Considering all of these variables it can be expected that comprehensive studies are needed to unravel the optimal conditions, in terms of foliar nutrition, for the different economic crops cultivated on the planet today.

With regard to maize, reports on the efficacy of foliar applied fertilizer were also inconsistent. Almost 30 years ago, Harder *et al.* (1982) did not observe any significant contribution of foliar applied fertilizer towards yield in maize. In this regard it must be noted that all applications with different fertilizers were executed late in the life cycle during the grain filling stage. What is important about this study is that it made a contribution in terms of sensitizing scientists towards the time of application. Interestingly, Barel and Black (1979) reported positive results in that foliar application of a polyphosphate (P) to maize at a late stage in the life cycle resulted in a yield

increase of 7.9% compared to the control. The authors also demonstrated that only 66% of the foliar applied fertilizer was absorbed by the maize leaf within 10 days while more than 87% of the absorbed fertilizer was translocated from the leaf to other plant parts within the same period of time. This study confirmed that inorganic fertilizer administered to leaves could be translocated to other plant parts where it could fulfil its function. However, recent trials have shown that foliar feeding in general can be 8 to 10 times more effective than soil feeding and that 90% of a foliar applied nutrient solution can be found in the smallest root of a plant within 60 minutes of application (Akanbi *et al.*, 2007). The efficiency of foliar fertilizers was also confirmed by O'Dell (2002), who stated that it was 95% more effective in getting nutrients into a plant versus an equivalent soil application.

Jaskulski (2004) reported that foliar fertilisation to maize at the 6 to 8 leaf growth stage resulted in increased grain yields. Kulczycki *et al.* (2008) concluded that the concentration at which foliar fertilizer is administered to a plant leaf is an important aspect that has to be considered. In a recent study, Sarakhsi *et al.* (2010) compared the response of maize to foliar application of nitrogen (N) (3, 5, 7%) at seven different individual or combined growth stages and concluded that a 3% N concentration significantly enhanced plant height as well as the number of kernels per ear when applied at all of the stages, while yield was the most increased when N was applied at this rate during tasseling. From this study it seems that the lower N concentration (3%) caused the best response in maize and that time of application played less of an important role. In the same year Amanullah *et al.* (2010) applied four urea concentrations (2, 4, 6 and 8%) to maize leaves at four different growth stages (V9, V12, VT and R1) and concluded that urea sprayed at a rate of 6% during the V12 growth stage improved the plant height, number of kernels per ear and also grain yield. The authors also found that grain yield and yield components were decreased when the same concentration was applied at the V9 or R1 growth stages, once again emphasizing the importance of application time.

The application of micro nutrients, as a foliar spray to plants is a more familiar practise used in agriculture, due to the fact that small quantities are needed to correct deficiencies and to stimulate plant growth and enhance yield (Grzebisz *et al.,* 2008). The authors reported that foliar application of zinc at an early vegetative growth stage resulted in a significantly higher grain yield, compared to the control, in maize. On the other hand, foliar fertilization with macro nutrients is a general practice in horticulture, especially by olive growers to correct frequent deficient levels of potassium (K) in

olive trees under rain fed conditions (Barranco *et al*., 2010). A cost effective fertilizer salt, containing both P and K, and that is often used in agriculture is monopotassium phosphate (MKP), also known as potassium dihydrogen phosphate (KH_2PO_4; Ankorion, 1995). Interestingly, almost three decades ago Neumann *et al.* (1981) found that minor differences in the chemical structure of applied foliar fertilizers to intact leaves had large effects on the threshold concentration above which damage was induced. They stated that the penetration of K_2HPO_4 and KH_2PO_4 through the intact cuticle of secondary leaves occurred at similar rates and that uptake was dependent on salt concentration.

According to Barranco *et al*. (2010) MKP is a fast source of plant absorbable P and K, when applied as a foliar spray, in order to correct nutrient deficiencies. In tomatoes, fruit development is often accompanied by the depletion of foliar K that is detrimental to both the plant and fruit quality (Chapagain and Wiesman, 2004). The authors foliar applied a 1% (w/v) solution of a MKP product called Nutri-Vant-PeaK containing 95% MKP and 5% Ferti-Vant, a non-destructive and long-lasting adjuvant, 40, 70 and 100 days after planting to tomato plants. They reported that chlorophyll, K, P, magnesium and iron contents in the leaves as well as marketable yield were significantly higher in treated plants compared to the control plants. The fact that MKP generally has a very low salt-index and electrical conductivity, is void of heavy metals, is slightly acidic, is high nutritious and is non hygroscopic, makes it very suitable for foliar application (Ankorion, 1997).

N, on the other hand, is an essential nutrient required by plants in large quantities (FSSA, 2003). Potassium nitrate (KNO_3) is a fertilizer salt containing relatively high contents of both N (N = 13%) and K (K = 38%), which makes it ideal for foliar application (Protea Chemicals, 2008). A three year trial conducted by Boman (2001) on 'Valencia' oranges showed that foliar application of KNO_3 resulted in treated trees having more fruit per tree (25-29%), more marketable boxes per tree (28%) and higher total pound solids per tree (25%), compared to control trees. According to the author's calculation, KNO_3 treated trees produced a 24% higher gross return in terms of processed fruits and a 28% higher gross return for packed fruits, compared to the control. On maize, Suwanarit and Sestapukdee (1989) did a study where a single 2.5% KNO_3 foliar spray was applied between 50% tasselling and 10 days after tasselling. They observed that applying the salt solution on the third day after 50% tasselling was the most effective time of application. A second application, more than 7 days after 50% tasselling suppressed the effects of the first spray, previously found to be most effective. The 2.5% KNO_3 spray solution administered to all aerial parts were the most effective in

increasing grain yield by 74%, versus spraying the ear leaf only (51%), spraying all leaves above the ear leaf (41%) and applying K to soil (23%). The authors concluded that the latter effect observed for foliar applied KNO_3, affected maize by stimulating chlorophyll synthesis and not by increasing leaf area.

In general, essential plant nutrients are mainly applied to soil for achieving maximum economic yields and this has become a common practice among maize producers over many years. However, according to a recent report by Fageria *et al.* (2009), soil versus foliar application as well as a combination of the two practices has become a matter of dispute lately and is a valid problem statement. This has lead to more research being done by scientists and fertilizer companies alike with the main objective to identify the most effective method of application, especially for elements needed by plants in higher amounts. According to the authors, foliar fertilization might, under certain circumstances, be more economic and efficient compared to soil application. Although Fageria *et al.* (2009) did not elaborate on the circumstances implied, one can speculate that soil factors in areas which is not conducive for the uptake of nutrients by crop plants, e.g. high clay content, dry conditions or pH, might favour foliar application as an alternative method. In this regard, and in the same year, the work of Li *et al.* (2009) contributed to our knowledge and speculative approach of the problem statement referred to higher up by noting that the response of dicotyledonous plants to foliar application of fertilizer seemed to be more positive than that of monocotyledons, probably because of different leaf types and structures of surface waxes. They additionally emphasized that, in order to achieve an economic advantage, it is essential to spray the correct fertilizers at the right growth stage.

Interesting work by Debreczeni (2000) on maize under irrigation revealed that the N-uptake of two maize hybrids was considerably higher from the NO_3-N source than from the NH_4-N one, and also noted that no NO_3^- accumulation could be detected in the kernels of treated plants. Unfortunately, no literature could be obtained regarding the foliar application of MAP to maize under rain fed conditions. Results published by Mudau *et al.* (2005) on oranges showed that a 1% MAP solution, applied 4 or 6 weeks after full bloom of oranges ('Shamouti' and 'Valencia Late') improved rind texture, but had no effect on juice and soluble solid contents.

In view of the potential attributed to foliar application of fertilizers, keeping in mind that relatively little research has been done in this regard on maize, the main aim of this study was firstly to

indentify the optimal foliar application time of fertilizers for maize (first season). A prerequisite was to schedule the application time of foliar fertilizers during those vegetative growth stages of the maize plant when a tractor sprayer could still be used. The second objective was to identify the optimal application dosage (second season) of relevant fertilizer salts used in this study, while the third objective (third season) was to identify the specific inorganic nutrient (element) mainly responsible for the observed growth and yield responses in maize.

3.2 Materials and Methods

3.2.1 Materials

In this study maize (*Zea mays* L.) was used as test crop to assess the possible benefits, or not, of foliar applied fertilizers during different growth stages. The maize cultivar DKC 78-15B, containing the YieldGard®-gene that makes the plant resistant towards the maize stork borer, *Busseola fusca* was chosen due to its good performance and popularity amongst farmers in the maize production triangle of the Republic of South Africa (RSA) (Monsanto, 2008a). The cultivar is a relatively short plant, highly prolific, does not produce a lot of leaves and tillers and is heat and drought tolerant. Days to 50% tassel development is 64-83, with 675 heat units necessary to reach 50% tassel development. Days to physiological maturity is 120-148 or 1360 heat units.

MKP used in the study was manufactured by Rotem Amfert Negev Ltd (Israel), a NovaPeak Division (LOT 2237). KNO_3 (Omni K), Reg No. K6887, and MAP (MAP 39), Reg No. K6892, was purchased from Omnia Fertilizer, a Division of Omnia Group (Pty) Ltd, Republic of South Africa.

3.2.2 Methods

3.2.2.1 Experimental layout and treatments

The trials were conducted over three growing seasons (2004/05, 2005/06 and 2006/07) in the Allanridge region (27°43'00.6"S; 026°42'37.1"E) of the Free State Province, RSA. The crop was established on a 1.5 m deep sandy soil, classified as an Avalon soil form belonging to the Woodburn family (Soil Classification Working Group, 1991). Plant rows were in a N-S direction, with an inter row spacing of 1.5 m and an intra row spacing of 35-40 cm, yielding a population of 16000 plants ha^{-1}. A complete randomized block design was used in all trials with each treatment being replicated four times. Soil samples were taken at a depth of 0-30 cm and 30-60 cm and analyzed to determine the soil fertility status (Table 3.1). Fertilizer applications were based on the soil analysis (Table 3.2). Based on the results obtained with specific treatments in a specific season, treatments were adapted for the following season resulting in different treatments in different seasons. The foliar treatments that were applied between 09:00 and 12:30 during each of the three seasons are shown in Table 3.3.

Table 3.1: The average soil fertility status over three seasons (Free State Department of soil analysis, National Department of Agriculture, Glen and SGS Agri-Laboratory Services)

Parameter	Unit	2004/05 0-30 cm	*2005/06 0-30cm	*2005/06 0-60cm	*2006/07 0-30cm	*2006/07 0-60cm	Norm
		A	B	B	B	B	
Clay & Silt (0-30cm)	%	10	10	14	12	14	
Sand (0-30cm)	%	90	90	86	88	86	
Effective depth	cm	200	200	200	200	180	
Conductivity	mS m^{-1}	18	21	18	16	16	200-500
pH (KCl)		4.47	4.5	4.22	4.2	4.54	5.5-7.5
Calcium (NH$_4$ OAc)	mg kg^{-1}	118	168	227	164	301	300-2000
Magnesium (NH$_4$ OAc)	mg kg^{-1}	46	75.5	87	71	109	80-300
Potassium (NH$_4$ OAc)	mg kg^{-1}	130	126.6	119	106	111	80-160
Sodium (NH$_4$ OAc)	mg kg^{-1}	13	3.25	4	1.1	4.23	100-500
Phosphorus (Olsen)	mg kg^{-1}	11 (Bray 1)	45	22	53	11	15-30
Ca:Mg		1.57	1.35	1.59	1.41	1.68	1.5-4.5
(Ca+Mg):K		2.91	4.52	6.07	5.17	3.15	10-20
Mg:K		1.13	1.92	2.34	2.15	8.45	3-4
Acid saturation	%	8.13			8.33		

A = average of individual samples taken all over the trial area and pooled together.
B = average of individual samples pooled together. Samples were taken on the plant rows after fertilizer application.
*Samples between rows were not acquired for the 2005/06 and 2006/07 growing seasons due to traffic control practices on this experimental farm where rows are kept in the same position year after year.

Table 3.2: Standard farm fertilizer applications for three seasons based on soil analysis and a grain yield potential of 4 ton ha^{-1}

Time	Rate (kg ha^{-1})	Type	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	S (kg ha^{-1})
2004/05						
At planting	210	3:1:0 (28)	44.1	14.7	0	0
Top dressing	100	LAN	28	0	0	0
TOTAL			**72.1**	**14.7**	**0**	**0**
2005/06						
Pre-planting	2000	Dolomitic lime	0	0	0	0
Pre-planting	100	MAP	11	22	0	0
At planting	200	21:8:0 (29) + 8% S	42	16	0	16
Top dressing	100	LAN	28	0	0	0
TOTAL			**81**	**38**	**0**	**16**
2006/07						
At planting	200	21:8:0 (29) + 8% S	42	16	0	16
Top dressing	100	LAN	28	0	0	0
TOTAL			**70**	**16**	**0**	**16**

Table 3.3: Treatments applied during the various growing seasons

Season	Foliar spray*	Application rate	Application growth stage	pH**	Nutrient content (g L^{-1})		
		(% w/v)		(25ºC)	N	P	K
2004/05	0 (control)	-	-	-	-	-	-
	KNO$_3$	3.0%	V3	6.14	3.90	-	11.40
	KNO$_3$	3.0%	V8	6.14	3.90	-	11.40
	MKP	4.0%	V3	5.12	-	9.04	11.28
	MKP	4.0%	V8	5.12	-	9.04	11.28
2005/06	0 (control)	-	-	-	-	-	-
	KNO$_3$	3.0% (1x)	V8	6.14	3.90	-	11.40
	KNO$_3$	6.0% (2x)	V8	6.79	7.80	-	22.80
	MKP	4.0% (1x)	V8	5.12	-	9.04	11.28
	MKP***	8.0% (2x)	V8	4.8	-	18.08	22.56
2006/07	0 (control)	-					
	KNO$_3$	3.0%	V8	6.14	3.90	-	11.40
	MKP	4.0%	V8	5.12	-	9.04	11.28
	MAP	3.4%	V8	4.96	4.11	9.14	-

*KNO$_3$ – Potassium nitrate (130g N kg^{-1}; 380g K kg^{-1}). MKP – Mono potassium phosphate (226g P kg^{-1}; 282g K kg^{-1}). MAP – Mono ammonium phosphate technical (121g N kg^{-1}; 269g P kg^{-1}). **Measured using a Crison MM40 multi meter. ***MKP (108 g) dissolved in 1.35 L water at 26 ºC despite the claim of Lide (1991) that its solubility is only 33 g kg^{-1} H$_2$O.

The knap sack sprayer was calibrated for each plot to deliver 1.35 L (100 L ha^{-1}) of foliar treatment or distilled water in the case of controls. A Solo® 475 knap sack sprayer (Solo Incorporated; Germany) delivering a pressure between 334.7 and 413.6 kPa was used in the experiments to apply the foliar fertilizer solution directly over the plant row. The sprayer was equipped with a flat fan nozzle spraying at a 40º angle (both sides of the perpendicular) covering a width of 1.1 m on the soil surface from a height of 90 cm above the ground. The gross plot had an area of 135 m^2 (9 m x 15 m) and consisted of six rows. The two outside rows of each plot were not harvested, serving as border rows to check possible contamination from adjacent plots. Yield and quality were determined from the net plot of 58.5 m^2 (4.5 m x 13 m) consisting of the three centre rows. Plants from the remaining row were used for morphological and physiological studies.

3.2.2.2   Agronomic practices

Primary soil tillage was executed with a ripper to an average depth of 90 cm. Lime was broadly applied and incorporated with an offset disc harrow to an average depth of 15-20 cm. Pre-plant fertilizer was band placed 20 cm below the soil surface and directly on the row approximately 30 days before planting. At planting fertilizer was band placed 5 cm below the seed and 5 cm to the side to prevent any negative salt effects on the seedling. Fertilizer applied as a side dressing commenced at the 10 to 12 leaf growth stage (V10-V12), approximately 15 cm to the side of each plant row and was slightly tilled into the soil. Seeds were planted with a mechanical planter approximately 5 cm below the soil surface. Weeds were controlled by hand.

3.2.2.3   Summary of sampling time and activities over three seasons

The growth stage of the plant, days after seeding (DAS), parameters measured and time of treatment application for the three seasons are summarized in Table 3.4:

Table 3.4: Growth stage of the plant, the DAS, parameters measured and time of treatment application for the various seasons

Date	DAS	Crop stage	Activity on the day
2004/05			
11/12/2004	0		Planting date
23/12/2004	12	V3	Treatment application & morphological measurements
13/01/2005	33	V8	Treatment application & morphological measurements
03/02/2005	54	V13	Morphological measurements
24/02/2005	75	V19	Morphological measurements
01/03/2005	79	Flowering	Physiological measurements
10/03/2005	89	Milk stage	Physiological measurements
26/03/2005	105	Soft dough	Physiological measurements
10/04/2005	119	Hard dough	Physiological measurements
26/04/2005	135	Physiological ripe	Physiological measurements
31/08/2005	232	Harvest	Yield and quality
2005/06			
05/12/2005	0		Planting date
12/01/2006	39	V8	Treatment application & morphological measurements
26/01/2006	53	V13	Morphological measurements
08/02/2006	66	V19	Morphological measurements
13/02/2006	71	Flowering	Physiological measurements
28/02/2006	86	Milk stage	Physiological measurements
14/03/2006	100	Soft dough	Physiological measurements
24/03/2006	110	Hard dough	Physiological measurements
13/04/2006	127	Physiological ripe	Physiological measurements
15/08/2006	254	Harvest	Yield and quality
2006/07			
09/12/2006	0		Planting date
12/01/2007	34	V8	Treatment application & morphological measurements
31/01/2007	53	V13	Morphological measurements
27/02/2007	80	V18	Morphological measurements
05/03/2007	86	Flowering	Physiological measurements
14/03/2007	95	Milk stage	Physiological measurements
28/03/2007	109	Soft dough	Physiological measurements
04/04/2007	116	Hard dough	Physiological measurements
12/04/2007	124	Physiological ripe	Physiological measurements
13/06/2007	186	Harvest	Yield and quality

The long-term climate can be described as semi-arid, with an average annual rainfall of 450 mm per annum. Climatic data, including temperature, rainfall and humidity over three seasons are supplied in Table 3.5.

Table 3.5: Average minimum and maximum temperatures, rainfall and relative humidity per month for the various growing seasons. (South African Weather Service 27°99'40" S 26°66'50" E, 1343 m above sea level)

MONTH	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	TOTAL
2004/05										
Temp max (°C)	32.5	31.9	31	28.4	24.6	22.6	21	21	23.9	
Temp min (°C)	18.5	18.8	18.3	15.1	11.4	6.1	3.7	2.1	5.5	
Rainfall (mm)	55.8	94.4	48.4	37.6	2.2	30	0	0.8	0	269.2
Humidity (%)	63.4	68.9	69.1	76.2	85.5	74.6	72.4	66.1	61.5	
2005/06										
Temp max (°C)	31.2	29.4	28.5	26.4	25.4	20.1	20.2	20.7	19	
Temp min (°C)	14.8	17.6	18.2	14.5	11	4.6	2.5	3.4	3.9	
Rainfall (mm)	23.6	52.2	100	101.2	18.8	12.8	0	0	39.2	347.8
Humidity (%)	59.2	79.3	81.4	77.3	80.4	78	73.4	61.6	71.1	
2006/07										
Temp max (°C)	31.8	33.3	33.8	31.1	26.6	22.4	16.5	20	22.2	
Temp min (°C)	16.7	17.1	16.8	14.4	11.7	4.5	2.5	2	4.1	
Rainfall (mm)	35.8	23.8	6	7.6	29	1	24.6	0	0	127.8
Humidity (%)	59.3	55.8	50	53.5	67.8	51.5	72.4	50.1	44.4	

3.2.2.4 Quantification of the vegetative growth and reproductive responses
3.2.2.4.1 General

Three plants per plot (randomly selected), taken from the row ear-marked for destructive testing represented a sample for quantifying the vegetative growth response of maize to foliar treatments at three growth stages (V8, V13 and V19). Parameters measured from the destructive testing included aerial fresh and dry mass. Additionally, non-destructive morphological measurements were taken from nine plants plot[-1] marked at the beginning of the season in the three rows allocated for yield determination. In case of the latter, the plant height as well as the number of tillers and tiller number expressed as a function of length (growth), were either counted or measured. The data were pooled and averages calculated. According to Russell and Stuber (1983) the total leaf number for maize varies from 10 to 29 and is influenced by the time of tassel initiation, temperature and photoperiod. Consequently leaf number was not determined for each replicate during the experiment.

At harvest the ears from the nine plants referred to above, were counted and subsequently collected to determine the total length of the ears (without ear leaves), the length of the ear up to the point where kernels was formed (ear filling height), the ear mass, the number of kernels per ear, as well as the kernel mass. In total, ears from seventy five plants per plot were harvested, including the nine plants used for non-destructive tests, to determine grain yield.

3.2.2.4.2   Vegetative growth parameters

The growth stage was determined by counting the number of leaves from the bottom upwards to the last fully developed leaf, as well as by cutting open the stem and counting the nodes. Plant height was measured from the soil surface up to the upper most V shape of youngest leaves as illustrated in Figure 3.1, using a measuring tape.
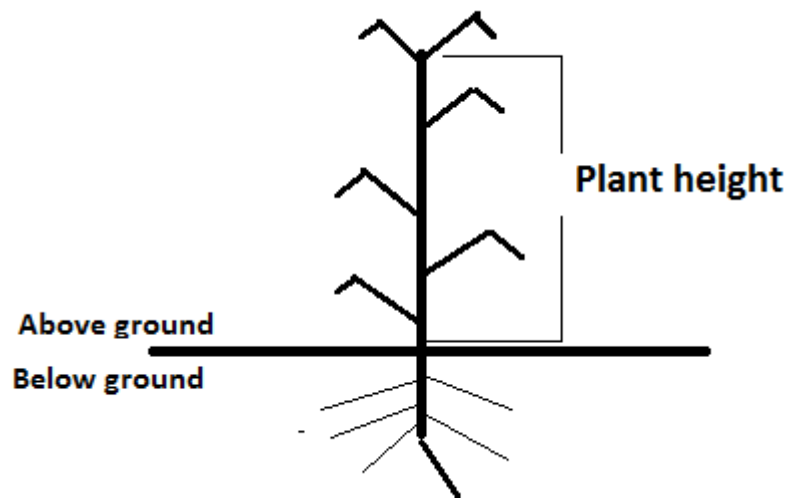
Figure 3.1: Method for measuring plant height.

The fresh mass of the aerial plant parts from three plants plot^{-1} was determined every three weeks from date of treatment. The plant material was then placed in paper bags, dried in an oven at 60°C for 102 h and the dry mass determined**.**

3.2.2.4.3   Reproductive parameters

Total ear length and filled length was measured as shown in Figure 3.2 for all ears from the nine plants per plot marked in the beginning of the season for non-destructive measurements.
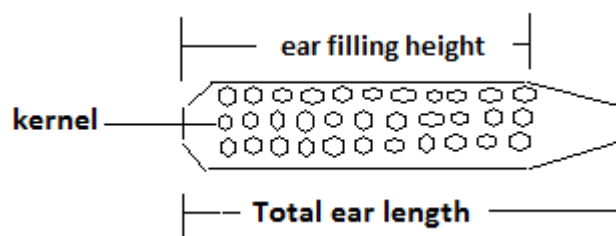
Figure 3.2: Method for measuring ear parameters.

The total ear mass was determined by weighing the ear without the husk leaves. Subsequently, the kernels were removed, counted and weighed in order to calculate the average number of kernels and kernel mass per ear as well as the final grain yield at harvest. Harvest took place when the moisture content was close to 12.5% for the field in general. A 100 g sample was taken from each replicate and pooled for calculating the average moisture content of kernels. Grain yield was expressed in ton ha^{-1} at a corrected moisture content of 12.5%.

3.2.2.5 Statistical analysis

Data presented were means of four replicates (vegetative growth parameters) and three replicates (yield parameters) along with standard deviations of means. Means of raw data and percentage differences from controls were subjected to analysis of variance using the NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA) statistical program. Treatment means of parameters showing significant differences ($P<0.05$) were separated using the Tukey-Kramer Multiple-Comparison Test (Steele & Torrie, 1980). The coefficient of variation (CV) was calculated for each parameter (raw data only) and included in the legend of the corresponding graph. Data calculated as percentage differences from control treatments, were statistically analyzed in order to verify interactions and main effects between factors (growth stage and concentration) which were not possible to calculate from the means of raw data. Percentage difference data were calculated as the percentage change from the control treatment for each replicate, prior to determining the mean of the percentage difference from control for the treatment.

3.3   Results

3.3.1   Vegetative growth parameters

3.3.1.1 Plant height

During the 2004/2005 growing season trials were conducted to identify the optimal application time for the nitrate, potassium and phosphate containing salts. None of the foliar treatments, contributed to significant differences in plant height when measured at either V8 or V13 (Figure 3.3A1). When plants were treated with MKP at the V8 growth stage and plant height measured at V13 a marked, but non-significant increase (+8.2%) was observed compared to the control (Figure 3.3A1). However, plant height measured at V19 (204 cm) was increased significantly by the V8 MKP treatment compared to the control (184 cm), but compared to the other treatments differences were not significant.   When data was expressed as a percentage difference from the control (Figure 3.3A2), it was only the measurement of plant height at V19, which revealed a significant main effect for the MKP salt versus the KNO_3 salt.

During the 2005/06 growing season, a trial was conducted to identify the optimal concentration for the KNO_3 and MKP salts, by applying the treatments only at the V8 growth stage. When KNO_3 and MKP were applied at single (1x) and double (2x) rates at V8, no statistically significant differences were observed, when plant height was measured at either V13 or V19. Both salts, applied at double rates tended to suppress plant height compared to the single rate (Figure 3.3B1). Based on this data it was decided to only apply foliar treatments at the single rate during the 2006/2007 season. Data plotted in Figure 3.3B2, in order to identify main effects or significant interactions between treatments, showed no significance.

During the 2006/2007 growing season a trial was conducted in order to verify P and/or K involvement by applying only the single dosage of salts MKP, KNO_3 and MAP (Table 3.3) at the V8 growth stage. The KNO_3 treatment applied at V8 but measured at V19 reduced plant height

Figure 3.3: Plant height of the main stem at growth stages V8, V13 and V19 during different growing seasons. A1 = 2004/05 season (sprayed at V3 and V8 in order to identify the optimal application time), B1 = 2005/06 season (sprayed only at V8, based on previous season results, in order to identify the optimal application concentrations) and C1 = 2006/07 season (sprayed only at V8, based on previous season results, in order to verify P and/or K involvement). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage; S = salt, GSxS = growth stage and salt interaction, C = concentration and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represent standard deviations between replicates from the mean for each treatment. A1 = V8: CV = 4.1%; V13: CV = 5.0%; V19: CV = 3.9%. B1 = V13: 4.1%; V19: CV = 2.2%. C1 = V13: 4.3%; V19: 2.9%.

slightly, but not significantly, when measured at both V13 and V19, while neither MKP nor MAP had any significant effect (Figure 3.3C1). However, when data was calculated and expressed as a percentage change from the control, it was found that the KNO_3 application caused a significant decrease in plant height measured at V19 and MKP a significant increase compared to the control (Figure 3.3C2). All other parameters measured and reported in this chapter were based on the three approaches for different seasons as outlined above and described in the legends of figures.

3.3.1.2 Number of tillers per plant

The maize cultivar DKC 78-15 B is known to fall within the group that genetically produces a medium number of tillers (Monsanto, 2008b). Both the KNO_3 and MKP treatments applied at V3 during the 2004/05 season, showed a marked increase (not significantly) in the number of tillers found at growth stage V13, namely 38% and 68% respectively (Figure 3.4A1). The same result was noted at V19, with an average increase in tiller number of 23% and 52%, respectively. However, compared to the earlier application stage (V3), foliar treatment with the these salts at V8, seemed to have either suppressed or had no effect on the number of tillers produced per plant, when counted at both V13 and V19 during the 2004/05 growing season. None of the rather substantial differences in tiller number observed between plants treated at the V3 or V8 growth stages were statistically significant, due to large standard deviations between replicates notable in Figure 3.4A1, as well as a coefficient of variation (CV) of 47.6% and 32.1% for the number of tillers measured at V13 and V19, respectively.

During the 2005/06 growing season, the tiller number at both V13 and V19 was unaffected by treatment at V8 (Figure 3.4B1, B2). The number of tillers produced during this season was on average lower compared to the first season (Figure 3.4A1).
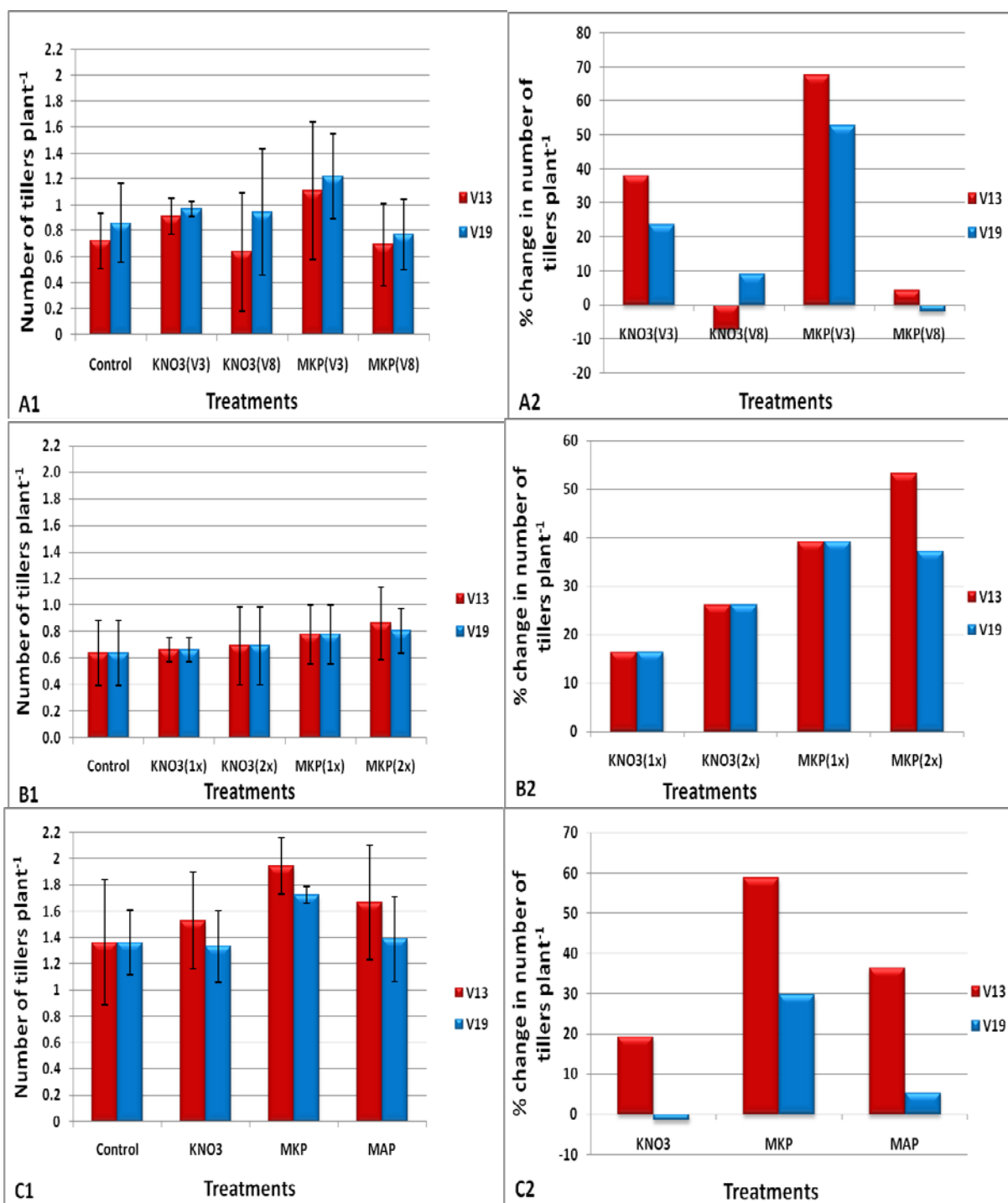
Figure 3.4: Number (n) of tillers produced at the V13 and V19 plant growth stages during different growing seasons. A1 = 2004/05 season (sprayed at V3 and V8 in order to identify the optimal application time), B1 = 2005/06 season (sprayed only at V8, based on previous season results, in order to identify the optimal application concentrations) and C1 = 2006/07 season (sprayed only at V8, based on previous season results, in order to verify P and/or K involvement). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage; S = salt, GSxS = growth stage, C = concentration and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represent standard deviations between replicates from the mean for each treatment. A1 = V13: CV = 47.57%; V19: CV = 32.14%. B1 = V13: CV = 33.5%; V19: CV = 32.3%. C1 = V13: CV = 16.6%; V19: CV = 13.9%.

MKP applied at V8 during the 2006/07 season (Figure 3.4C1), as was the case during the 2005/06 season, showed no significant differences (large standard deviations between replicates) increasing the tiller number per plant at both V13 and V19 when compared to the control. It was, however, interesting to note that the number of tillers was reduced between crop stage V13 and V19, which was not the case during the first two growing seasons (Figure 3.4A1 & B1), except for the double MKP treatment during the 2005/06 season.

The expression of data as a percentage difference from the untreated control, in terms of number of tillers per plant, revealed neither significant interactions between factors, nor any significant main effects for any of the three growing seasons (Figure 3.4A2, B2 and C2).

3.3.1.3 Total length of tillers per plant expressed as a function of the number of tillers

As a considerable amount of assimilates and energy are used for tiller growth, it can be expected that tiller growth might have an impact on other growth parameters, such as main stem plant height. It was, therefore, considered important to quantify this aspect. During the 2004/05 growing season (Figure 3.5A1), both the KNO_3 and MKP foliar treatments applied at V3 showed a tendency to contribute to an increase in total tiller length, expressed as an average for the number of tillers per plant, when measured at V19. Although the increase in tiller length was rather marked for these salts, it was not statistically significant due to a CV of 30%. Applying salts at V8 had no significant effect on tiller length expressed as a function of the tiller number per plant.

Application of KNO_3 and MKP at the single rate at V8 (Figure 3.5B1) during the second season (2005/2006) also had no significant effect on the average tiller length as was the case in the previous season. Interestingly, KNO_3 applied at the double rate contributed to a decrease in tiller length when measured at V19, while MKP applied at the same rate (2x) had the opposite effect and, although not significant, markedly enhanced the tiller length to number ratio by 23% compared to the control.
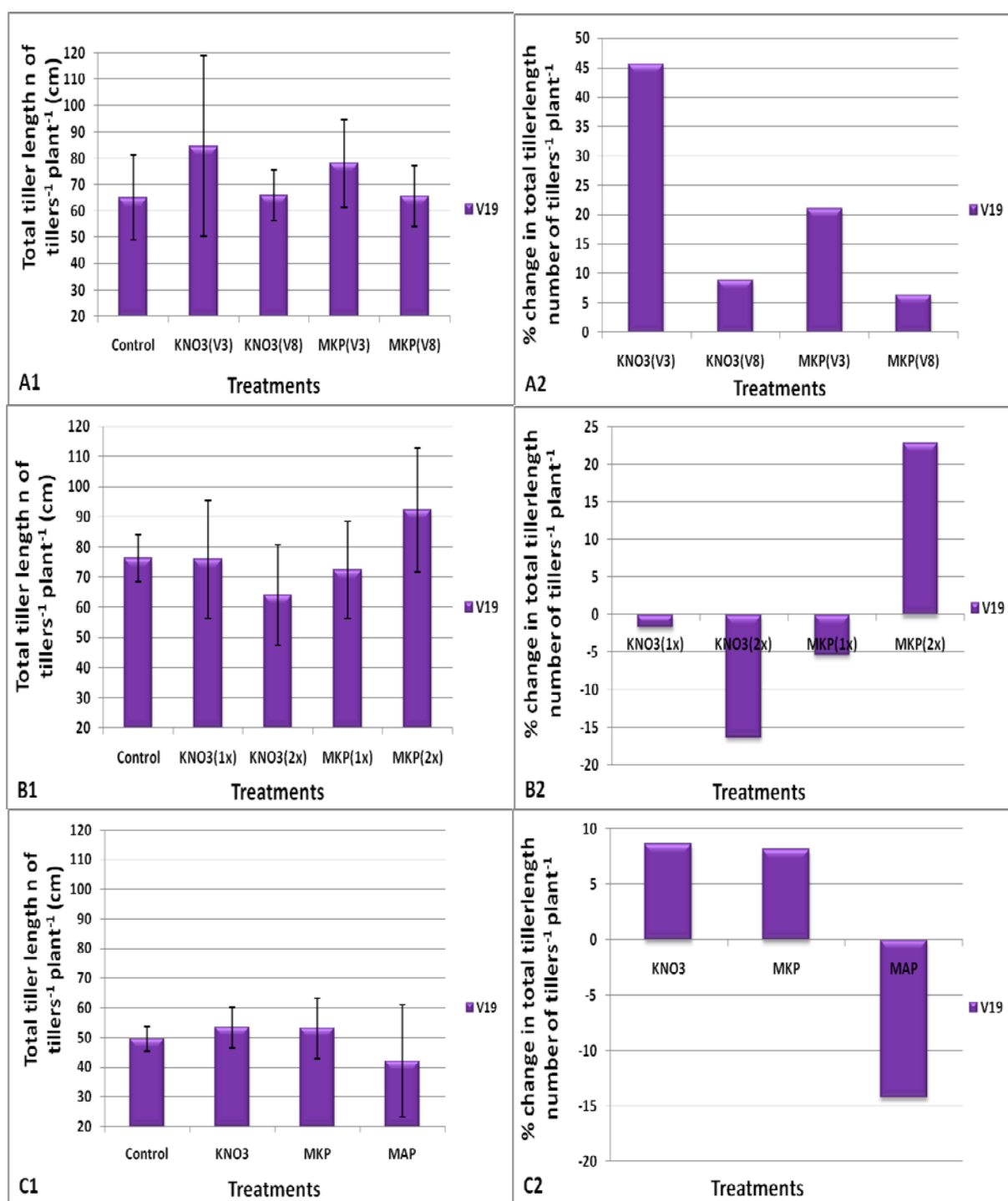
Figure 3.5: Total tiller length expressed as a function of the number (n) of tillers per plant at crop growth stage V19 during different growing seasons. A1 = 2004/05 season (sprayed at V3 and V8 in order to identify the optimal application time), B1 = 2005/06 season (sprayed only at V8, based on previous season results, in order to identify the optimal application concentrations) and C1 = 2006/07 season (sprayed only at V8, based on previous season results, in order to verify P and/or K involvement). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage; S = salt, GSxS = growth stage and salt interaction, C = concentration and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represent standard deviations between replicates from the mean for each treatment. A1 = V19: CV = 29.95%. B1 = V19: CV = 20.4%. C1 = V19: CV = 26.2%.

During the third season (Figure 3.5C1), both the KNO_3 (1x) and MKP (1x) treatments applied at V8 showed a negligible tendency towards enhancing the average tiller length as a function of tiller number per plant, when measured at V19. In contrast, applying MAP (1x) at the same growth stage tended to decrease this parameter by almost 15%, but this was not statistically different from the control at V19.

No significant interactions or main effects in terms of total tiller length as a function of the number (n) of tillers per plant could be detected at V19 in any of the seasons (Figure 3.5A2, B2 and C2).

3.3.1.4 Fresh mass (FM)

None of the treatments had any significant effect on aerial plant FM measured at any of the growth stages (V8, V13 and V19) when applied at either V3 or V8 growth stages during the 2004/05 season (Figure 3.6A1). Based on percentage differences between treatments from the control, it was found that the mass of plants treated with MKP was significantly lower than those treated with KNO_3 when measured at the V8 stage (Figure 3.6A2).

During the 2005/06 growing season (Figure 3.6B1) none of the treatments, either at single or double rates, had any significant effect on plant FM when measured at growth stage V13 and compared to the control. However, a significant interaction between the salt and the application concentration was observed, with the KNO_3 at single rate enhancing the aerial plant FM when applied at V8 and measured at V19, compared to all other treatments and the control (Figure 3.6B1/B2).

During the third growing season (2006/07; Figure 3.6C1) no significant differences in FM between treatments applied at V8 and the control were noted at growth stage V13. However when measured at V19, the MAP treatment significantly reduced plant FM by approximately 19%, compared to both the control and the MKP (1x) treatment (Figure 3.6C1). When data were expressed as a percentage change from the control, it was found that the MAP application significantly reduced plant FM at growth stage V19 compared with the MKP application (Figure 3.6C2).
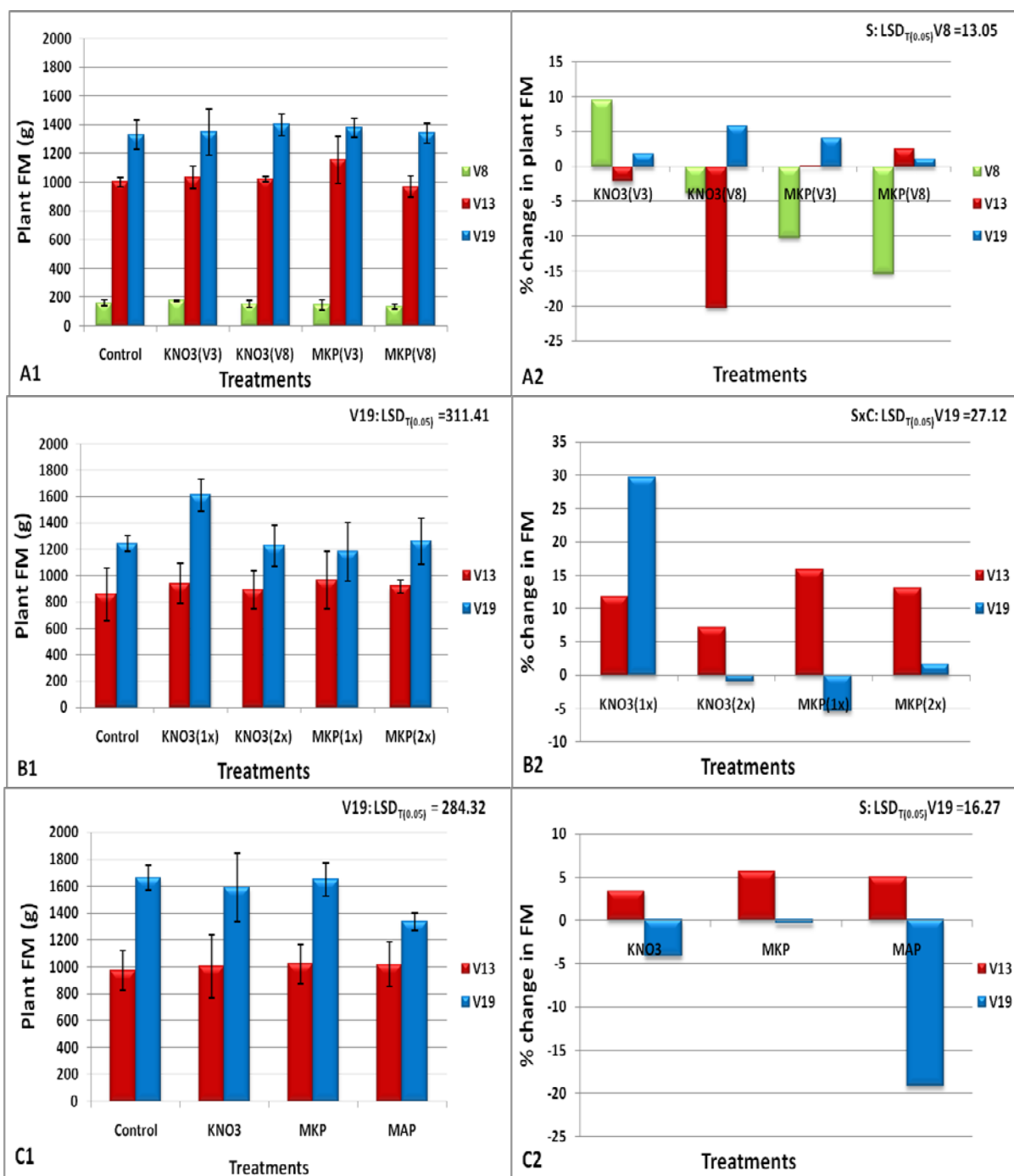
Figure 3.6: Aerial fresh mass (FM) of plants at crop growth stages V8, V13 and V19 during different growing seasons. A1 = 2004/05 season (sprayed at V3 and V8 in order to identify the optimal application time), B1 = 2005/06 season (sprayed only at V8, based on previous season results, in order to identify the optimal application concentrations) and C1 = 2006/07 season (sprayed only at V8, based on previous season results, in order to verify P and/or K involvement). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage; S = salt, GSxS = growth stage and salt interaction, C = concentration and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represent standard deviations between replicates from the mean for each treatment. A1 = V8: CV = 11.3%; V13: CV = 8.4%; V19: CV = 8.1%. B1 = V13: CV = 15.7%; V19: CV = 3.4%. C1 = V13: CV = 11.9%; V19: CV = 8.3%.

3.3.1.5 Dry Mass (DM)

Results from the first growing season (Figure 3.7A1) showed no significant differences in DM between treatments measured at any of the growth stages (V8, V13 or V19). However, when raw data was expressed as a percentage change from the control (Figure 3.7A2) it was found that the DM of plants treated with MKP was significantly lower than those treated with KNO_3 at the V8 growth stage. Conversely, the opposite effect was noted at V19, where the DM of plants treated with MKP increased significantly compared with that of plants treated with KNO_3, irrespective of when application took place (Figure 3.7A2).

During the second season (Figure 3.7B1) neither the single nor double concentration applications of the two salts at V8 contributed to any significant differences in plant DM when measured at either V13 or V19, compared to the control treatments. Expressing data as a percentage change from the control showed that the application of KNO_3 enhanced plant DM significantly at V19, while MKP reduced it, when applied at stage V8 (Figure 3.7B2).

The results in Figure 3.7C1 include an MAP foliar treatment applied at V8 and at a single concentration rate in order to verify P and/or K involvement. The MAP treatment applied at V8 (Figure 3.6C1) again tended to reduce the aerial FM at growth stage V19, albeit non-significantly when compared to the control (Figure 3.7C1), as was the case with FM. The single concentration MKP treatment applied at V8 again had no significant effect on plant DM as was observed during the first season (Figure 3.7A1). No significant differences could be detected when DM data were expressed as a percentage difference from the control treatments during the 2006/07 season (Figure 3.7C2).

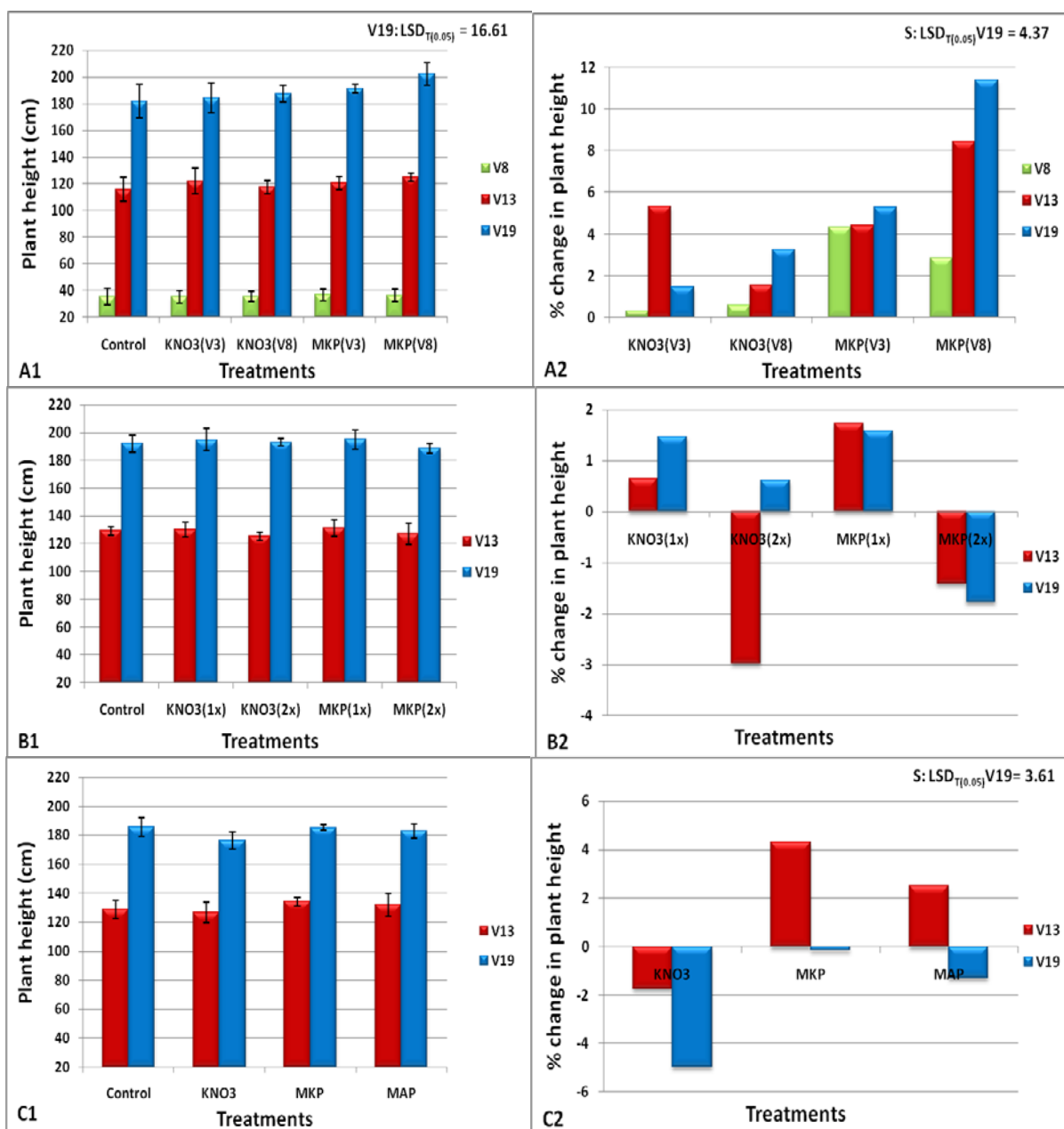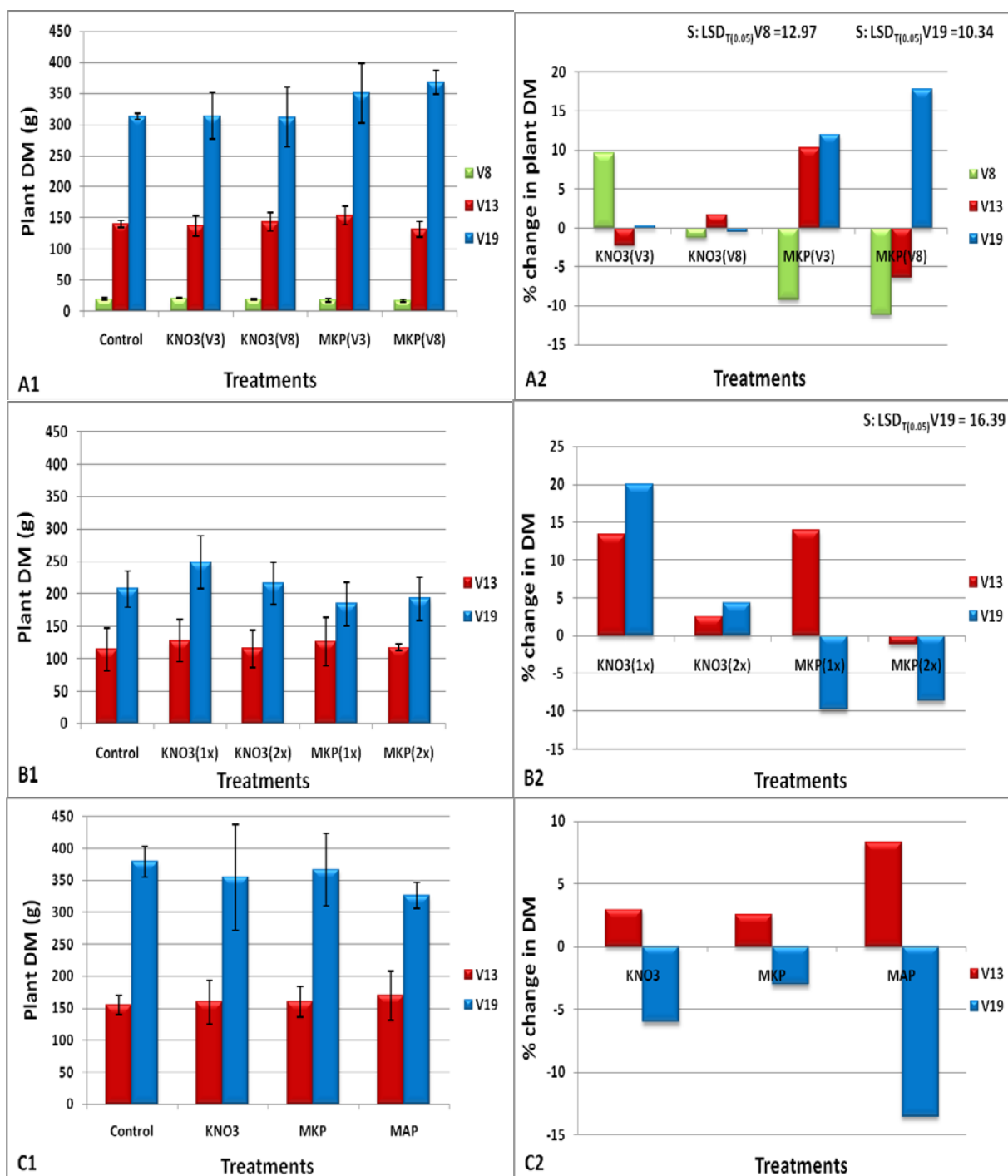**Figure 3.7:** Aerial dry mass (DM) of plants at crop growth stages V8, V13 and V19 during different growing seasons. A1 = 2004/05 season (sprayed at V3 and V8 in order to identify the optimal application time), B1 = 2005/06 season (sprayed only at V8, based on previous season results, in order to identify the optimal application concentrations) and C1 = 2006/07 season (sprayed only at V8, based on previous season results, in order to verify P and/or K involvement). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage; S = salt, GSxS = growth stage and salt interaction, C = concentration and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represent standard deviations between replicates from the mean for each treatment. A1 = V8: CV = 11.75%; V13: CV = 9.0%; V19: CV = 7.2%. B1 = V13: CV = 20.3%; V19: CV = 12.7%. C1 = V13: CV = 11.9%; V19: CV = 11.9%.

3.3.2   Yield parameters

3.3.2.1 Ear length and ear filling height

In this study ear length partially represents ear size while ear filling height represents the degree to which kernel filling towards the ear tip has been realized. Both parameters were used in order to ascertain their role in eventually interpreting the measurement of final kernel yield. Potassium nitrate, applied at growth stage V3 increased both total ear length (+1.03 cm; significantly) and ear filling height (+1.09 cm; non-significantly) of the first ear over that of the control at harvest (Figure 3.8A1/A2). This effect also prevailed in the second ear, although not as marked, but was not the case when KNO_3 was applied later at the eight leaf growth stage. On the other hand, MKP applied at V3 had no effect on either ear parameter in the first ear (Figure 3.8A1). This did, however, show a positive effect, although not significant, to increase both the ear length and ear filling height for the second ear. More or less the same effect to increase the total ear length as well as ear filling height for both first and second ears (+1.8 cm on average) over that of the control was observed for the MKP treatment applied at V8 during the 2004/05 growing season (Figure 3.8A1). By expressing data as a percentage change from the control, ear filling height of the second ear was significantly improved by the application of MKP irrespective of application growth stage compared with that of the KNO_3 application (Figure 3.8A2).

During the 2005/06 growing season none of the treatments affected either of the ear parameters for the first ear significantly (Figure 3.8B1). It was, however, interesting to note that for both KNO_3 and MKP applied at double rates, reductions (non-significant) in both total ear length and ear filling height of the first ear occurred when compared to the single rates of KNO_3 and MKP. The second ear exhibited a similar tendency, with the double concentration having very little effect on both parameters, compared to the single concentration treatment. The application of MKP at the single rate increased the ear filling height for the second ear significantly (+11.8%), compared to the control. Expressing the data as a percentage change from the control, verified the significant effect of MKP in increasing ear filling height for the second ear, whether applied at 1x or 2x rates, compared to the KNO_3 salt (Figure 3.8B2).
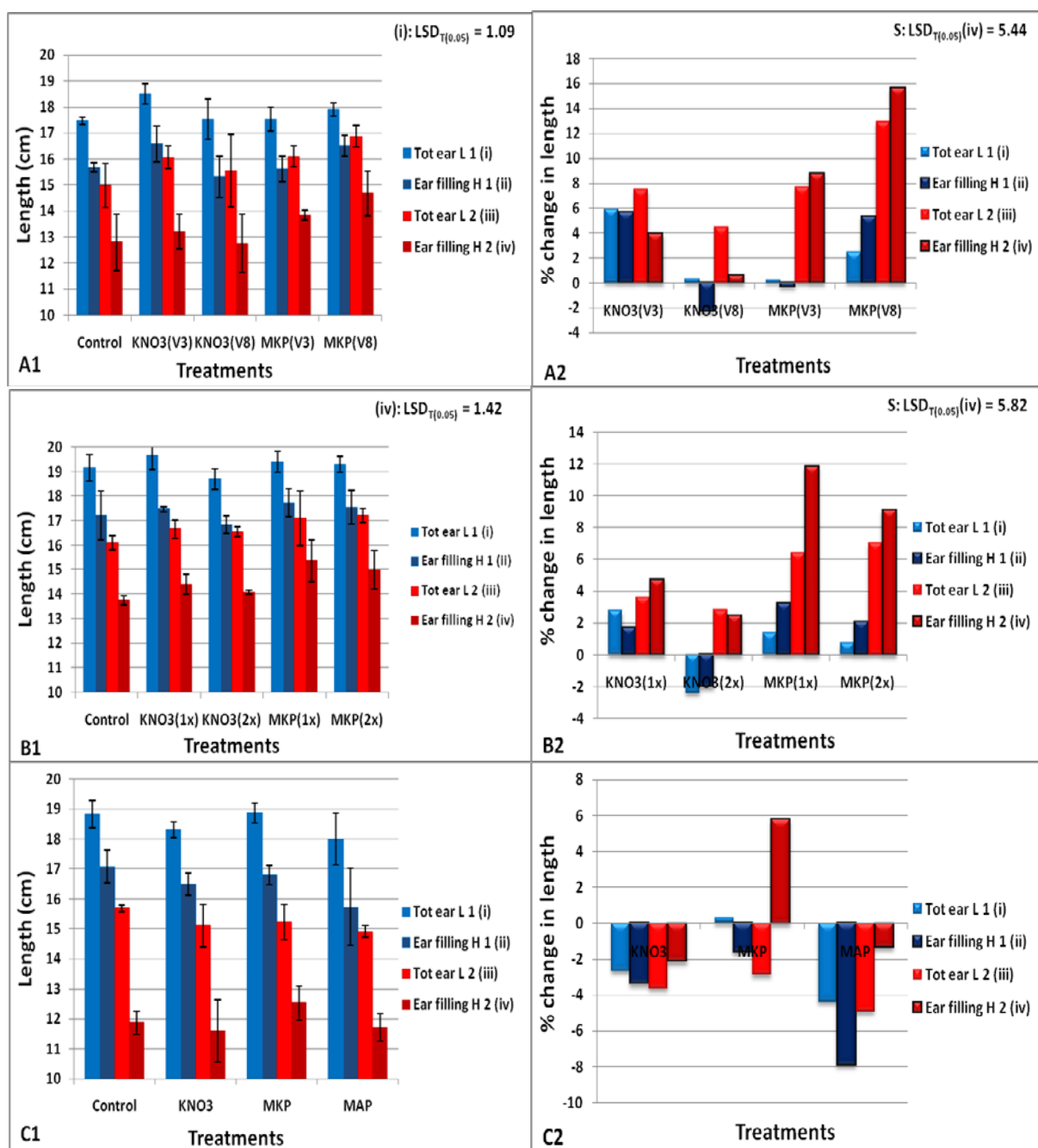
Figure 3.8: Total ear length (L) and ear filling height (H) for the first (oldest) and second (second oldest) ears at harvest during different growing seasons. A1 = 2004/05 season (sprayed at V3 and V8 in order to identify the optimal application time), B1 = 2005/06 season (sprayed only at V8, based on previous season results, in order to identify the optimal application concentrations) and C1 = 2006/07 season (sprayed only at V8, based on previous season results, in order to verify P and/or K involvement). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage; S = salt, GSxS = growth stage and salt interaction, C = concentration and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represent standard deviations between replicates from the mean for each treatment. (i) Total ear length for the main ear, (ii) ear filling height for the main ear, (iii) total ear length for the second oldest ear, (iv) ear filling height for the second oldest ear. A1 = (i) CV = 2.0%; (ii) CV = 3.1%; (iii) CV = 5.6%; (iv) CV = 6.5%. B1 = (i) CV = 2.2%; (ii) CV = 2.9%; (iii) CV = 3.1%; (iv) CV = 3.5%. C1 = (i) CV = 2.8%; (ii) CV = 4.4%; (iii) CV = 3.4%; (iv) CV = 5.4%.

No statistically significant differences between foliar treatments in terms of measured ear parameters were observed during the drier 2006/07 growing season (Figure 3.8C1) for either the first or second ear. However, MKP applied at growth stage V8 positively affected (non-significantly) ear filling height of the second ear while MAP consistently resulted in a non-significant reduction in ear length and filling height for both ears. In contrast to MKP, KNO_3 had either no effect or a slight reducing effect on ear length and ear kernel length for both ears (Figure 3.8C1). No significance in terms of total ear length and ear filling height, when data was expressed as a percentage change from the control, was observed during the third season (Figure 3.8C2).

3.3.2.2 Total ear mass and kernel mass

Total ear and kernel mass (Figure 3.9) were determined in addition to ear length and ear filling height. Results obtained during the first season showed that KNO_3 applied at the eight leaf stage as well as MKP applied at the three leaf stage tended to decrease both the total ear and kernel mass for the first ear, compared to the control (Figure 3.9A1). Expression of data as a percentage change from the control, revealed no significant interaction between the salt and application growth stage, with the KNO_3 (V8) treatment tending to reduce total ear mass and kernel mass for the first ear (Figure 3.9A2). No other treatment had any effect on the ear or kernel mass of the first ear. MKP application increased both the total ear mass and kernel mass of the second ear at harvest, compared to the control (Figure 3.9A1). Although no significant interaction between the salt and application growth stage for the MKP (V8) treatment was calculated in terms of total ear mass for the second ear, a significant main effect was noted for the MKP salt versus KNO_3. However, the kernel mass of this ear was significantly increased (approximately 20%) by MKP applied at the V8 stage compared with other treatments (Figure 3.9A2).

Data collected during the 2005/06 season, revealed that none of the treatments, either at single or double rates, had any significant effect on either total ear or kernel mass of the first ear (Figure 3.9B1). In terms of the second ear, all treatments showed a tendency to increase both total ear and kernel mass, compared to the control, but none were statistically significant.
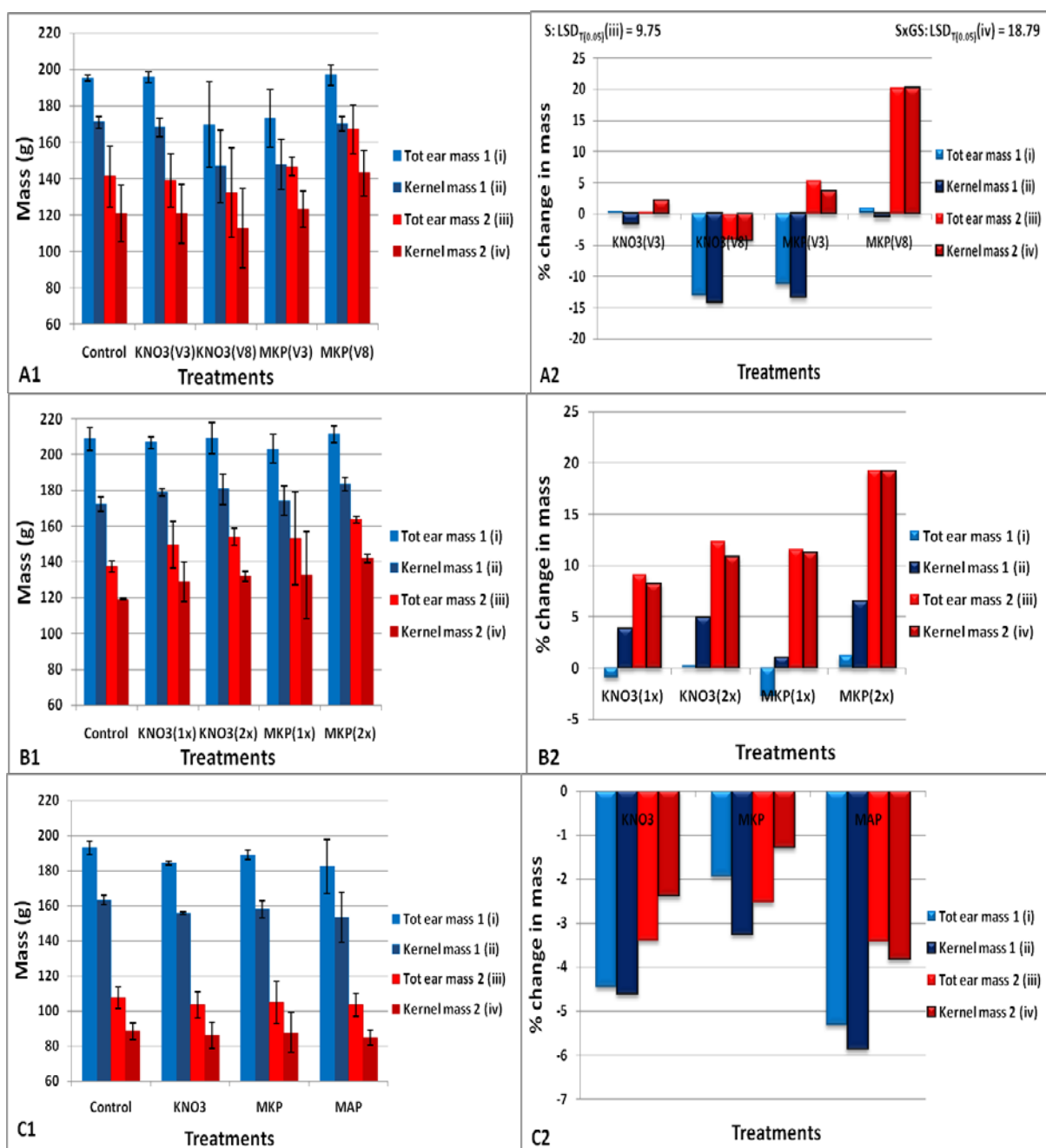
Figure 3.9: Total ear mass and kernel mass for the first (oldest) and second (second oldest) ear per plant at harvest during different growing seasons. A1 = 2004/05 season (sprayed at V3 and V8 in order to identify the optimal application time), B1 = 2005/06 season (sprayed only at V8, based on previous season results, in order to identify the optimal application concentrations) and C1 = 2006/07 season (sprayed only at V8, based on previous season results, in order to verify P and/or K involvement). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage; S = salt, GSxS = growth stage and salt interaction, C = concentration and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represent standard deviations between replicates from the mean for each treatment. i) Total ear mass for the main ear, ii) kernel mass for the main ear, iii) total ear mass for the second oldest ear, iv) kernel for the second oldest ear. A1 = (i) CV = 6.3%; (ii) CV = 6.8%; (iii) CV = 11.1%; (iv) CV = 12.2%. B1 = (i) CV = 3.4%; (ii) CV = 3.6%; (iii) CV = 8.8%; (iv) CV = 8.9%. C1 = (i) CV = 4.6%; (ii) CV = 5.1%; (iii) CV = 9.0%; (iv) CV = 9.8%.

The results in Figure 3.9C1 (2006/07 season) indicate that foliar treatments with KNO_3, MKP and MAP had a tendency to affect total ear and kernel mass negatively. In neither the second nor third seasons (Figure 3.9B2 and C2) could any significant interactions or main effects between salts, growth stages and/or concentrations be detected when data was expressed as a percentage change from the control.

3.3.2.3 Number of kernels per ear

The kernel number per ear for the first ear (Figure 3.10A1) was lower in plants treated with KNO_3 than that of plots treated with MKP at the eight leaf stage. No significant interaction between the salt and growth stage was obserbved with the KNO_3 (V8) treatment, tending to decrease the number of kernels in the first ear when expressed as a percentage change from the control (Figure 3.10A2). In terms of the second ear, the MKP (V8) treatment significantly increased the number of kernels compared to that of the KNO_3 (V8) treatment (Figure 3.10A1). Expressing the data as a percentage change from the control revealed a significant interaction between salt and growth stage. In this case the MKP (V8) treatment significantly increased the number of kernels compared to other treatments (Figure 3.10A2).

At harvest of the 2005/06 season, both KNO_3 and MKP applied at the single rate, as well as MKP applied at a double rate, increased the number of kernels produced in the first ear by 22.4, 2.6 and 15.1 kernels respectively, compared to the control (Figure 3.10B1). The number of kernels produced in the second ear was positively influenced by all treatments, i.e. KNO_3 (1x), KNO_3 (2x), MKP (1x) and MKP (2x), in that the increase over the control was 42.2, 33.1, 24.5 and 37.6 kernels, respectively. However, neither of these measured differences were statistically significant nor was any significant interactions or main effects observed (Figure 3.10B1, B2).

In terms of both the first and second ear, no significant differences in kernel number were observed between treatments during the 2006/07 season (Figure 3.10C1) nor were any significant interactions or main effects noted when expressed as a percentage change compared to the control (Figure 3.10C2).
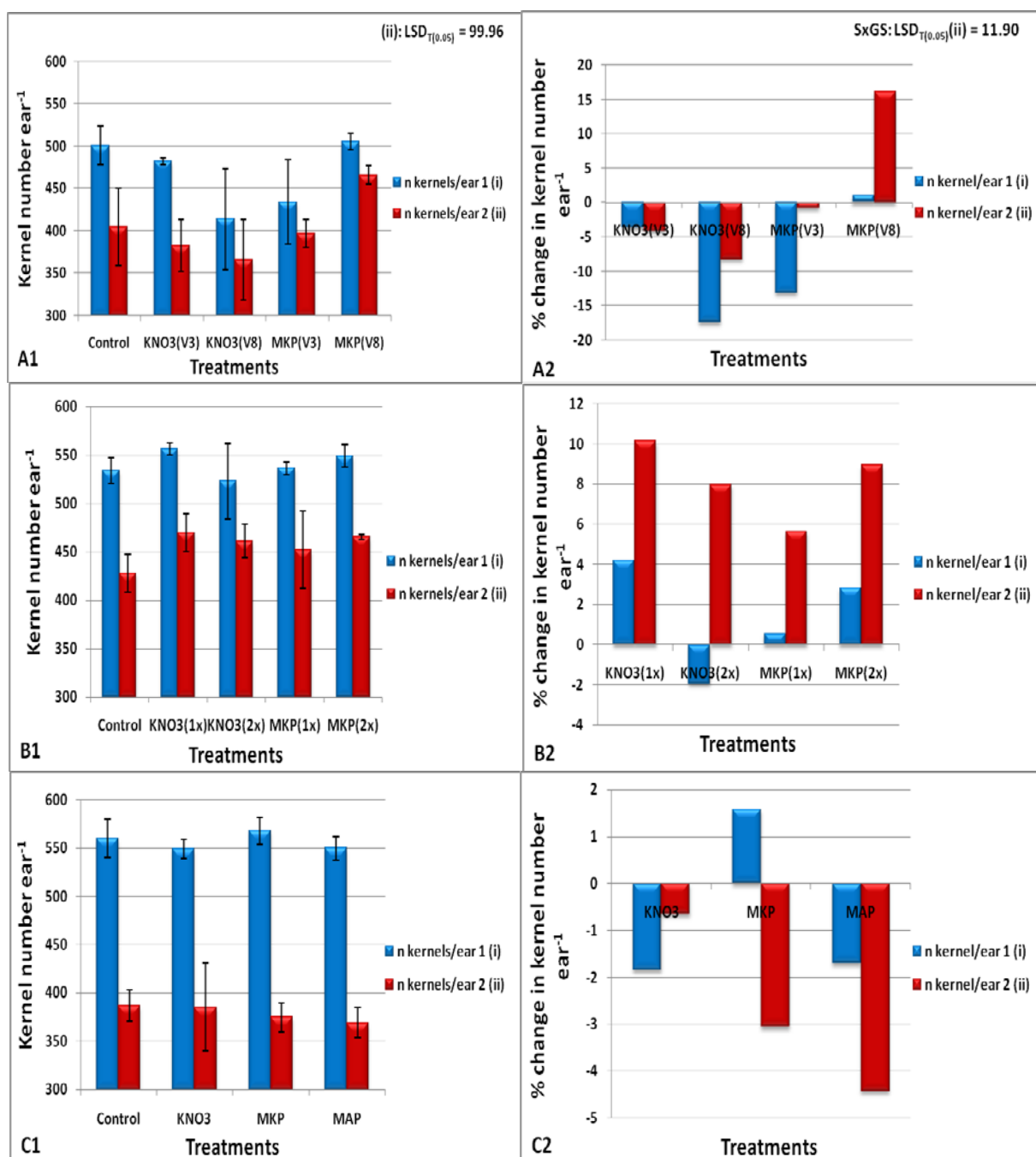
Figure 3.10: Number of kernels per ear for the first (oldest) and second (second oldest) ear per plant at harvest during different growing seasons. A1 = 2004/05 season (sprayed at V3 and V8 in order to identify the optimal application time), B1 = 2005/06 season (sprayed only at V8, based on previous season results, in order to identify the optimal application concentrations) and C1 = 2006/07 season (sprayed only at V8, based on previous season results, in order to verify P and/or K involvement). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage; S = salt, GSxS = growth stage and salt interaction, C = concentration and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represent standard deviations between replicates from the mean for each treatment. i) Number of kernels for the first (oldest) ear, ii) number of kernels for the second (second oldest) ear. A1 = (i) CV = 7.2%; (ii) CV = 8.8%. B1 = (i) CV = 3.7%; (ii) CV = 5.5%. C1 = (i) CV = 2.4%; (ii) CV = 7.2%.

3.3.2.4 Final grain yield

Results from the first season revealed that application of KNO_3 at the eight leaf stage reduced kernel yield while MKP applied at this stage showed an increased yield of 489.56 kg/ha compared to the control (Figure 2.11A1). Although, when a percentage difference from the control was calculated, neither KNO_3 nor MKP applied at V3 and V8 had any significant effect on grain yield (Figure 2.11B1).

All foliar treatments applied at V8 during the 2005/06 season, increased the final grain yield (Figure 3.11B1). This increase in kernel yield was the same for KNO_3, irrespective of the application rate (1x or 2x). MKP application at double rate slightly outperformed the single rate in terms of final grain yield, although this difference was not significant. Due to large standard deviations, none of the treatments contributed to statistically significant grain yield increases during the 2005/06 season, nor were any significant interactions between factors or main effects noted (Figure 3.11B1, B2).

The 2006/07 growing season was an extremely dry one and, although not statistically significant, all treatments showed a marked decrease in kernel yield by an average of 500 kg ha^{-1} (Figure 3.11C1) when compared to the control. No significant interactions or main effects were observed when data was expressed as a percentage difference from the control for the 2006/07 season (Figure 3.11C2).
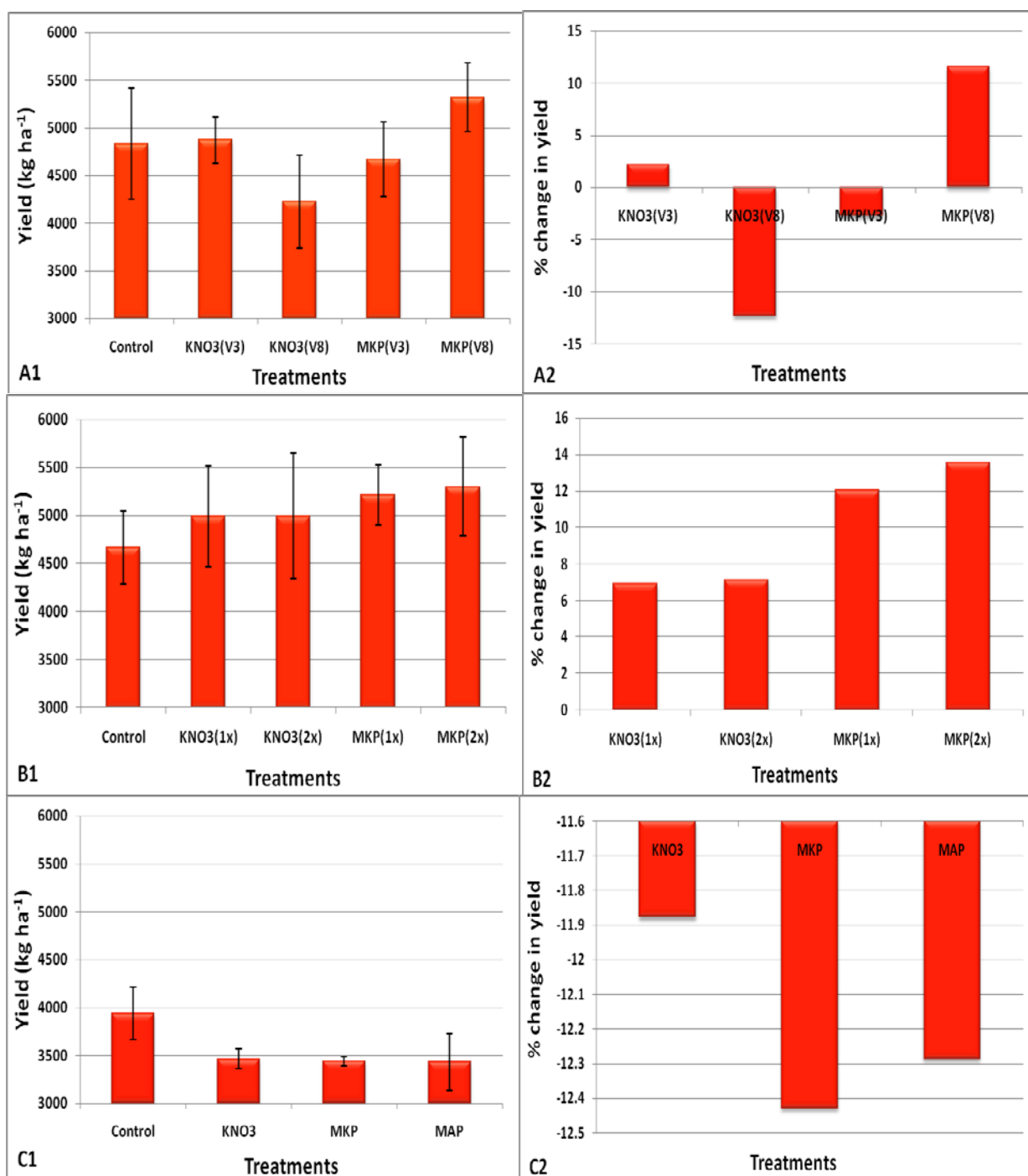
Figure 3.11: Kernel yield, expressed as kg ha^{-1}, at harvest during different growing seasons. A1 = 2004/05 season (sprayed at V3 and V8 in order to identify the optimal application time), B1 = 2005/06 season (sprayed only at V8, based on previous season results, in order to identify the optimal application concentrations) and C1 = 2006/07 season (sprayed only at V8, based on previous season results, in order to verify P and/or K involvement). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage; S = salt, GSxS = growth stage and salt interaction, C = concentration and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represent standard deviations between replicates from the mean for each treatment. A1: CV = 9.1%. B1: CV = 7%. C1: CV = 6.5%.

3.4   Discussion

Final yield remains the most important aspect for a maize farmer, particularly from an economic perspective. This does not gainsay the interest of the farmer in vegetative growth as the season progresses, for this remains a strong indication of what can be expected in terms of the ultimate yield at the end of a season. Secondly, it is generally accepted that all maize farmers following traditional production practices are believed to be interested in new ways and means to assist them in achieving their most important goal, namely sustainable production. Therefore, quantifying both vegetative growth and final yield by using a series of parameters is of great importance in order to interpret the relationship between the two main events, growth and yield, especially in case of new endeavours to improve either one or both events. In this light, the main objective was to compare the growth and yield response of maize to foliar applied N, P and K containing salts in this chapter. Treatments were applied foliar at different growth stages and at different concentrations. Standard agronomic practices were followed at planting using the cultivar, DKC 78-15B. Potassium nitrate (KNO_3), mono potassium phosphate (MKP) and mono ammonium phosphate (MAP) were used as treatments. From the onset, it was important to clarify three aspects namely a) optimal time of application (2004/05 season), b) optimal dosage (2005/06 season) and c) whether P or K contributed most towards the crop's response when applied foliar (2006/2007 season).

Literature confirms that plant height positively correlates with yield (Guatum *et al*., 1999). Results obtained with only foliar applied MKP at single dosage (4% solution) at V8 showed a significant increase (+11.1%) in plant height, when measured at V19 during season one, compared to the untreated control. The fact that MKP significantly increased plant height compared to KNO_3, whether applied at V3 or V8, corresponds for example with findings of Chapagain and Wiesman (2004) who indicated that Nutri-Vant-PeaK (95% MKP and 5% adjuvant) administered as a foliar spray at 1% (w/v) concentration, increased plant height of greenhouse tomatoes markedly. Although not significant, the same tendency was observed for the MKP treatment when measured at V13 in this experiment. The same tendency prevailed for the single rate KNO_3 treatment applied at V8, although this was not as marked. KNO_3 applied during growth stage V8 during the 2006/07 season at a 3% solution, however, significantly reduced plant height from that of the control. This tendency was also noted when a 6% KNO_3 solution was applied at stage V8 and measured at V13. A possible reason for the reduction of plant height by KNO_3 might be explained by its salt index (74), which might have contributed to dehydration of growing tissue at the higher application rate (6% solution)

as was experienced during the rather dry third growing season. Based on the grain yield data obtained during the 2004/05 season, it was decided that the optimal application growth stage for foliar treatments on maize will be at V8.

During the 2005/06 season, the same salts as were used in season one, were applied at both single (1x) and double (2x) rates during V8 in order to identify the optimal application rate, while plant measurements were taken at V13 and V19. Neither application rate had any effect, confirming that no differences between salt concentrations were apparent. In order to verify P or K involvement, MAP was included in the third season while the single application concentration and V8 treatment time was standardized. However, no differences between treatments were observed, complicating any interpretation by using only plant height as a standard. No clear pattern could be deduced as differences in plant height were insignificant. However, MKP foliar applied at a 4% concentration at V8, while the response was measured at V13, had a consistent tendency to increase plant height over consecutive seasons.

For all other parameters used to quantify the crop's response, the same treatments as outlined above were followed in each growing season. Although the main aim of this chapter was to quantify the growth and yield response of maize to foliar treatment with the different inorganic salts, the anticipated objective was also to gain information on the possible relationship between growth and yield parameters.

Tiller development of a maize plant is a function of its genetic potential, coupled with environmental factors, such as soil fertility and moisture availability, as well as plant population density. Extensive tillering in commercial maize hybrids is mostly undesirable (Duncan, 1975). Surprisingly, very little information on tillering in recent maize hybrids is available in modern literature. However, Kapanigowda *et al.* (2010) carried out experiments on dry land maize using two planting methods namely clump vs. equidistant spacing, finding that clump spaced plants produced fewer tillers than those at equidistant spacing. It was also observed that tillers accounted for 10% of the stover biomass in equidistant spaced plants, but contributed less than 3% to grain yield. Clump spaced plants, which produced fewer tillers, generated significantly higher grain yields compared to equidistant spaced plants under these conditions. Interestingly, in terms of water use efficiency (WUE), clump spaced plants had a lower evapo-transpiration (ET) threshold in favour of grain initiation at a later stage (Kapanigowda *et al.,* 2010).

Extensive research results obtained by the ARC-GCI (2008) with 50 trials conducted over three seasons, using the cultivar DKC 78-15B, revealed that this particular cultivar produced on average 23.22 tillers per 100 plants. In this study, both KNO_3 and MKP sprayed at the three leaf stage had a marked effect on the number of tillers formed by both the V13 and V19 growth stages, compared to the later application of both salts at V8. The number of tillers was more than three times that reported by the ARC-GCI (2008), and more or less in line with the findings of Kapanigowda *et al.* (2010). The difference in the number of tillers observed between the ARC-GCI (2008) and Kapanigowda *et al.* (2010) might possibly be influenced by differences in factors such as row spacing, plant density, fertility and climate.

Tillers are initiated at an early vegetative crop stage, indicating that the application of foliar nutrients at the early growth stage (V3) could have contributed towards increased fertility, which in turn stimulated tillering, confirming the findings of Duncan (1975). The application of double (2x) rates of both salts during the 2005/06 growing season at the eight leaf stage slightly stimulated tiller formation, compared to the single (1x) rates while the tiller number remained constant between V13 and V19, as was the case during the first season. Generally MKP applied at V8 showed the most consistent potential to increase tiller number during the second and even the third drier season, while plants that received MAP tended to abort tillers between V13 and V19. However, during the dry 2006/07 season, compared to the first two seasons (Table 3.4), maize plants reduced the number of tillers between the V13 and V19 growth stages. Samarah (2005) reported a similar reduction in tiller number for barley under drought stress conditions.

Under rain fed conditions maize production can be limited by sparse and erratic precipitation that might result in severe water stress, particularly during grain filling. When plant populations are reduced to conserve soil water for use during grain filling, tillers often form during the vegetative growth phase and negate the expected economic benefit in terms of grain yield (Kapanigowda *et al.*, 2010). All treatments applied in 2006/07 stimulated tiller formation during the vegetative phase and therefore also water use. However, rainfall was significantly lower in this season, so soil water was probably more depleted in the foliar treated plots and could therefore have caused reproductive parameters measured in this study to be negatively affected. It is well documented that crops in their reproductive phase is highly sensitive to drought injury (Saini and Westgate, 2000; Mahajan and Tuteja, 2005).

Whether the growth or development of tillers contributes positively towards final yield by increasing photosynthetic capacity or negatively by decreasing yield through greater evapo-transpiration under rain fed conditions, and whether a harvestable ear is produced on tillers or not, is still controversial ([1]personal communication; A.J. Steyn, 2010). Earlier applications (V3) of both KNO_3 and MKP had the most pronounced effect on the total tiller length expressed per number of tillers, compared to the later application (V8), as was the case with the number of tillers. The relationship between the number of tillers produced per plant on crop yield in maize cv. DKC 78-15B, was evaluated by means of correlations. For the 2004/05 growing season, a non significant correlation between the number of tillers produced per plant and yield was obtained ($R^2 = 0.216$, data not shown). However, where MKP was applied at the double rate, it resulted in a 23% increase in average tiller length at V19 compared to those plants that received either no foliar treatments or the single rate application. The double rate N salt (KNO_3) tended to inhibit tiller growth which was not expected, as N usually increases vegetative growth (FSSA, 2003).

It is well documented that N occurs in plants as a component of organic compounds such as amino acids, proteins, enzymes, adenosine triphosphate (ATP), dioxiribose nucleic acid (DNA), ribonucleic acid and phospholipids as well as structures such as membranes and cell walls (FSSA, 2003). From this it is clear that N not only plays a role in plant growth, but also in processes such as photosynthesis, respiration, reproduction and the maintenance of genetic identity. N deficiencies were noted in the oldest leaves of some plants at the point where ears formed, which indicated a possibility of severe plant N shortages (Figure 3.12) correlating with the description supplied by the FSSA (2003). Although this occurred during all seasons, a photograph from the 2005/06 season was used to illustrate this phenomenon (Figure 3.12) During the 2006/07 season, leaf samples taken at the time when ears formed, just after flowering, confirmed N shortages (data not shown). To a large extent, KNO_3 applied during all three seasons had no effect on the total tiller length expressed as a function of the number of tillers. This was possibly due to the transportation and incorporation of N (plant mobile) into younger leaves higher up on the main stem, in order to be used in bio-chemical process rather than growth. The fact that a high percentage of K^+ (38% K) is present in KNO_3 and K^+ is known to enhance the movement of N in plants (FSSA, 2003), might further assist in explaining these results.

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Figure 3.12: Photograph of an older maize leaf during the grain filling stage of the 2005/06 season showing a typical inverted V pattern along the midrib, a clear indicator of N deficiency.

P is well known to induce root growth (FSSA, 2003) when applied to the soil at planting and, in its inorganic form (Pi) plays an extensive role in energy distribution (Lea and Leegood, 1999) via its function in the conversion of adenylate species (ATP, ADP and AMP). It is postulated that P supplied directly to the leaves could be related to higher availability of ATP that might have resulted in increased tiller growth as proposed by Panigrahy *et al.* (2009). However, the fact that the MAP foliar treatment reduced tiller growth rather substantially (-15%) contradicts this (Figure 3.5C1). The possibility exists that MAP containing nitrogen in the ammonium form together with phosphate, might point towards antagonism either during the uptake through leaves or metabolic action when arriving in leaf cells simultaneously. Alternatively, but not exclusively, a number of factors could have contributed to the results obtained by the application of MAP. These include i) the dry spell experienced during the 2006/07 season, ii) subsequent fast evaporation of the spray droplets from the leaf surface causing an osmotic gradient and iii) the rather high application rate used. According to Wenkem (2010), a concentration of not higher than 0.5% (w/v) should be applied to young leaves and a concentration not higher than 1% (w/v) to older leaves. In this study the reduction in the number of tillers between V13 and V19 was largest for the MAP treatment. Plants treated with MAP

showed a reduction in biomass accumulation later during vegetative growth, as expressed in terms of FM and DM accumulation at V13 and V19. This tendency was specifically observed for MAP, as opposed to MKP (also containing P) and could possibly be due to the influence (signalling role) of N on reproduction and maintenance of genetic identity (FSSA, 2003). Maize plants growing in a medium with low P show a decrease of up to 40% in the FM of aerial parts within a period of 24 days after planting (Usuda and Shimogawara, 1991).

Stamp and Geisler (1980) reported that the lack of K impeded shoot growth in C_4 plant types. In this study KNO_3 applied at V8 increased plant FM at V19 during both the first and second seasons as opposed to MKP. However, FM has been criticized as an expression of plant growth in the past due to the fact that water might screen real tissue growth (Liu *et al.*, 2010). As a result DM accumulation has been accepted as a better indicator of tissue expansion and, therefore, an indicator of average metabolic activities in the plant. For this reason DM accumulation was quantified. Compared to FM that remained unchanged, a slightly different picture emerged during the first season for MKP when looking at the DM. The MKP treatment, in contrast to the KNO_3 treatment, significantly reduced plant DM at V8, irrespective of application stage. However, at V19 the opposite effect was observed, where MKP contributed to a significant increase in biomass. In fact, MKP applied at either the V3 or V8 growth stage increased the DM significantly by 12.2% and 17.8% respectively, over that of the control.

During the 2004/05 season foliar application of KNO_3 had no effect on DM accumulation. However, during the 2005/06 season the KNO_3 application contributed towards increasing aerial part DM by 20%, when measured at V19, while the effect of MKP observed in the first season was not repeated. The observed responses could also be due to seasonal responses. Quantitatively, the MKP treatment contributed to a significant reduction in plant DM at V19, compared to the KNO_3 treatment. This inconsistency makes it difficult to reach a conclusion, particularly as neither of these salts had any significant effect on DM during the 2006/07 season. Furthermore, both KNO_3 and MKP applied at double rates either had no effect or reduced plant DM. This is difficult to explain but might be associated with an osmotic effect due to the high concentration, as this was much higher than the standard rate for foliar applied salts.

With regards to the latter postulate that involves concentration and a possible osmotic effect, information in the literature was limited to reports of MKP treatment by Reuveni *et al.* (1998) on green peppers and Arquero *et al.* (2002) on olive trees. The work of Arquero *et al.* (2002), in

particular, is notable, as they tested MKP concentrations (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%) in foliar application on olive trees. They found the 2 to 4% MKP solutions to be most effective in increasing leaf P content. Although no visible leaf damage was observed in maize, the osmotic effect might have culminated in tissue damage, particularly that of the stomatal complex, an aspect that will be dealt with in a later chapter due to its involvement in nutrient uptake applied foliar (Eichert and Burkhardt, 2001). At this stage, it must be stated that foliar applied N, P and K did not consistently contribute to significant changes in DM accumulation. The application of MAP did also not allow for distinguishing between P and K.

Despite inconsistencies and controversial observations made in the vegetative response of maize to foliar applications with N-, P- and K-containing salts, the ultimate yield is the culmination of all growth and physiological events that take place throughout the season and must be regarded as the final indicator of whether foliar applied nutrients have a positive or negative influence on the crop. In this regard a number of yield parameters were quantified. Rafique *et al.* (2004) reported a positive correlation between ear length, the extent to which kernels are filled over the length of the ear and final grain yield. They reported that ear length determines the potential for grain filling and, subsequently, grain yield. In this study, both the first and second ear was measured to ascertain whether any of the treatments contributed to ear length and filling of kernels towards the ear tip.

The application of KNO_3 at V3 proved to be more beneficial in terms of ear length and kernel filling in both ears, compared to application at V8, except during the drier 2006/07 season. In contrast, MKP applied at V8 markedly increased ear length and improved kernel filling over three consecutive seasons in both the first, but especially the second ear. Interestingly, application of both salts at double rates negatively influenced ear length and kernel filling height in both ears, compared to the single rates. The application of MAP during the 2006/07 season did not supply sufficient evidence to reach a conclusion regarding N and P involvement in determining ear length and kernel filling. Nevertheless, from the results obtained it is speculatively postulated that the K and P combination, as in MKP, rather than the N and P combination, as in MAP, contributed positively towards these two yield parameters, possibly due to a synergistic effect. This is supported by the more consistent results obtained when MKP is applied at V8. The increase in total ear mass obtained with this treatment during the first season while the increase in kernel mass was significant during both of the first two seasons, particularly in the case of the second ear. Further support of the postulate that the possible combined synergistic effect of K and P might be part of the explanation

for MKP's more consistent positive influence on the mentioned two yield parameters, is the tendency of KNO_3 to significantly reduce total ear and kernel mass, in particularly the first ear. Interestingly, both KNO_3 and MKP applied at the double rate tended to further enhance ear and kernel mass of both ears, over that obtained at the single rate, while having a negative effect on vegetative growth as well as ear length and kernel filling.

The number of kernels per ear is genetically controlled, but it may be influenced by environmental and nutritional factors, and is directly related to grain yield (Tahir *et al*., 2008). KNO_3 applied at V8 reduced the number of kernels during the 2004/05 season for the first ear. However, during the second season KNO_3 applied at V8 and at the single rate had the opposite effect. MKP, on the other hand, contributed to a significant increase (16%) in kernel number, particularly for the second ear during both the 2004/05 and 2005/06 seasons. Importantly, these tendencies for the two salts were confirmed when applied at the double rate during the 2005/06 season.

Foliar application of a 4% MKP (1x) solution at growth stage V8 was the only treatment to consistently improve yields during the first two seasons (11 and 12% respectively). This result was not repeated during the very dry third season probably due to the sensitivity of reproductive organs to drought (Mahajan and Tuteja, 2005). The 8% MKP solution resulted in a further yield improvement of almost 2%, although this was not statistically significant. Conflicting results were obtained with foliar application of KNO_3 at V8 as it resulted in a substantial decrease in yield during season one (12%), while not affecting yield significantly during season two.

MAP was added as a second P-containing salt together with MKP and KNO_3 in order to verify K and P involvement in the vegetative growth and yield response of maize towards foliar applications of these salts under rain fed conditions. However, MAP did not affect vegetative growth or yield parameters in the same way as MKP. In fact, the response of maize plants to foliar applications of MAP was generally opposite to treatment with MKP. It is, therefore, difficult to conclude whether the K-moiety or the P-moiety or synergism between the two elements contained in MKP was responsible for the observed reaction of maize to treatment with this salt. The response to foliar treatments with KNO_3 did not assist in solving this problem due to the inconsistent results obtained. It was therefore decided to analyze selected physiological parameters in an attempt to shed light on the possible metabolic role of P and K after being applied to the leaves (chapter 4).

In summary, despite the rather inconsistent results obtained during the third season, MKP applied foliar during the eight leaf stage and at a single (1x) dosage rate most consistently contributed towards improvement of most of the vegetative and yield parameters measured during the first two seasons compared to the control and the two other fertilizer salts. Before reaching final judgment on the less convincing influence of KNO_3 and MAP, the physiological response of maize to treatment with the three fertilizer salts was followed in chapter 4.

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CHAPTER 4

Physiological response of *Zea mays* L. to foliar treatment with inorganic nutrients

Abstract

Field studies were conducted over three seasons, 2004/05, 2005/06 and 2006/07, in order to assess the physiological response of *Zea Mays* L., cv. DKC 78-15B to foliar applied mono potassium phosphate (MKP), potassium nitrate (KNO_3) and technical mono ammonium phosphate (MAP) under rain fed conditions. During the first season 4% (w/v) MKP and 3% KNO_3 solutions were applied at growth stages V3 and V8 and the physiological response of maize followed. Physiological parameters included ear leaf chlorophyll content and chlorophyll *a* fluorescence as well as sugar and starch content in kernels at different grain filling stages. Treatment at V3 with both salts had a reducing effect on leaf chlorophyll content while treatment at V8 had no effect. However, treatment with MKP at V8 contributed to an enhanced probability for an absorbed photon to move an electron into the electron transport chain (φ_E) that lead to improvement of the overall performance output (PI_{abs}) of photosystem II. Concomitantly, the latter treatment resulted in the detection of higher sugar and starch levels in kernels, corresponding with an increase in yield during the first season and confirming its superiority over treatment at V3. During the second season both MKP and KNO_3 were additionally applied at double rates namely 8% and 6%, respectively, in order to identify the best concentration to be used. The higher application rates for both salts did not differ significantly from the lower dosage rates in affecting measured physiological factors, leading to the decision of applying only the lower dosage rate. During the third season MAP technical was added and applied at 3.4% in order to verify the involvement of either P or K or both in influencing the physiological response measured in maize. MAP had no significant effect eliminating the purpose of its use. Additionally, the effect of different foliar applied MKP concentrations (2%, 4% and 8%) on stomatum structure was qualitatively followed under a light microscope while P and K uptake by leaves was quantified. The 8% MKP solution caused a severe disruption in stomatum structure while significantly more P was detected in leaves treated with the 2% solution, 24 h after application.

Keywords: maize, foliar fertilizer, MKP, KNO_3, MAP, chlorophyll *a* fluorometry, chlorophyll *a*, *b* and *c* content, sugar and starch content, stomata complex

4.1   Introduction

Improving cereal starch production has become an important objective for scientists and farmers alike. The main reason being that starch comprises 55 to 75% of daily human food intake, is the main source of food for domestic animals and contributes towards final yield (Pan, 2000). Maize (*Zea mays* L.), with its high kernel starch content, is widely cultivated and is a major component of human diets in many developing countries. Maize is, further, considered as one of the crops with the highest potential for bio-fuel production where high starch content is required (McLaren, 2005). From this it becomes apparent that the manipulation of metabolic events involved in starch production must be considered an important research activity.

Ling and Silberbush (2002) claimed that foliar application of fertilizer to crops is an agricultural practice that has been used for many years. They further explained the application of nitrogen (N), phosphorous (P) and potassium (K) containing fertilizers to have a positive effect on not only yield, but also quality aspects in certain crops. However, limited references linking additional application of N, P and K through leaves to kernel quality in maize were found in the literature. The latter encouraged monitoring the physiological events leading to starch production following foliar treatment of maize with N, P and K containing salts using photosynthesis as well as sugar and starch production parameters as indicators. Chlorophyll (Chl) *a* fluorescence and Chl content were used to quantify photosynthesis activity in this study.

One of the few reports found in literature was that of Ankorion (1995) who claimed that late foliar application of MKP to crops, whether fruit or seed bearing, enhanced sugar as well as starch accumulation in the harvestable products. According to the author, MKP is ideal for applying K and P to crops at higher than normal rates due to a rather low salt index. This is in concert with a technical report by Haifa Chemicals Ltd (2011) stating that a 5% (w/v) MKP solution has a pH of 4.4 and electrical conductivity (EC) of 33.5 mS cm^{-1} ensuring safe use on plants even at a high rate. However, contradicting results were published by Sawyer and Barker (1999) who reported that the foliar application of MKP (with surfactant) to maize at various growth stages, namely V6-V8, V12-V14 and 50% VT, at a solution of 10% (w/v) or a rate of 5 lb acre^{-1} (approx. 5.6 kg ha^{-1}) dissolved in 20 gallons (approx. 91 L) of water slightly reduced yields by 2.8% on average and based on two trial localities in one season. Although the authors noted no significant differences in terms of either ear leaf Chl readings measured at growth stage R4 or kernel starch content, they concluded that it would

be important to discern possible reasons for the results they obtained since no visible leaf damage was observed as well as to verify whether similar results would be repeated across multiple growing seasons.

In terms of the other salt used in this study, KNO_3 applied foliar at a 2.5% solution (w/v) between 50% tasseling and 10 days after tasseling on sweet corn maize in a pot trial, increased the reducing sugar as well as sucrose contents during early grain filling stages (Suwanarit and Sestapukdee, 1989). The authors also found that the 2.5% KNO_3 treatment affected maize by stimulating Chl synthesis, but not by increasing leaf area.

It is generally accepted that numerous factors, including nutrient availability, influence crop yields, while high productivity is primarily associated with the ability of plants to produce large amounts of photosynthate (Ding *et al.*, 2004). Photosynthesis, but more specifically increased dry matter accumulation through the enhancement of the process, remains one of the most important aspects linked to grain yield (Hu *et al*., 1998). Chl *a* fluorescence was used by Veberic *et al*. (2007) to further investigate the effects of P and K foliar fertilization on photosynthesis in apple trees. Measurements of Chl fluorescence by the authors were performed at short intervals after foliar treatment in order to ascertain whether the applied P and K caused any form of stress on the plants. No significant effects on photosynthesis (fluorescence) were detected and, based on this parameter, it was assumed that the plants were not exposed to any form of stress and that photosystem II (PSII) exhibited good photochemical efficiency (Veberic *et al*., 2007).

Mechanical damage to adaxial leaf cells or, more specifically, stomatal damage that can influence plant development, is an aspect that must be taken into account when foliar fertilizer applications are considered. Gamble and Emino (1987) treated maize plants foliar with a 120 g N L^{-1} urea solution and discovered that a 4 µL drop applied to the adaxial leaf surface caused darkening and wrinkling in the epidermal cells as well as sunken stomata within two hours after application. They further observed that the epidermis was desiccated, sunken and discoloured, forming lesions on the leaf surface, after eight hours. They concluded that events associated with visual damage appear to be related to water loss since epidermal and mesophyll cells become desiccated.

In view of the potential benefits attributed to foliar fertilizer application to crops, keeping in mind that relatively little research has been done in this regard on maize, the main objective of this study was to quantify the possible influence of foliar applied macro nutrient inorganic fertilizers on selected physiological events in the maize plant. The selected metabolic events, of which all are associated with starch formation and ultimately yield, included photosynthesis capacity via Chl content and Chl *a* fluorescence measurements as well as sugar and starch content in kernels during early grain filling stages as well as at harvest. As was the case in chapter 3, different N, P and K containing salts were applied at different growth stages (first season) and concentrations (second season) while MAP was used during the third season in an attempt to distinguish between K and P involvement in evoking physiological responses to the foliar treatments. Results were expressed by plotting both raw data and the calculated percentage difference from controls while statistical analyses were performed on both data sets. During the fourth season a commercial trial was conducted to follow the yield response to a 4% MKP foliar treatment only when applied at V8 and at the single (4%) rate. Additionally, the P and K content in leaves were quantified 24 h after application under glasshouse conditions while the effect of different MKP concentrations on stomatal structure was qualitatively viewed under a light microscope.

4.2   Materials and Methods
4.2.1   Materials

A test combination kit (UV method Cat. Nr. 10 716 260 035), purchased from Boehringer Mannheim (Germany), was used to determine sucrose, D-glucose and D-fructose content in kernels during different development stages. A test combination kit (Cat. Nr. K-TSA 01/05), purchased from Megazyme International Ltd (Ireland), was used to determine the starch content in kernels at physiological maturity (AOAC method 996.11). All other chemicals were of the purest quality available and purchased from either Merck (Germany) or Sigma (Germany). MKP (LOT 2237), manufactured by Rotem Amfert Negev Ltd (Israel), a NovaPeak Division as well as KNO_3 (Omni K), Reg No. K6887 and MAP (MAP39), Reg No. K6892, was purchased from Omnia Fertilizer, a Division of Omnia Group (Pty) Ltd, Republic of South Africa. Lactofenol (Product Nr. 61335), a blue dye used for microscopically studying the stomatal complex, was purchased from Sigma Aldrich.

4.2.2   Methods

4.2.2.1 Experimental layout and treatments

Experimental layout and treatments have been explained in chapter 3 (see 3.2.2.1). In all laboratory analyses treatments were replicated three times. Methodology of the commercial trial conducted in the fourth season is supplied in 4.2.2.6.

4.2.2.2 Chlorophyll *a* fluorescence

Chl *a* fluorescence spectroscopy, a rapid and non-invasive screening test, was used to detect differences in the leaf photosynthetic rate of the maize plants. *In vivo* and *in situ* determinations were made using a Portable Hansatech Plant Efficiency Analyser (Type: PEA MK2) at the flowering stage of the maize plants. The fluorescence intensity was measured at 0.05, 0.1, 0.3, 2 and 30 ms. Illumination was provided by an array of six high intensity light emitting diodes (LEDs), providing red light at a peak wavelength of 650 nm. The maximum photosynthetic photon flux density was 3000 µmol m^{-2} s^{-1}. Initial fluorescence (F_0) was recorded on leaves adapted to a dark period of five minutes by means of three leaf clips (white plastic with integral shutter and foam pad; 4 mm diameter illuminated area) attached to the middle of the leaf (between the leaf margin and middle lamella) for the leaf below the uppermost (oldest) ear as illustrated in Figure 4.1.
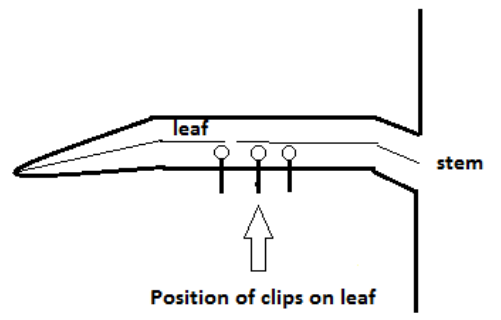
Figure 4.1: Position of clips on leaf for measurement of chlorophyll *a* fluorescence on the leaf directly below and attached to the oldest (uppermost) ear.

Three plants per plot (three of the same plants used for morphological measurements in chapter 3) were selected realizing a total of nine measurements per replicate. Captured data were transferred to the Hansatech Winpea 32 v1.04 software program where parameters such as F_o, F_m, F_v, F_v/F_m were calculated (see chapter 2). The Biolyzer v.3.0.6 software program (Maldonado-Rodríguez, 2000) was used to load the full fluorescence O-J-I-P transients and to calculate the O-J-I-P parameters from variable fluorescence values at $F_{50µs}$, $F_{100µs}$, $F_{300µs}$, F_{2ms} and F_{30ms}, according to the equations of the

JIP-test (Strasser and Govindjee, 1992; Strasser and Tsimilli-Michael, 2001). Absorbance (ABS) refers to the absorption of photons by the chlorophyll molecules in the antenna complex. Part of the absorbed energy is trapped (TR_O) by the reaction centre of PSII (P_{680}) while the remainder is dissipated (DI_O) in the form of heat and fluorescence. Of the trapped energy, part of it is converted to redox energy by electron transport (ET_O) through Q_A and Q_B (Strasser *et al.*, 2000). In this study the following specific fluxes were calculated, namely i) ABS/RC – absorbance of photons by the antenna pigments (Chl), ii) TRo/RC – channelled energy to the antenna pigments, iii) DIo/RC – excitation energy dissipated as heat or fluorescence at the antenna pigments (quantum efficiency), iv) ETo/RC – the converted excitation energy to redox energy by reducing the electron acceptor Q_A to Q_A^-, creating an electron transport that leads ultimately to CO_2 fixation. The quantum photosynthetic yield was also determined and expressed as ETo/ABS (or φ_{Eo}) defined as the probability for an absorbed photon to move an electron into the electron transport chain. In Figure 4.2 a simplified summary of the processes involved with regards to the specific fluxes and quantum photosynthetic yield are supplied. Performance Index (PI_{abs}) which quantifies and includes the main steps in PSII photochemistry namely light energy absorption, energy trapping and conversion of excitation energy into electron flow was also calculated by means of the following equation (Thach *et al.*, 2007):

$PI_{abs} = (RC/ABS) (\varphi_{Po/(1-} \varphi_{Po})) (\Psi_{Eo}/(1- \Psi_{Eo}))$ **……………………[1]**
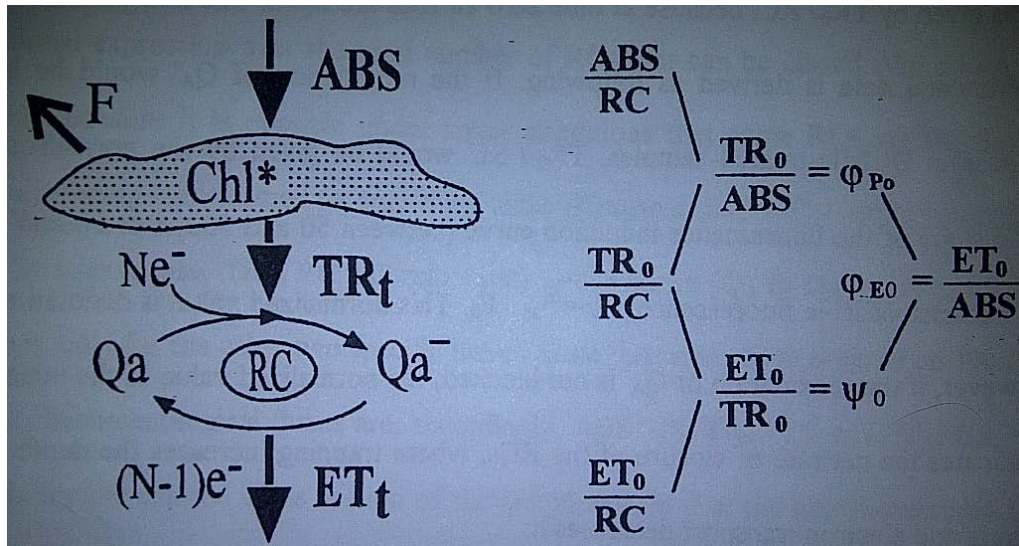
Figure 4.2: A highly simplified working model of energy fluxes in the photosynthetic apparatus, from the point where photons are absorbed by the Chl antenna pigment to the point where excitation energy is converted to redox energy (Strasser *et al.*, 1999). (F = fluorescence emission, ABS = absorbance of photons, Chl* = antenna pigment, TRt = trapping flux, Qa = electron acceptor, RC = reaction centre, ETt = electron transport). TRo/ABS and ETo/TRo were not calculated.

4.2.2.3 Chlorophyll content in leaves

Chl was destructively measured by cutting out a 1 cm diameter leaf disk in the middle of the leaf (between the leaf margin and middle lamella), using a special clipper, from the leaf below the uppermost (oldest) ear for nine plants per plot at flowering, as illustrated in Figure 4.3.
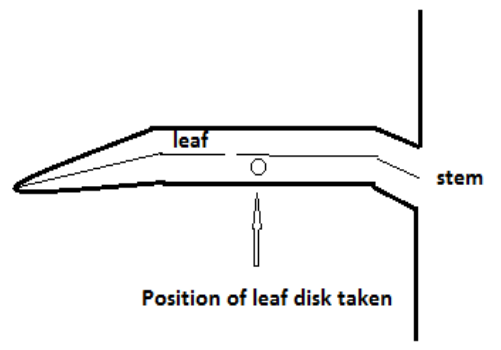
Figure 4.3: Position on leaf where 1cm disk was taken for Chl analysis directly below and attached to the oldest (uppermost) ear.

The nine leaf disks were immediately placed in liquid nitrogen (N) for 1 minute and stored at -20°C until Chl content could be determined. Before analysis the leaf discs were thawed and weighed, whereafter it was homogenized in 15 ml 100% acetone until discs were colourless and Chl totally removed. A Shimadzu UV/VIS PharmaSpec spectrophotometer, equipped with a 10 mm light path cell, was used to detect the absorbance at 665, 645 and 630 nm. Chl *a, b* and c were calculated according to the method of Parsons and Strickland (1965) by means of the following equations:

$$C_a = 11.6\ D_{665} - 1.31\ D_{645} - 0.14\ D_{630}$$
$$C_b = 20.7\ D_{645} - 4.34\ D_{665} - 4.42\ D_{630}$$
$$C_c = 55.0\ D_{630} - 4.64\ D_{665} - 16.3\ D_{645}$$

C = the Chl content in mg L^{-1} and D = absorbance at different wavelengths. The mass of the nine leaf disks were included in the calculation and data expressed as mg g^{-1} fresh mass (FM).

4.2.2.4   Carbohydrate sugar content

4.2.2.4.1   Extraction procedure for sugars

The content of two monosaccharide sugars (glucose and fructose) and one oligosaccharide (sucrose) was determined in the kernels of the oldest ear, selected from three plants per plot, at the milk -, soft dough-, hard dough and physiological ripe stages. The middle five cm of each ear was cut out immediately after it was removed from the plant, placed in a pre-heated 80% (v/v) aqueous ethanol solution, boiled for 10 minutes in order to stop all enzymatic activity and frozen at -20ºC until sugar content could be determined. Prior to analysis, the middle part of ears was thawed and 1 g kernels per ear (total of 3 g for three ears) were removed. Only the kernel endosperm was homogenized to a fine paste using an oscillating Milll MM 400 (Germany) and diluted with 21 ml (7 ml g^{-1}) distilled water. A one ml aliquot homogenate was transferred to an Eppendorff vial and centrifuged (Selecta P, Spain) at 12 000 rpm for 5 minutes. The supernatant was transferred to clean marked Eppendorff vials. The Boehringer Mannheim enzymatic technique (Bergmeyer and Brent, 1974) was employed to spectrophotometrically determine the sugar content in kernels using a Biorad Microplate reader (Germany) equipped with GEN 5 software. The absorbance of a 10 µL aliquot from supernatant of each replicate was measured at 340 nm.

4.2.2.4.2   Sugar content determination: Principle of the Boehringer Mannheim enzymatic technique for determining sucrose, D-glucose and D-fructose content in solid tissue

The D-glucose concentration is determined before and after the enzymatic hydrolysis of sucrose. D-fructose is determined subsequent to the determination of D-glucose.

Determination of D-glucose before inversion:

At pH 7.6 the enzyme hexokinase (*HK*) catalyses the phosphorylation of D-glucose by adenosine-5'-triphosphate (ATP) with the simultaneous formation of adenosine-5'-diphosphate (ADP) [1].

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\textit{HK}} \text{Glucose-6-phosphate} + \text{ADP} \quad ……………………[2]$$
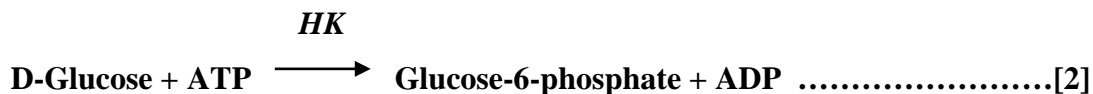
In the presence of *glucose-6-phosphate dehydrogenase* (*G-6-PDH*), the D-glucose-6-phosphate (G-6-P) formed is specifically oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH $+H^+$) [2].

$$\text{Glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\textit{G-6-PDH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}^+ \text{ ....[3]}$$

The NADPH formed in this reaction is stoichiometric to the amount of D-glucose and is measured by means of its absorbance at 340 nm.

Determination of D-fructose:

Hexokinase (*HK*) also catalyzes the phosphorylation of D-fructose to fructose-6-phosphate (F-6-P) in the presence of ATP [3].

$$\text{D-Fructose} + \text{ATP} \xrightarrow{\textit{HK}} \text{Fructose-6-phosphate} + \text{ADP} \text{ ………………………[4]}$$
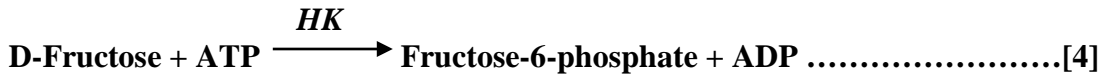
On completion of the reaction [3], **F-6-P** is converted by *phosphogluco isomerase (PGI)* to glucose-6-phosphate (G-6-P) [4].

$$\text{Fructose-6-phosphate} \xrightarrow{\textit{PGI}} \text{Glucose-6-phosphate} \text{ ………………………… [5]}$$

G-6-P reacts again with NADP to form gluconate-6-phosphate and NADPH [2]. The amount of NADPH formed is now stoichiometric with the amount of D-fructose.

Enzymatic inversion:

At pH 4.6, sucrose is hydrolyzed by the enzyme *β–fructosidase (invertase)* to D-glucose and D-fructose [5].

$$\text{Sucrose} + \text{H}_2\text{O} \xrightarrow{\textit{β–fructosidase}} \text{D-glucose} + \text{D-fructose} \text{ ....[6]}$$

The determination of D-glucose after inversion (total D-glucose) was carried out according to the principle outlined above. The sucrose content was calculated from the difference of the D-glucose concentrations before and after enzymatic inversion.

Procedure:

The directions of the suppliers (Boehringer Mannheim/R-Biopharm) were followed and the sugar content calculated by means of the following equation:

$$c = \frac{V \times MW}{\epsilon \times d \times v \times 1000} \times \Delta A \ g\,L^{-1}$$

Where:
c = concentration
V = final volume (ml)
v = sample volume (ml)
MW = molecular weight of the substance to be assayed (g mol^{-1})
d = light path (cm)
ϵ = extinction coefficient of NADPH at 340 nm (= 6.3)

It follows for sucrose:

$$c = \frac{3.02 \times 342.3}{\epsilon \times 1.0 \times 0.1 \times 1000} \times \Delta A_{sucrose} = \frac{10.34}{\epsilon} \times \Delta A_{sucrose} \ g\,L^{-1}$$

for D-glucose:

$$c = \frac{3.02 \times 180.16}{\epsilon \times 1.0 \times 0.1 \times 1000} \times \Delta A_{D\text{-}glucose} = \frac{5.441}{\epsilon} \times \Delta A_{D\text{-}glucose} \ g\,L^{-1}$$

for D-fructose

$$c = \frac{3.04 \times 180.16}{\epsilon \times 1.0 \times 0.1 \times 1000} \times \Delta A_{fructose} = \frac{5.477}{\epsilon} \times \Delta A_{fructose} \ g\,L^{-1}$$

Sucrose, D-glucose and D-fructose content was expressed as g 100g^{-1} dry mass (DM). The total sugar content was derived from the sum of sucrose, D-glucose and D-fructose and expressed as g 100g^{-1} DM.

4.2.2.5 Starch content

The Megazyme total starch analysis procedure (AA.AMG) was used to measure the total starch in maize kernels of the oldest ear detached from three plants per plot for the milk, soft dough and hard dough stages as well as at harvest. The principle of this procedure is based on starch hydrolysis that proceeds in two phases namely a) partial hydrolysis and solubilization and b) quantitative hydrolysis of starch dextrins to glucose by the enzyme *amyloglucosidase*.

4.2.2.5.1   Pre-treatment of samples potentially containing glucose and malto-saccharides

Each sample, 100 mg of finely ground maize flour excluding the testa, was suspended in 5 ml 80% aqueous ethanol (v/v) in a test tube and incubated at 80-85ºC for 5 minutes. Only the middle area of the ear was taken as representative of ear starch content after Seebauer *et al.* (2009) who reported that the accumulation of kernel starch was uniform along the ear. The contents were mixed on a vortex mixer, further diluted with 5 ml 80% aqueous ethanol and centrifuged for 10 minutes at 3000 rpm. The supernatant was discarded. The pellet was re-suspended in 10 ml, 80% aqueous ethanol and homogenized using a vortex mixer. Subsequently, samples were centrifuged again and supernatants discarded.

4.2.2.5.2   Modification of the standard procedure for samples potentially containing resistant starch

Sample pellets were re-drenched with 0.2 ml, 80% aqueous ethanol (v/v) to aid dispersion and stirred thoroughly using a vortex mixer. Immediately, 2 ml dimethyl sulphoxide (DMSO) was added to the sample, stirred on vortex mixer and incubated in a boiling water bath for 5 minutes for complete solubilization and dextrinisation of samples.

4.2.2.5.3   Starch assay procedure

Three ml of thermo stable α-amylase in MOPS buffer (50 mM, pH 7.0) was added to the sample and vigorously stirred on a vortex mixer. The samples were incubated in boiling water for 6 minutes and, subsequently, transferred to a water bath at 50 ºC. Four ml sodium acetate buffer (200 mM, pH 4.5) was added, followed by 0.1 ml amyloglucosidase (20 U). The samples were stirred on a vortex mixer, incubated further at 50ºC for 30 minutes, individually transferred to volumetric flasks and the volumes adjusted to 10 ml with distilled water. Samples were then centrifuged at 3000 rpm for 10 minutes. For samples potentially containing 10-100% starch (soft dough, hard dough and at harvest) a 1 ml aliquot was further diluted to 10 ml with distilled water. Duplicated aliquots (0.1 ml) of each treatment was transferred to glass test tubes, 3 ml GOPOD reagent was added and incubated at 50ºC

for 20 minutes. Glucose controls consisted of 0.1 ml glucose standard solution (1 mg ml^{-1}) and 3.0 ml GOPOD reagent. Reagent blank solution consisted of 1.0 ml of water and 3 ml of GOPOD reagent. Both the glucose control and reagent blank solution was incubated at 50ºC for 20 min. The absorbance was measured at 510 nm for each sample and the glucose control was read against the reagent blank.

4.2.2.5.4   Starch content calculation

The following equations were used to calculate the starch content of each sample:

Starch $= \Delta_E$ x F x 1000 x 1/1000 x 100/W x 162/180

$\qquad = \Delta_E$ x F/W x 90

where: Δ_E = absorbance read against the reagent blank

$\qquad$ F = 100 µg glucose / absorbance of 100 µg glucose

$\qquad$ 1000 = volume of correction (0.1 ml aliquot taken from 100 ml)

$\qquad$ 1/1000 = conversion from micrograms to milligrams

$\qquad$ 100/W = factor to express starch as a percentage of flour weight

$\qquad$ W = the weight in milligrams of the flour analyzed

$\qquad$ 162/180 = adjustment from free glucose to anhydrous glucose

Starch % (DM basis) = starch % (as is) x 100/[100 - moisture content (% w/w)]

4.2.2.6   Commercial trial: Growing season 2007/2008

A commercial trial was conducted during the 2007/08 growing season using the same maize cultivar, DKC 78-15B on a trial site located in the Odendaalsrus region, Free State Province, Republic of South Africa. The crop was established on a 1.5 m deep sandy soil, classified as an Avalon soil form belonging to the Woodburn family (Soil Classification Working Group, 1991). Primary soil tillage was done with a till-ripper to an average depth of 90 cm. At planting fertilizer was band placed approximately 5 cm below the seed and 5 cm to the side to prevent any negative salt effects on the seedling. Seeds were planted mechanically with a commercial planter 5 cm below the soil surface. Fertilizer was applied as a top (side) dressing at the V10 growth stage, approximately 15-20 cm from the row, and incorporated into the soil by means of a tiller. Weeds were controlled chemically

according to standard farm practices using Suprazine 600 SC® (Atrazine 300g L⁻¹ and Terbuthylazine 300g L⁻¹) at 2.1 L ha⁻¹. Soil samples were taken at a depth of 0-30 cm between rows at the beginning of the trial and analyzed to determine the soil fertility status (Table 4.1).

Table 4.1: Some chemical characteristics of the Avalon soil form at the study site (2007/08). Individual samples were pooled together and a representative sample was analyzed

pH (KCl)	P (Bray 1) (mg kg⁻¹)	K (mg kg⁻¹)	Ca (mg kg⁻¹)	Mg (mg kg⁻¹)	Na (mg kg⁻¹)	CEC (cmol$_c$kg⁻¹)
4	20.1	142.7	269	83.9	1.8	3

Based on the soil analysis, fertilizer was applied according to commercial agronomic practices as summarized in Table 4.2.

Table 4.2: Standard fertilizer applications for a 4 ton ha⁻¹ grain yield potential in the commercial trial during the 2007/08 growing season

Time	Rate (kg ha⁻¹)	Type	N (kg ha⁻¹)	P (kg ha⁻¹)	K (kg ha⁻¹)	S (kg ha⁻¹)
At planting	150	3:1:0 (28)	31.4	10.5	0	0
Top dressing	150	LAN	42	0	0	0
TOTAL			**72.1**	**14.7**	**0**	**0**

The long-term climate can be described as semi-arid, with an average annual rainfall of 450 mm per annum. Climatic data, including temperature, rainfall and humidity during the season (Table 4.3).

Table 4.3: Average minimum and maximum temperatures, rainfall and relative humidity per month for the 2007/08 growing season. (Climate data for the field trial was obtained from the South African Weather Service situated approximately 15 km from the trial site)

MONTH	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	TOTAL
Temp max (°C)	28.9	29.2	30.6	26.2	24.9	22.3	19.3	19.6	23.6	
Temp min (°C)	15.7	16.7	17	13.9	8.1	7.6	3.3	1.6	5.2	
Rainfall (mm)	86.6	137.4	7.2	100.4	3.2	35.2	4	0	1.4	375.4
Humidity (%)	67.5	70.3	64.1	78	67.2	77	79.7	68.3	50	

Plant rows were in a north-south direction, with an inter-row spacing of 1.5 m and intra-row spacing of 35-40 cm, yielding a population of 16000 plants ha^{-1}. The cultivar was DKC 78-15B. The plot size was 13.5 m x 498 m = 6723 m^2. The water volume applied, using an Agritech 2000 L chemical tractor sprayer, was 200 L ha^{-1}. A split plot design layout was followed, with each treatment being replicated three times.

Based on the results obtained with specifically the MKP foliar treatment in previous seasons during this study (2004 to 2007), the following treatments were applied during the 2007/08 growing season. MKP was foliar applied at a 2% (0.5x) solution rate in order to evaluate the grain yield response of maize to this lower concentration:

1.   Control (standard agronomic practices, as described above, were applied).
2.   Mono potassium phosphate (MKP) (KH$_2$PO$_4$) (0.5x) was applied as a foliar spray at the eight leaf growth stage (V8). Application rate was 4 kg MKP 200 L^{-1} water ha^{-1}, equal to a 2% (w/v) solution. MKP contains 226 g kg^{-1} P and 282 g kg^{-1} K.

All spray applications were made between 9:00 and 12:30. Replicated plots for each treatment of the commercial trial (2007/08 growing season) were harvested with an Agritech 172 combine harvester. The moisture grain content for both the control and MKP treated plants were 12.5% at harvest.

4.2.2.7 Effect of different MKP concentrations, applied foliar, on stomatal structure under glasshouse conditions

Plastic containers with an area of 0.126 m^2 (volume = 34.02 L) were used and filled with sieved soil. The field water holding capacity was determined 48 hours after flooding the soil with distilled water. The difference between the weight of the soil in the container after 48 hours and the dry soil was 7.1 kg and was taken as field water capacity (FWC). Containers were maintained at 50% FWC during the trial period. A soil sample was taken and analyzed values are supplied in Table 4.4.

Table 4.4: Some chemical characteristics of the pot trial soil

pH (KCl)	P (Bray 1) (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	CEC cmol$_c$kg^{-1}
4.8	14.9	89	369	166.3	3.6	3.9

Fertilizer was applied at 3.78 g per container (as indicated in Table 4.5) and mixed with the soil to a depth of 10 cm.

Table 4.5: Standard fertilizer application for the glasshouse trial based on soil analysis figures

Time	Rate (kg 0.126m^{-2})	Type	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	S (kg ha^{-1})
At planting	3.78g	3:1:0 (28)	63	21	0	0
TOTAL			**63**	**21**	**0**	**0**

Three seeds per container, of the cultivar DKC 78-15B were planted 5 cm below the soil surface. Only one plant was left per container after emergence. At the V8 crop growth stage, foliar treatments were applied with a Solo® 475 knap sack sprayer (Solo Incorporated; Germany) delivering a volume equal to 135 L ha^{-1} at a pressure between 345 and 414 kPa. The sprayer was equipped with a flat fan nozzle spraying at a 40º angle (both sides of the perpendicular) covering a width of 1.1 m on the soil surface from a height of 90 cm above the ground. Application of treatments were done between 14:00 and 15:00, and calibrated to deliver the same volume of water per running meter as used in field trials. The dry and wet bulb temperature was measured at 24°C and 20ºC respectively with a thermo-hygrograph and humidity calculated to be 78%. Four treatments were applied during the glasshouse trial:

1. Control (standard agronomic practices)
2. Mono potassium phosphate (MKP) (0.5x) was applied as a foliar spray at the eight leaf growth stage (V8). A 2% (w/v) solution was applied. Spray solution pH 4.71 @ 24.3ºC (Crison MM40 multi meter).
3. Mono potassium phosphate (MKP) (1x) was applied as a foliar spray at the eight leaf growth stage (V8). A 4% (w/v) solution was applied. Spray solution pH 4.62 @ 23.8ºC (Crison MM40 multi meter).
4. Mono potassium phosphate (MKP) (2x) was applied as a foliar spray at the eight leaf growth stage (V8). An 8% (w/v) solution was applied. Spray solution pH 4.55 @ 23.4ºC (Crison MM40 multi meter).

MKP contains 226 g kg^{-1} P and 282 g kg^{-1} K. The knapsack sprayer was calibrated to deliver the same volume per area as was used during field trials.

Twenty four hours after treatment, the top four unfolded leaves were cut-off and immediately dipped in a 0.01% HCl solution to remove all salts/nutrients that may have remained on the leaf surface. The adaxial epidermal layer of the last unfolded leaf, (leaf number eight) was immediately removed with a lab knife and placed in a lactofenol solution. The leaf stomatal complex was investigated under a light microscope at 100x magnification and photographed. The remaining fresh leaf material for each treatment was immediately sent to the ARC-ISCW for analysis of specifically P and K content.

4.2.3   Statistical analysis

Data presented were means of three replicates along with standard deviations of means. Means of raw data and percentage differences from controls were subjected to analysis of variance using the NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA) statistical program. Treatment means of parameters showing significant differences ($P<0.05$) were separated using the Tukey-Kramer Multiple-Comparison Test (Steele & Torrie, 1980). The coefficient of variation (CV) was calculated for each parameter (raw data only) and included in the legend of the corresponding graph. Data calculated as percentage differences from control treatments, were statistically analyzed in order to verify interactions and main effects between factors (growth stage and concentration) which were not possible to calculate from the means of raw data. Percentage difference data were calculated as the percentage change from the control treatment for each replicate, prior to determining the mean of the percentage difference from control for the treatment.

4.3 Results

4.3.1 Chlorophyll *a* fluorescence fluxes

KNO_3 applied at both V3 and V8, during the 2004/05 season showed a positive tendency to enhance all Chl *a* fluorescence fluxes, including the energy dissipated per reaction centre (DIo/RC) compared to the control (Figure 4.4A1). This was, however, non-significant in all cases. MKP applied at either V3 or V8 had no significant effect on any of the measured flux values. However, MKP applied at V8 was the only treatment that showed a tendency to elevate (+10.6%) the conversion of absorbed energy to redox energy (ETo/RC) when compared to the control.

Significantly lower ABS/RC and TRo/RC values were observed between the control and the MKP (2x) treatment during the 2005/06 season (Figure 4.4B1). By expressing the data as a percentage change from the control, MKP reduced the ABS/RC, TRo/RC and ETo/RC parameters significantly, independent of the concentration applied versus KNO_3 (Figure 4.4B2).

Fluorescence measurements during the 2006/07 season showed the MAP treatment tended to increase all parameters ABS/RC, TR0/RC, DI0/RC and ETo/RC by 9%, 8%, 5.6% and 12.4% respectively, compared to the control (Figure 4.4C1). When data was expressed as a percentage change from the control, the MAP treatment only tended to enhance TRo/RC and ETo/RC, compared to all other salts when applied at V8 (Figure 4.4C2).
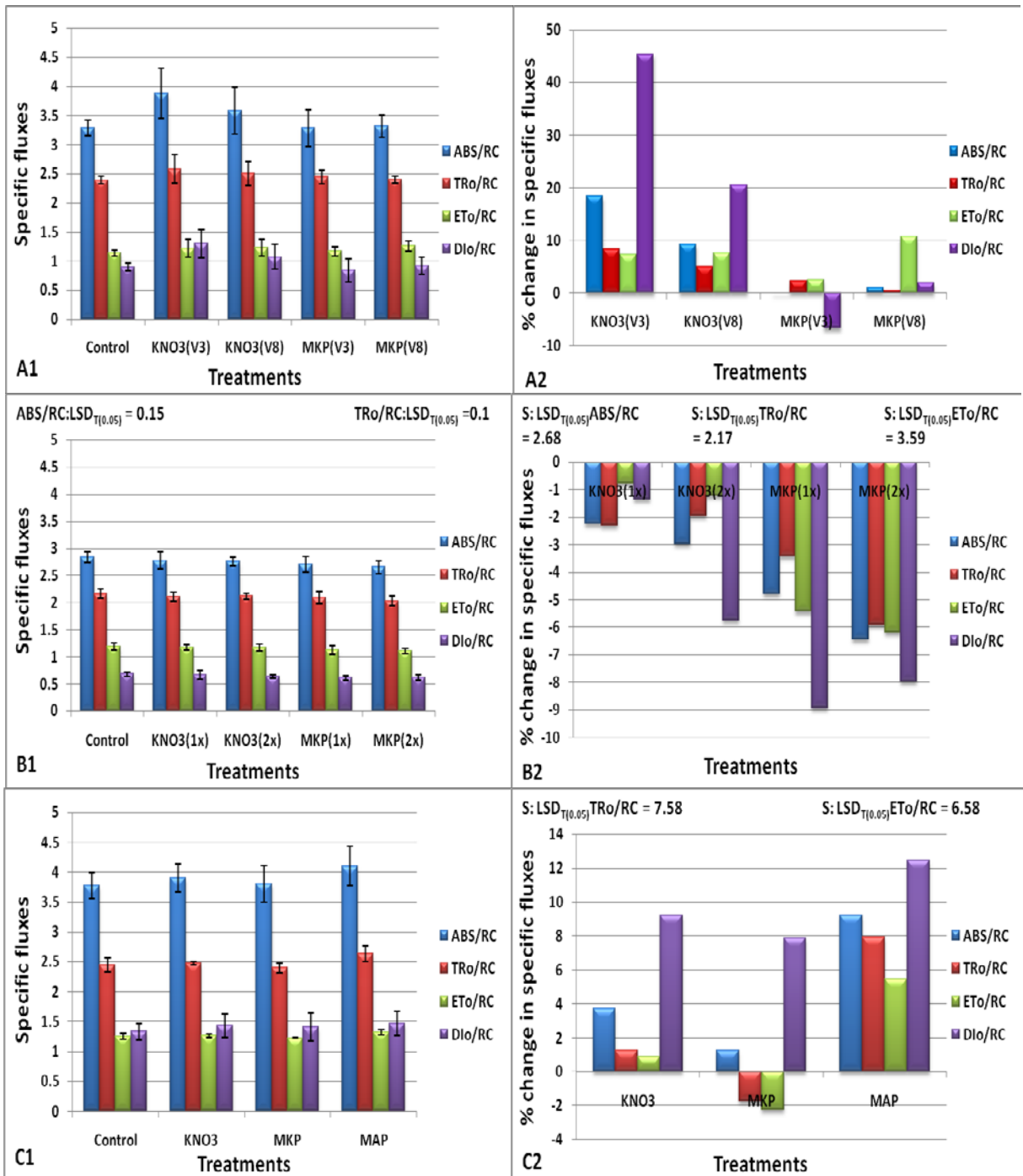
Figure 4.4: Chlorophyll *a* fluorescence response, in terms of fluorescence fluxes, of maize to foliar applied nutrients at different growth stages and concentrations over three seasons. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1 (ABS/RC): CV = 10.03 %; A1 (TRo/RC): CV = 7.12%; A1 (ETo/RC): CV = 5.08%; A1 (DIo/RC): CV = 18.57%. B1 (ABS/RC): CV = 2.49%; B1 (TRo/RC): CV = 2.13%; B1 (ETo/RC): CV = 3.79%; B1 (DIo/RC): CV = 7.98%. C1 (ABS/RC): CV = 6.41%; C1 (TRo/RC): CV = 3.52%; C1 (ETo/RC): CV = 2.67%; C1 (DIo/RC): CV = 13.16%.

4.3.2   Chlorophyll *a* fluorescence quantum efficiency (yield)

The probability that an absorbed photon will move an electron into the electron transport chain (quantum efficiency (φ_{Eo}) calculated by ETo/ABS) was enhanced by the MKP treatment when applied at V3, but more so when applied at V8 during the 2004/05 season (Figure 4.5A1). MKP applied at V8 enhanced φ_{Eo} by approximately 10% compared to the control, although no significant interactions or main effects were observed between application growth stages and salt types (Figure 4.5A2).

Although the φ_{Eo} values were higher for all treatments on average during the 2005/06 season compared to season one, no significant differences between treatments were observed (Figure 4.5B1). Moreover, no significant interactions or main effects between plants treated with either of the two salts KNO_3 and MKP at any concentration revealed to have affected φ_{Eo} (Figure 4.5B2).

During the 2006/07 season, being significantly drier than the first two seasons, all treatments applied at V8 decreased φ_{Eo}, although not significantly (Figure 4.5C1). The expression of data as a percentage change from the control also showed no significant interactions or main effects between treatments (Figure 4.5C2).
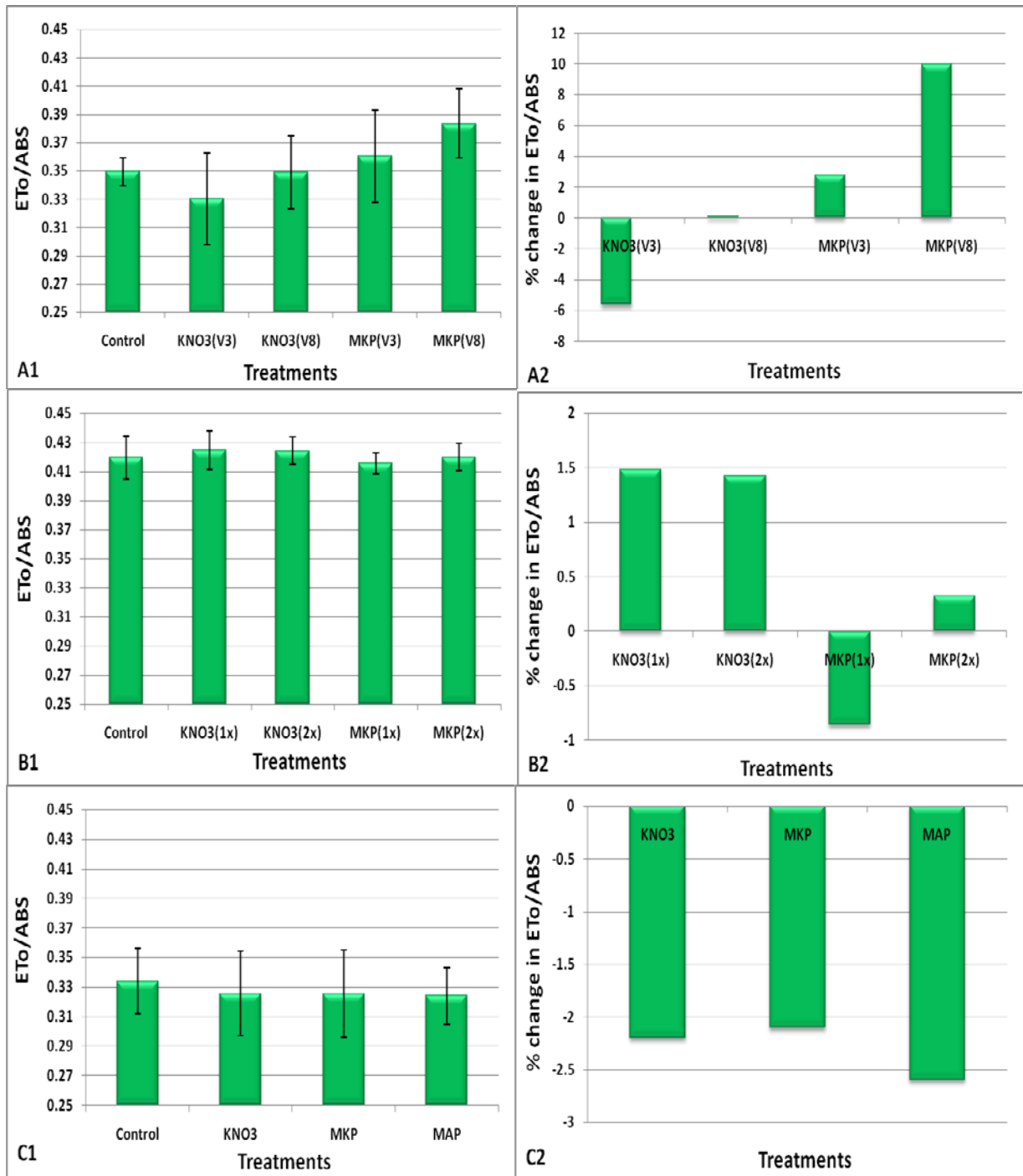
Figure 4.5: Chlorophyll *a* fluorescence response of maize, in terms of quantum efficiency yield (φ_{Eo} or ETo/ABS), to foliar applied nutrients at different growth stages and concentrations over three seasons. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1: CV = 5.74%; B1: CV = 4.37%; C1: CV = 6.79%.

4.3.3 Chlorophyll *a* fluorescence performance index (PI_abs)

MKP applied at both V3 and V8, tended to enhance the performance index (PI_{abs}) by 12% and 22% respectively, compared to the control during the 2004/05 season (Figure 4.6A1). Conversely, plants treated with KNO_3 at V3 tended to reduce PI_{abs}, while application at V8 had no effect. This was, however, non-significant in all cases.

During the 2005/06 season a similar tendency of MKP to enhance PI_{abs} by 5% and 8%, respectively, compared to the control when applied at single (4%) and double (8%) rates and at V8, was observed (Figure 4.6B1). In contrast to the first season, KNO_3 applied at both single (3%) or double (6%) and at V8 tended to enhance PI_{abs} slightly versus the control (Figure 4.6B1). None of the treatments showed any significant effect for PI_{abs} during the second season compared to the control.

Plants treated with KNO_3 and MAP at single rates during the V8 growth stage of the 2006/07 season, tended to reduce PI_{abs} when compared to control plants, while MKP showed an omissible effect on PI_{abs} (Figure 4.6C1). When data were expressed as a percentage change from the control, neither a significant main effect nor interaction was noted for any of the treatments during neither of the seasons for PI_{abs} (Figure 4.6A2, B2, C2).
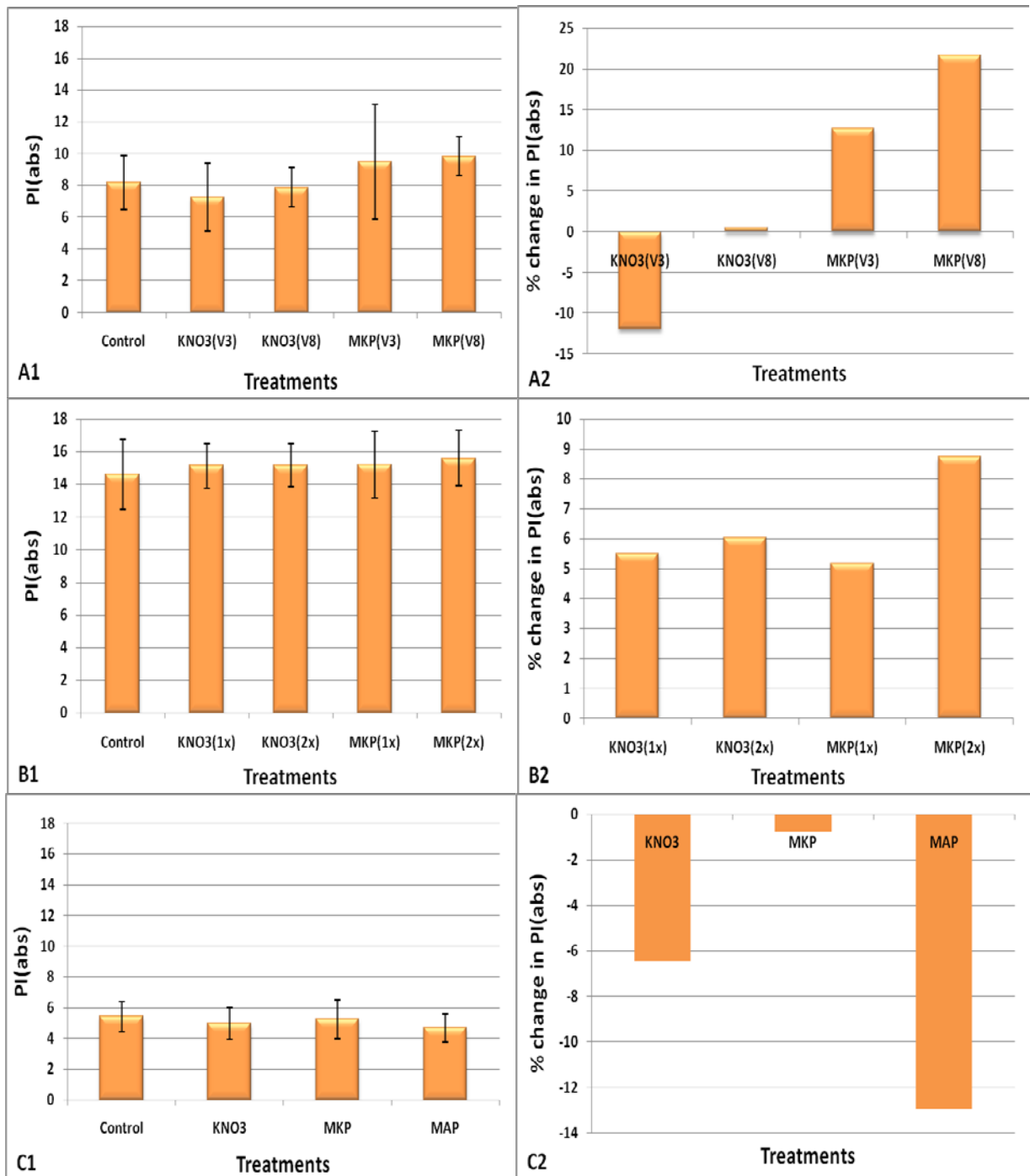
Figure 4.6: Chlorophyll *a* fluorescence response of maize, in terms of performance index (PI$_{abs}$), to foliar applied nutrients at different growth stages and concentrations over three seasons. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1: CV = 20.43%; B1: CV = 11.7%; C1: CV = 21.15%.

4.3.4 Chlorophyll content

No treatment had any significant effect on the alteration of Chl *a*, *b* or *c* content in leaves when measured at the flowering stage during the 2004/05 season (Figure 4.7A1), compared to the control. However, significant differences did occur when data was pooled to obtain a value for total Chl content. Both KNO_3 (-18%) and MKP (-14%) applied at V3, reduced the total Chl content significantly compared to the control. Although the same tendency was observed for treatment with both nutrients at V8, the differences were not significant. When data was expressed as a percentage change from the control, significant main effects in terms of application growth stage was noted for both Chl *a* and total Chl content to be reduced by treatment at V8, compared to treatment at V3 (Figure 4.7A2).

Results obtained during the 2005/06 season followed a different pattern in terms of Chl *b* content compared to the first season (Figure 4.7B1). KNO_3 applied at V8 and at single rate reduced the Chl *b* content significantly when compared to the control, but this was not the case when KNO_3 was applied at double the rate. MKP applied at double rate significantly decreased the Chl *b* content, compared to the KNO_3 (2x) treatment as well as the control when both was applied at V8 (Figure 4.7B1). Chl data expressed in terms of a percentage change from the untreated control for the second season, showed a significant interaction between the salt and concentration for the KNO_3 (1x) treatment to reduce Chl *b* and total Chl content (Figure 4.7B2). No Chl *c* was detected in the leaves during this season.
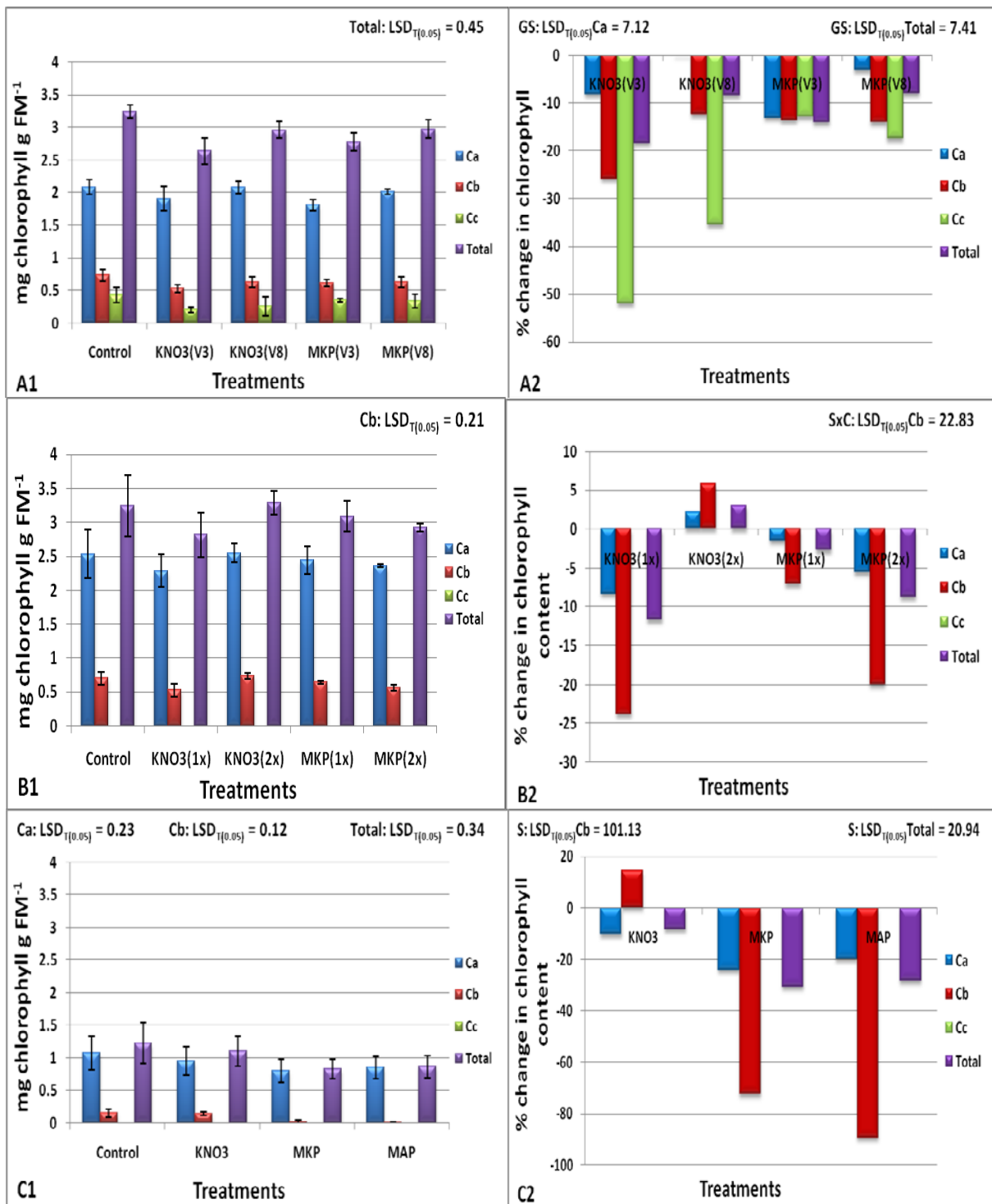
Figure 4.7: Chlorophyll *a* (Ca), *b* (Cb) and *c* (Cc) content, as well as total chlorophyll content, in the leaf directly below and attached to the oldest (upper most) ear at the flowering stage of maize. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1(Ca): CV = 5.18%; A1(Cb): CV = 11.79%; A1(Cc): CV = 29.74%; A1(Total): CV = 5.49%. B1(Ca): CV = 10.05%; B1(Cb): CV = 11.52%; B1(Total): CV = 10.1%. C1(Ca): CV = 8.81%; C1(Cb): CV = 48.35%; C1(Total): CV = 11.92%.

Based on the total value, Chl content in leaves was threefold lower in the third season compared to the previous two seasons (Figure 4.7C1). All treatments applied during the 2006/07 season decreased Chl *a* content, as was the case during the first two seasons. MKP applied at a single rate during V8 significantly reduced the Chl *a* content of the main ear leaf at flowering, compared to the control (Figure 4.7C1). Chl *b* content was significantly reduced in plants treated with either MKP (1x) and MAP treatments, compared to both the KNO_3 (1x) treatment and control. Further, the total Chl content was also significantly lower in the ear leaf for all plants treated versus the control. When Chl data was expressed as a percentage change from the control during the 2006/07 season, it confirmed results expressed in Figure 4.7C1 with regards to Chl *b* and total Chl contents (Figure 4.7C2). No Chl *c* was detected in any of the differently treated plants during the 2006/07 growing season, as was the case in the second season.

4.3.5   Glucose, fructose and sucrose content in maize kernels at the milk stage

In order to follow the translocation of carbohydrate from the source (leaves) to the sink (kernels) under the influence of different foliar treatments, the results are presented separately for each kernel development stage and for each season. The disaccaharide sucrose is the form in which carbohydrate is translocated from the source to the sink, but is rapidly converted to its monosaccharide moieties, glucose and fructose, where after starch is synthesised in kernels. For this reason glucose and fructose levels were also determined in an attempt to additionally follow the conversion prior to starch formation in kernels at different development stages (Fernie *et al*., 2002). During the 2004/05 season both KNO_3 and MKP applied at V3, tended to contribute markedly towards increasing the sucrose content in kernels by 82% and 98% respectively, at the ear milk stage, compared to the control (Figure 4.8A1). The later application of KNO_3 and MKP at V8 had no significant effect on sucrose content at the milk stage. The two monosaccharides, glucose and fructose, were more or less stoichiometric in all cases. However, only KNO_3 applied at both V3 and V8 during the first season contributed to significant increases of both monosaccharides compared to the control, as was the case with sucrose. Once again MKP applied at both growth stages had a significant reducing effect on glucose content compared to KNO_3 applied at V3 (Figure 4.8A1). A significant interaction effect occurred between the salt and growth stage for the KNO_3 (V3) treatment in terms of increased glucose content during the milk stage (Figure 4.8A2). Due to large standard deviations between replicates for sucrose content at the milk stage, the mean percentage differences for each treatment varies from that of mean values for raw data.
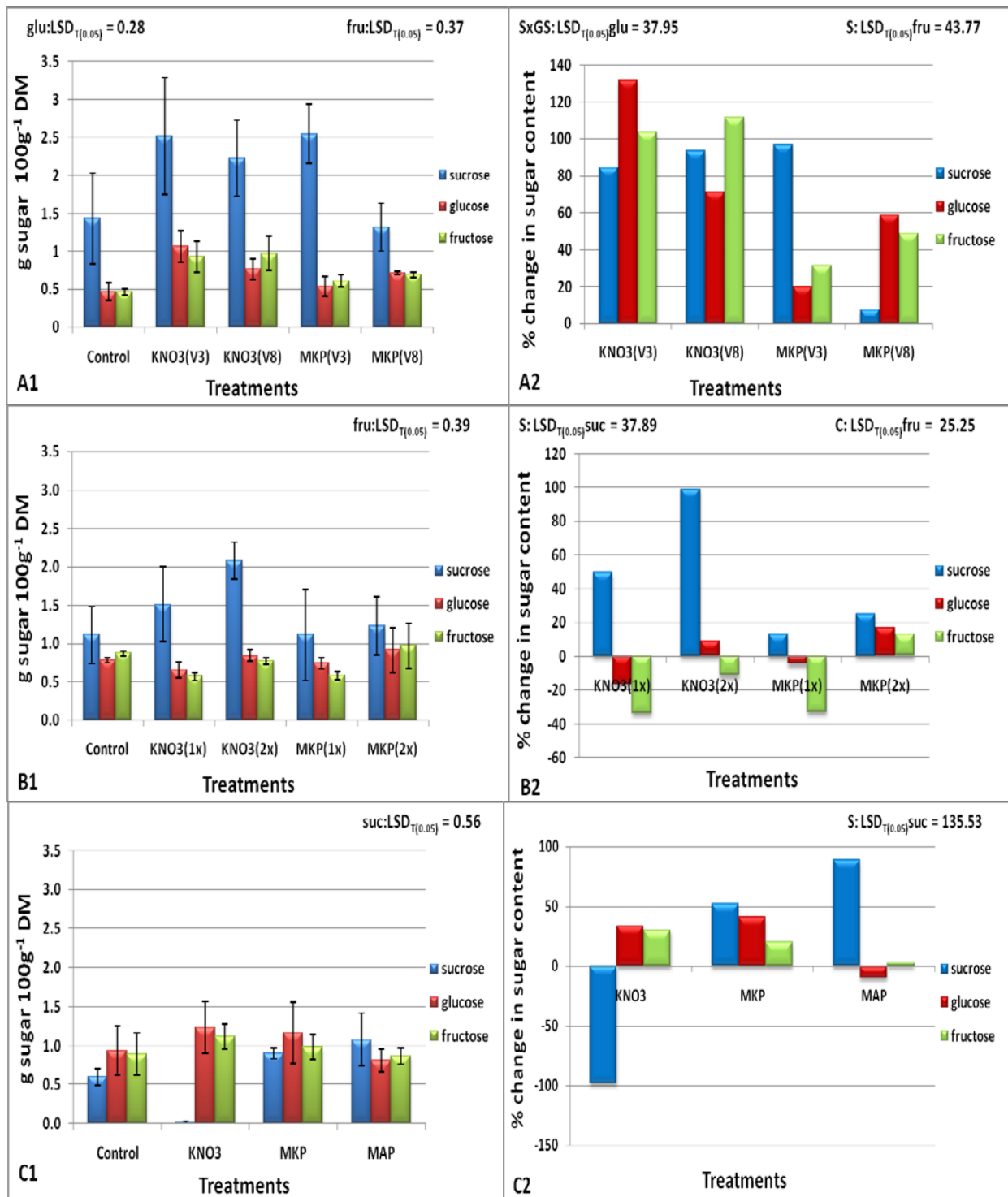
Figure 4.8: Sucrose, glucose and fructose content in maize kernels from the oldest (upper most) ear during the milk stage. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1(suc): CV = 25.85%; A1(glu): CV = 14.02%; A1(fru): CV = 17.72%; B1(suc): CV = 26.34%. B1(glu): CV = 17.36%; B1(fru): CV = 18.7%; C1(suc): CV = 30.4%. C1(glu): CV = 21.34%; C1(fru): CV = 17.58%.

KNO$_3$ significantly increased the kernel fructose content compared to MKP, independent of the application growth stage (Figure 4.8A2). More or less the same trend for sucrose content in kernels at milk stage, as was seen in the previous season under the influence of different treatments, was observed during the 2005/06 growing season (Figure 4.8B1). The KNO$_3$ treatment contributed to a significant enhancement of sucrose content compared to MKP, whether applied at the single or double rates during V8 (Figure 4.8B2). A tendency towards an interaction was observed between salt and concentration applied, whereby the KNO$_3$ (2x) treatment increased sucrose content during the milk stage, when applied at V8 (Figure 4.8B2). Again the two monosaccharides were more or less stoichiometric but, compared to the single application rate, it was only the double MKP (2x) treatment that contributed to a significant elevation of only the fructose content (Figure 4.8B1). The results in Figure 4.8B2, however, revealed a significant concentration main effect where the single rate for both salts suppressed the fructose content significantly compared to the double application rates when applied at V8 and measured during the milk stage (Figure 4.8B2).

During the 2006/07 season, no sucrose was detected in the kernels of KNO$_3$ treated plants at the milk stage and this was concomitant with high levels of the monosaccharide sugars in kernels from control plants as well as the other two treatments which did not differ significantly from each other (Figure 4.8C1). Expressing data as a percentage change (Figure 4.8C2) from the untreated control confirmed the observation made from raw data (Figure 4.8C1) in terms of sucrose content and the significant decreasing effect foliar treatment with KNO$_3$ had when applied at V8 and measured during the milk stage period of ear filling.

4.3.6   Glucose, fructose and sucrose content in maize kernels at the soft dough stage

In general, sugar levels were substantially lower in kernels during the soft dough stage compared to the milk stage. As was the case at the milk stage, all treatments contributed to markedly higher, but non-significantly different, sucrose content in kernels at the soft dough stage in the 2004/05 season (Figure 4.9A1). However, in contrast to the milk stage, both the KNO$_3$ and MKP foliar treatments applied at V8 contributed to higher, but not significantly different, sucrose levels compared to the earlier foliar applications of the two salts at V3. Application of these two salts at V3, nevertheless, also contributed to marked elevation of sucrose content compared to the control. Interestingly, a significant reduction in glucose content was observed in kernels obtained from all differently treated plants at the soft dough stage when compared to the control (Figure 4.9A1). When data was

expressed as a percentage change from the control, a significant interaction effect was observed between the salt applied and the time of application. The MKP (V3) treatment contributed to a significant decrease in glucose content of kernels in the main ear at the soft dough stage, compared to all other treatments (Figure 4.9A2). No significant differences or interactions in fructose content between treatments and the control were observed. Due to large standard deviations between replicates for sucrose content at the soft dough stage, the mean percentage differences for each treatment varies from that of mean values for raw data. The fact that only three replicates for each treatment was analyzed for all quality parameters in chapter 4, as well as the fact that plants are not homogeneous, reflected in the standard deviations calculated in Figure 4.9A1. Although it is hard to believe that more than a 100% difference in main ear kernel sucrose content between some of the treatments was not statistically significant, this was confirmed by a coefficient of variation of 38.85%.

During the 2005/06 season, as was seen at the milk stage (Figure 4.8B1), the double rates applied for both the KNO_3 and MKP treatments showed a more pronounced tendency towards elevating the sucrose content in kernels at the soft dough stage compared to the effect provoked by the single rates (Figure 4.9B1). This difference was only significantly different from the control in the case of the MKP (2x) treatment (Figure 4.9B1). Expression of the data as a percentage change from the control, confirmed the significant interaction between the salt applied and concentration for the MKP (2x) treatment in terms of sucrose (Figure 4.9B2). In comparing the double rates of the two salts, MKP and KNO_3, the former contributed to a significant decrease in kernel glucose content at the soft dough stage, indicating a significant interaction between salt and concentration for the MKP (2x) treatment (Figure 4.9B1 and B2). This tendency was not observed where the two salts were applied and the single rates. In terms of kernel fructose content at the soft dough stage the KNO_3 treatment revealed a significant main effect in contributing towards elevated levels, compared to MKP and independent of the concentration, during the second season when data was expressed on a percentage change basis (Figure 4.9B2).

Figure 4.9: Sucrose, glucose and fructose content in maize kernels from the oldest (upper most) ear during the soft dough stage. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1(suc): CV = 36.86%; A1(glu): CV = 33.71%; A1(fru): CV = 34.12%; B1(suc): CV = 17.12%. B1(glu): CV = 25.51%; B1(fru): CV = 23.38%; C1(suc): CV = 33.95%. C1(glu): CV = 29.40%; C1(fru): CV = 26.72%.

Sucrose content measured in control kernels at the soft dough stage during the 2006/07 season (Figure 4.9C1) was exceptionally high compared to the first two seasons (Figures 4.9A1 and 4.9B1). As a result, the sucrose levels in kernels from plants treated with KNO_3, MKP and MAP at V8 and at the single rate appeared to be at a much lower level compared to the control, notwithstanding the fact that it compared favourably with the results obtained during the first season (Figure 4.9A1). However, what needs to be emphasized is that treatment with both KNO_3 and MKP seemed to have contributed consistently towards accelerated breakdown of sucrose into its two monosaccharide moieties based on the distinct increase in glucose and fructose levels in kernels (at the soft dough stage) from plants treated with these two salts (Figure 4.9C1 and C2). This did not seem to be the case where plants were treated with MAP as the monosaccharide levels were almost the same as that of the control. However, the differences between treatments and control mentioned above were neither significant when mean raw data (Figure 4.9C1) was compared nor when percentage changes from the control (Figure 4.9C2) were calculated.

4.3.7   Glucose, fructose and sucrose content in maize kernels at the hard dough stage

Interestingly, during the hard dough stage of season one (2004/05) the sucrose content (Figure 4.10A1) measured in kernels remained at approximately the same level for all treatments as at the soft dough stage in the same season (Figure 4.9A1). Further, the pattern of sucrose breakdown to its monosaccharide moieties was also very similar. However, differences in sucrose content between treatments were significant at the hard dough stage in contrast with non-significant differences at the soft dough stage. KNO_3 applied either at V3 or V8, both contributed to a marked elevation of sucrose content, but this was only significant in the case of treatment at V8, compared to the control (Figure 4.10A1).   For the MKP treatment, it was the opposite as treatment at V3 resulted in significant and treatment at V8 to marked, but insignificant, elevation in sucrose content compared to the control (Figure 4.10A1). In terms of glucose and fructose content in kernels at the hard dough stage during season one, KNO_3 applied at V3 and V8 as well as MKP applied at V3 contributed to a significant decrease in content of both monosaccharide sugars compared with the control (Figure 4.10A1). However, concomitant with a reduction in sucrose content, foliar MKP application at the V8 growth stage resulted in significant elevated levels of both monosaccharide sugars in kernels at the hard dough stage when compared to all other treatments except the control (Figure 4.10A1). Expression of data as a percentage difference from the control revealed a significant
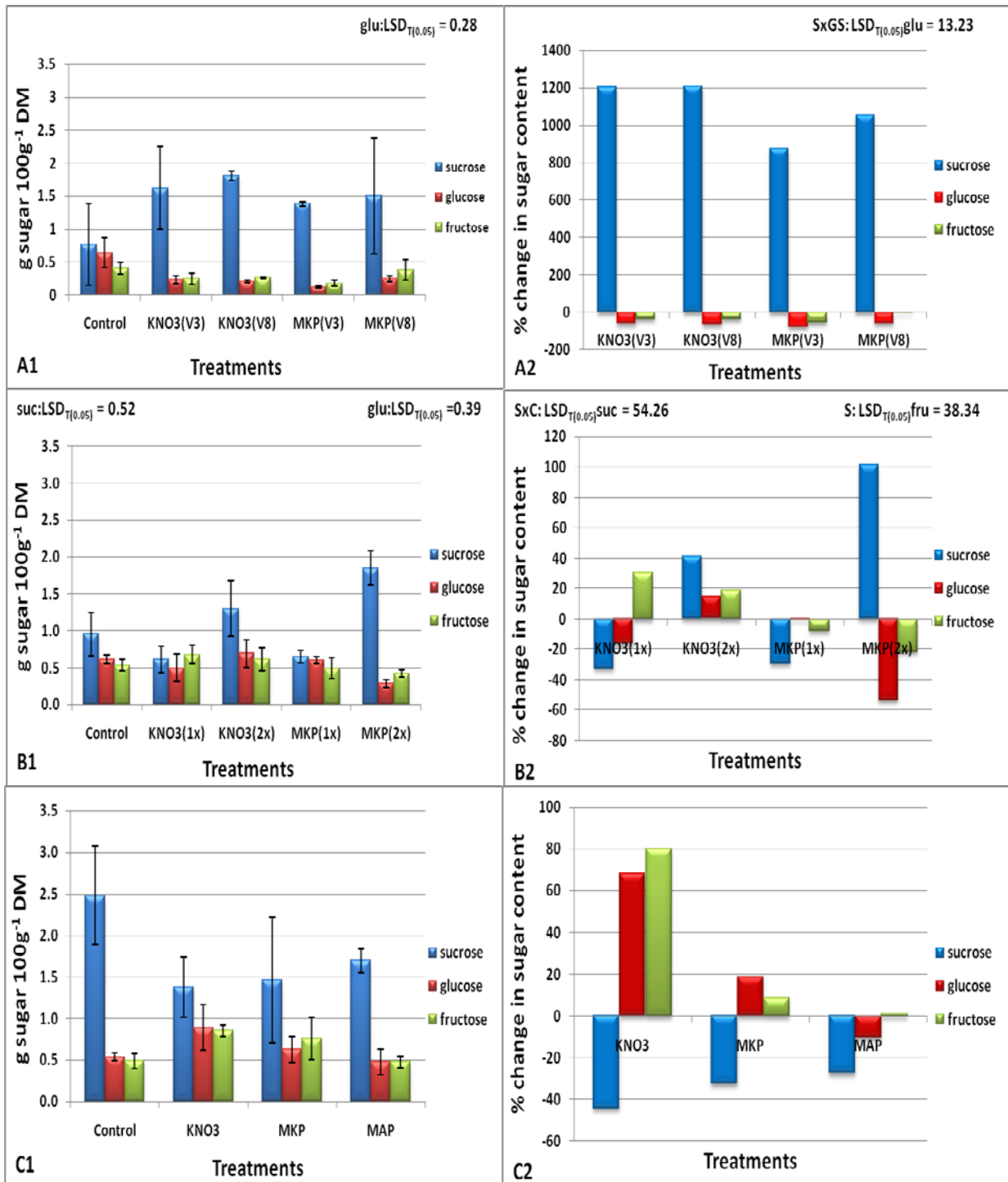
Figure 4.10: Sucrose, glucose and fructose content in maize kernels from the oldest (upper most) ear during the hard dough stage. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1(suc): CV = 30.73%; A1(glu): CV = 31.86%; A1(fru): CV = 26.77%; B1(suc): CV = 48.24%. B1(glu): CV = 22.5%; B1(fru): CV = 21.42%; C1(suc): CV = 39.46%. C1(glu): CV = 13.8%; C1(fru): CV = 16.21%.

Interaction effect between salt and time of application in terms of both glucose and fructose content in kernels at the hard dough stage during season one. In this regard MKP specifically applied at growth stage V8 contributed to significantly higher monosaccharide content in kernels at the hard dough stage compared with the other salt as well as time of application (Figure 4.10A2). Due to large standard deviations between replicates for sucrose content at the hard dough stage, the mean percentage differences for each treatment varies from that of mean values for raw data.

During the 2005/06 season the sucrose levels were at least twofold and for some treatments more than threefold lower in kernels from control and treated plants at the hard dough stage (Figure 4.10B1), compared to the first season results (Figure 4.10A1). Alternatively, the monosaccharide content in kernels from especially treated plants, except for the MKP treatment at V8, was at least twofold higher during the second compared to the first season at the same kernel development stage. However, no significant differences between treatments or interactions between factors, whether applied at the single or double rate, for either sucrose or monosaccharide content were observed during the hard dough stage in kernels for the 2005/06 growing season (Figure 4.10B1 and B2).

Sucrose content measured in kernels at the hard dough stage during the 2006/07 season (Figure 4.10C1), except for a marked lower level in kernels from plants treated with KNO_3 (1x) at V8, tended to follow the same patterns as during the first season. Based on the third season results only, the sucrose content in kernels from plants treated with both MKP and MAP at V8 was non-significantly higher compared to the control and the KNO_3 treatment. During the second season, both the glucose and fructose content was markedly higher than what was measured during the first season. Moreover, where MKP and MAP were applied to plants at the single rate and at the V8 growth stage, significantly higher glucose content was measured in kernels at the hard dough stage, compared to the control, while no significant differences were apparent between treatments in terms of fructose content (Figure 4.10C1). No significant salt or concentration effects were observed between treatments when data was expressed as a percentage difference from the control during the 2006/07 season (Figure 4.10C2).

Interestingly, during the hard dough stage the glucose and fructose content was never close to being stoichiometric and for all treatments the glucose content was higher than the fructose content. The latter is probably related to a change in metabolism at this kernel development stage. This aspect will be attended to in the discussion section.

4.3.8   Glucose, fructose and sucrose content in maize kernels at harvest

In general, and as could be expected, the sugar content in terms of both the disaccharide sucrose and the monosaccharide moieties thereof was markedly lower in kernels at harvest compared to the earlier kernel development stages. Based on results obtained in specific seasons, however, statistically significant differences were still observed in some cases (Figure 4.11A1).

Although no significant differences in sucrose content between treatments as well as the control occurred during the first season (Figure 4.11A1), sucrose tended to be less abundant in kernels from all treated plants compared to the control. Insignificance also applied in terms of differences between treatments for measured fructose content. Only the MKP treatment applied at V8 contributed to a significantly higher glucose content in kernels at harvest, compared not only to the control, but also to KNO_3 applied at both V3 and V8 (Figure 4.11A1). When data was expressed as a percentage change from the control, a significant interaction effect was observed between the salt and application growth stage for the MKP (V8) treatment to significantly contain more glucose in the kernels at harvest compared to KNO_3 applied at the same growth stage (Figure 4.11A2). Due to large standard deviations between replicates for sucrose content at the hard dough stage, the mean percentage differences for each treatment varies from that of mean values for raw data.

Both MKP and KNO_3 applied at V8 during the 2005/06 season at the double rate contributed to significantly higher sucrose content in kernels at harvest compared to the single rate treatments and the control (Figure 4.11B1). Interestingly, the control as well as the single rate treatments of both salts that contributed to the lowest sucrose content in kernels at harvest, consistently had the highest glucose and fructose content. The latter is a clear indication of a concentration dependant metabolic influence that will be dealt with in the discussion section. Expression of sugar data as a percentage change from the control, revealed a significant concentration main effect whereby the double rates of both KNO_3 and MKP contributed to a significant elevation of sucrose content in kernels at harvest compared to the single rates (Figure 4.11B2). MKP applied at the double rate during V8, on the other hand, showed a significant interaction effect to reduce glucose content in kernels at harvest, versus the KNO_3(2x) and MKP(1x) treatments. In terms of fructose content in kernels at harvest, the KNO_3 (2x) treatment contained more fructose compared to the MKP salt also applied at the double rate, although not significantly (Figure 4.11B2).

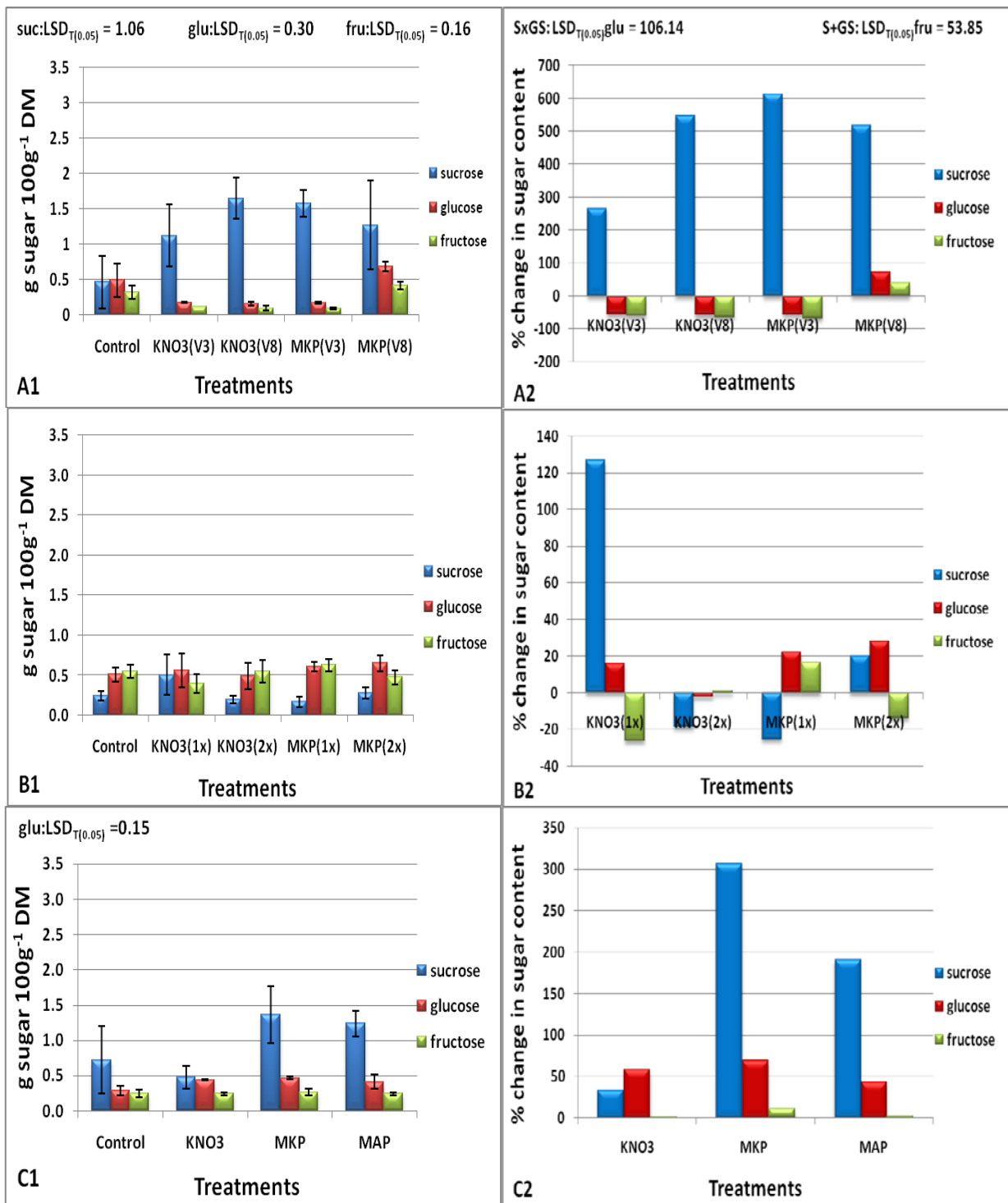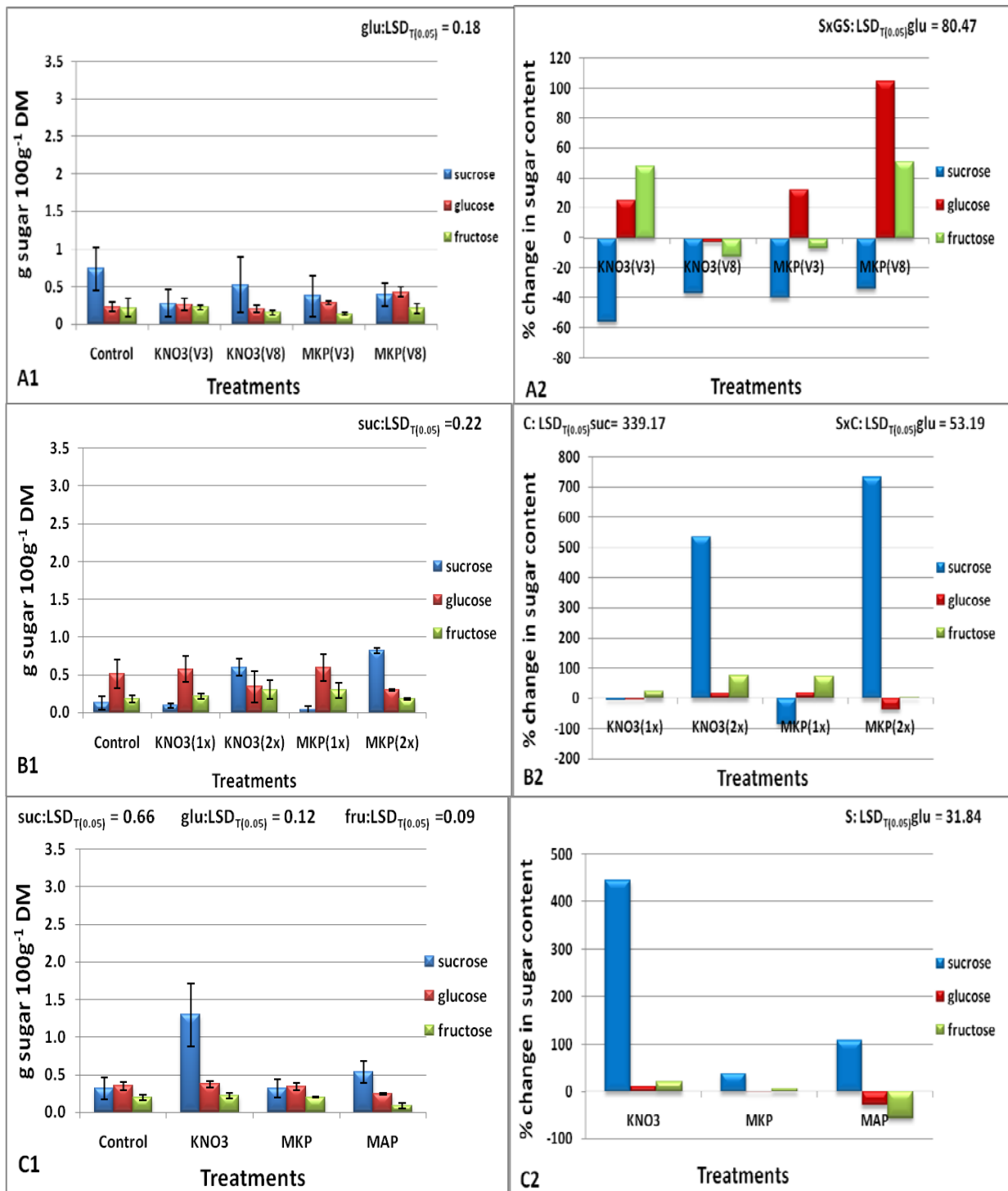**Figure 4.11:** Sucrose, glucose and fructose content in maize kernels from the oldest (upper most) ear at harvest. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1(suc): CV = 57.2%; A1(glu): CV = 22.56%; A1(fru): CV = 37.45%; B1(suc): CV = 23.41%. B1(glu): CV = 22.36%; B1(fru): CV = 26.81%; C1(suc): CV = 37.82%. C1(glu): CV = 13.01%; C1(fru): CV = 19.1%.

During the 2006/07 season rather contrasting results were obtained with the 3% (1x) foliar application of KNO_3 applied at V8, compared to the previous two seasons, in terms of kernel sucrose content at harvest (Figure 4.11C1). This treatment was the only one that contributed to significantly higher sucrose in kernels compared to all other treatments and the control. Moreover, a significant reduction in glucose and fructose content in kernels of plants treated with MAP was observed compared to the control and all other treatments at harvest (Figure 4.11C1). The expression of data as a percentage change from the control revealed that the MAP (V8) treatment applied during the third season contained significantly less glucose and tended to significantly contain less fructose in kernels analyzed at harvest, compared to all other treatments (Figure 4.11C2). It is acknowledged that sugar content constantly changes in kernels as they develop towards maturity. The main reason is the conversion of sugars to starch. In this light sugar data for the disaccharide sucrose and its two monosaccharide moieties, glucose and fructose, was pooled in order to compare total sugar content with starch production in the following section.

4.3.9 Total sugar content in maize kernels at the milk, soft dough and hard dough stages as well as at harvest

As expected the total sugar content decreased as kernels developed towards maturity and, although not in exactly the same fashion for the different treatments, this was observed in all seasons. In season one (2004/05) application of KNO_3 contributed towards significantly higher total sugar content at the milk stage of kernel development when applied at both V3 (+95%) and V8 (+80%), compared to the control and MKP applied at V8 (Figure 4.12A1). The same trend was observed for the two MKP treatments at the milk stage, but this was not significant when applied either at V3 or V8. The latter treatment, however, tended towards significance. In expressing the data obtained at the milk stage as a percentage difference from the control, the above observations from raw data was supported for both the KNO_3 and MKP treatments while a significant interaction between salt and growth stage for the KNO_3 (V3) treatment was confirmed (Figure 4.12A2). Although the total sugar content continued to be at a higher level in kernels at the soft dough stage for all treatments, compared to the control, the differences were not statistically significant whether expressed as mean raw data (Figure 4.12A1) or as a percentage difference from the control (Figure 4.12A2). Interestingly, the kernels of plants treated with MKP at V8, contained a markedly higher amount of total sugars at the hard dough stage of grain filling during the first season (Figure 4.12A1), compared to the control (Figure 4.12A2). At harvest, although the total sugar content in kernels was less than in the control
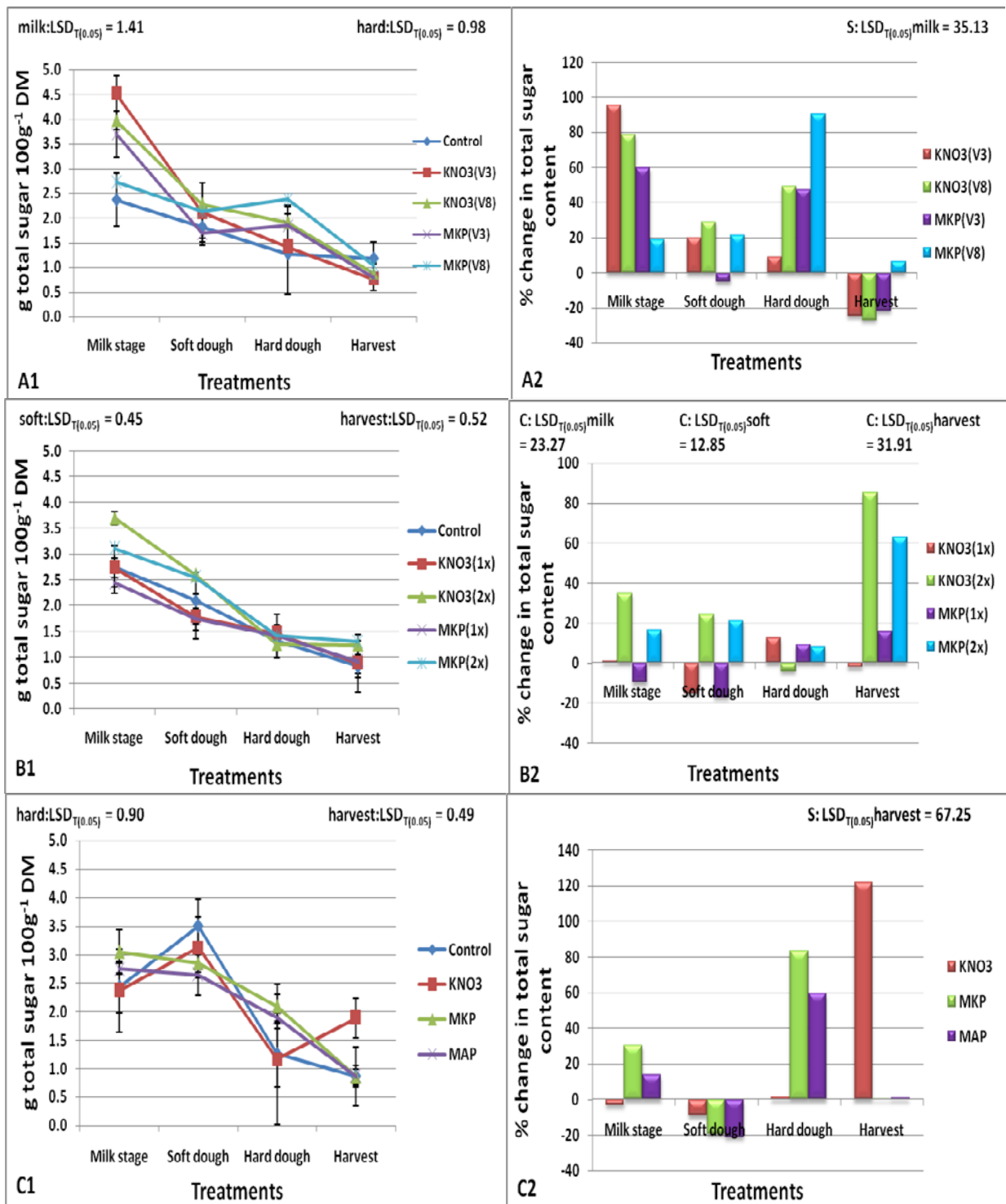
Figure 4.12: A-C) Total sugar content of the kernels for the milk, soft dough, hard dough stages and harvest of the maize plant. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1(milk): CV = 14.43%; A1(soft): CV = 20.35%; A1(hard): CV = 19.81%; A1(harvest): CV = 33.53% B1(milk): CV = 16.15%; B1(soft): CV = 7.4%; B1(hard): CV = 11.14%; B1(harvest): CV = 17.44%; C1(milk): CV = 17.24%; C1(soft): CV = 28.38%; C1(hard): CV = 19.98%; C1(harvest): CV = 15.43%

for all treatments except MKP applied at V8, the differences were not statistically significant during season one (Figure 4.12A1 and Figure 4.12A2).

During the 2005/06 season, application of KNO_3 at V8 and at the single rate did not contribute to exceptionally high total sugar levels at the milk stage as was the case during the previous season (Figure 4.12B1). However, application of this salt at double the rate tended towards the same trend, as did the MKP (2x) treatment, but differences were not significant in either of the two cases (Figure 4.12B1), compared to the control (Figure 4.12B2). At the soft dough stage, the same trend prevailed for both KNO_3 and MKP applied at V8 and at double rates (Figure 4.12B1) while the differences were significant in both cases confirming the main effect in terms of concentration (Figure 4.12B2). At the hard dough stage, no significant difference between treatments (Figure 4.12B1) as well as between treatments expressed as a percentage change for the control (Figure 4.12B2) were observed in terms of total sugar content. Expression of data as a percentage change from the control confirmed the significant main effect in terms of concentration at the double (2x) rate for both salts versus the corresponding single (1x) rates (Figure 4.12B2).

Compared to the control, none of the treatments at the V8 growth stage contributed to significant differences in the total kernel sugar content at both the milk and soft dough stages during the 2006/07 season (Figure 4.12C1). Although foliar application of both MKP and MAP increased the total sugar content markedly in kernels at the hard dough stage with 81% and 60% respectively, the difference from the control was only significant in case of the MKP treatment (Figure 4.12C1). However, expressing data as a percentage change from the control revealed no significance for any treatment in terms of total sugar content at either the milk, soft or hard dough stages (Figure 4.12C2). At harvest, only kernels from KNO_3 treated plants contained significantly higher total sugar contents (Figure 4.12C1), while a significant effect for KNO_3 applied at 1x during V8 in terms of total sugar content was confirmed (Figure 4.12C2).

The breakdown of the disaccharide sugar (sucrose) to its monosaccharide moieties (glucose and fructose), and the conversion of the latter into starch is the ultimate metabolic process responsible for increasing kernel mass and indirectly yield. In 4.3.10 the latter was further investigated.

4.3.10 Total starch content in maize kernels at the milk, soft dough and hard dough stages as well as at harvest

Compared to the control, foliar application of both KNO_3 and MKP at V3 as well as KNO_3 at V8 contributed to significantly higher starch content in kernels at the milk stage during 2004/05 (Figure 4.13A1). The latter was more accentuated for the earlier (V3) than the later applications (V8), an aspect that was confirmed when data was expressed as a percentage change from the control (Figure 4.13A2) where a significant main effect for early treatments (V3) versus later (V8) treatments with regards to starch content was observed. A second significant main effect in terms of salt was also noted, whereby KNO_3 contained significantly more starch in kernels at milk stage versus MKP treatment (Figure 4.13A2). At the soft dough stage, the foliar application of KNO_3 at both V3 (significantly) and V8 (significantly) as well as application of MKP at V3 (significantly) and V8 (non-significantly) contributed to lower kernel starch content (Figure 4.13A1). When data was expressed as a percentage change from the control a significant interaction effect between treatment and growth stage, to lower starch content, was again confirmed in case of KNO_3 (V3), KNO_3 (V8) and MKP (V3) treatments (Figure 4.13A2). At the hard dough stage of ear development no significant differences in kernel starch content between treatments (Figure 4.13A1&A2) were observed. Interestingly, at harvest all the foliar fertilizer treatments contributed towards significantly higher starch content in kernels irrespective of salt or application time (Figure 4.13A1), but significance was not confirmed when data was expressed as a percentage change from the control (Figure 4.13A2).
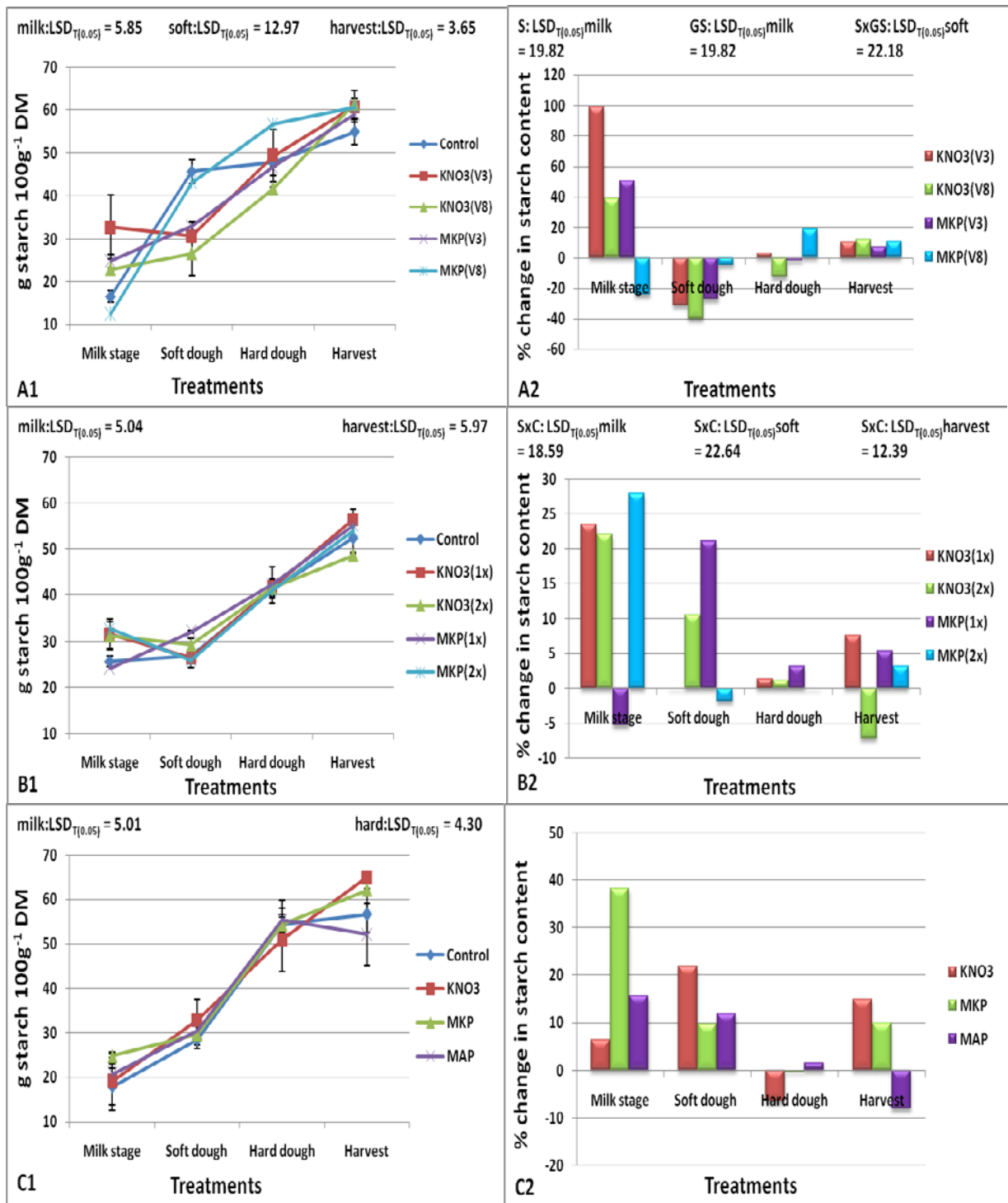
Figure 4.13: A-C) Kernel starch content of the oldest (upper most) ear at the milk, soft dough, hard dough stages and harvest of the maize plant. A1 = 2004/05 season (sprayed at V3 and V8), B1 = 2005/06 season (sprayed only at V8) and C1 = 2006/07 season (sprayed only at V8). A2, B2 and C2 are corresponding Figures based on statistical analysis of percentage differences from controls. GS = growth stage main effect; S = salt main effect, GSxS = growth stage and salt interaction, C = concentration main effect and SxC = salt and concentration interaction. Error bars in Figures A1, B1 and C1 represents standard deviations between replicates from the mean for each treatment. LSD values for different treatments are indicated in graphs. A1(milk): CV = 9.48%; A1(soft): CV = 12.83%; A1(hard): CV = 12.28%; A1(harvest): CV = 2.18% B1(milk): CV = 6.16%; B1(soft): CV = 9.49%; B1(hard): CV = 4.96%; B1(harvest): CV = 3.97%; C1(milk): CV = 8.57%; C1(soft): CV = 18.66%; C1(hard): CV = 2.83%; C1(harvest): CV = 7.9%.

During the 2005/06 season, foliar application at V8 of both KNO_3 and MKP at double rates contributed to noticeably higher starch contents at the milk stage of kernel development (Figure 4.13B1) and this was statistically significant in both cases when compared to the control (Figure 4.13B2). The same significant difference in starch content at the milk stage was observed between KNO_3 applied at the single rate and the control while this was not the case for MKP applied at the single rate both when means of raw data (Figure 4.12B1) and calculated percentage difference data (Figure 4.13B2) were plotted. By expressing data as a percentage change from the control (Figure 4.13B2), a significant interaction was only noted for the MKP (2x) treatment to increase starch content during the milk stage. During the soft dough stage of ear development, no significancant differences in starch content were found, compared to the control (Figure 4.13B1). However, when data was expressed as a percentage change from the control, a significant interaction between salt and concentration, in terms of starch content, was acquired for the MKP (1x) treatment compared to the same salt applied at 2x and the control (Figure 4.13B2). At harvest, treatment with both KNO_3 and MKP at the single rate contributed to a significantly higher starch content in kernels compared to the response of the double KNO_3 treatment (Figure 4.13B1). A significant interaction was confirmed for the KNO_3 (2x) treatment between the salt and concentration in terms of the decrease in starch content at harvest, compared to both the KNO_3(1x) and MKP(1x) treatments (Figure 4.13B2). Although not significant, the starch producing response to treatment with MKP at the double rate was markedly lower compared to application at the single rate (Figure 4.13B2).

During the third (2006/07) growing season all foliar treatments at V8 showed a positive tendency towards elevating starch content in kernels at the milk stage of development, compared to the control, but this was only significant for the MKP (1x) treatment (Figure 4.13C1). No significant differences between treatments in terms of starch content were observed at either the soft dough stage of kernel development as well as at harvest (Figure 4.13C1; 4.13C2). Only during the hard dough stage, did MAP applied at V8 result in kernels containing a significantly higher starch content, compared to the KNO_3(1x) treatment also applied at V8 (Figure 4.13C1).

In summary, results discussed in the previous chapter (chapter 3) as well is in this chapter strongly indicated that MKP, foliar applied to maize at the V8 growth stage and at a concentration of 4% (w/v; 1x), more consistently tended towards positively influencing morphological and physiological aspects related to final yield in at least two of the three test seasons when compared to other treatments. As a result a commercial field trial, additional to statistical field trials over three seasons

(chapter 3), was conducted. However, based on a report by Chapagain *et al.* (2004) where MKP applied to tomatoes three times together with an adjuvant at a 1% (w/v) concentration during the growing season increased plant height, leaf Chl content and mineral content while enhancing yield, MKP was applied to maize once at V8 and at a 2% (w/v; 0.5x) solution. Only final yield was followed and reported in the next section.

4.3.11 The effect of a 2% (w/v) foliar application of MKP on the average kernel yield of maize by means of a commercial field trial during the 2007/08 growing season

Foliar application of MKP at a 2% concentration disclosed a positive tendency towards increasing kernel yield by 589 kg ha^{-1}, compared to the control. However, due to large standard deviations between replicates for the control this difference was not significant (Figure 4.14).
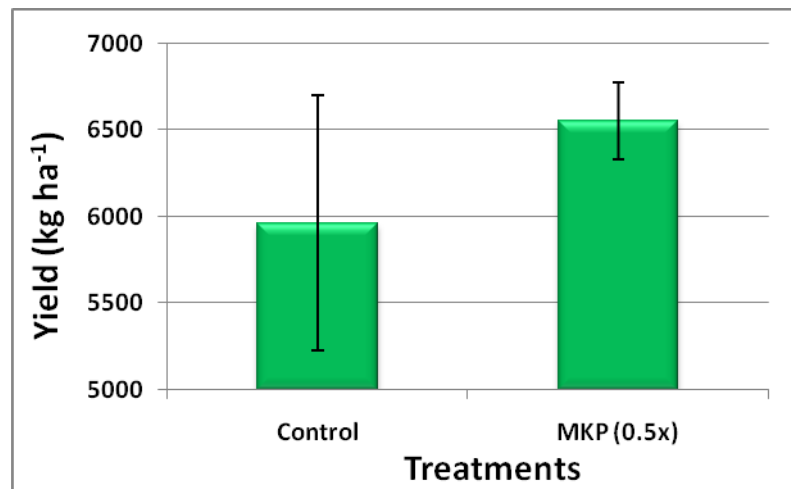
Figure 4.14: Maize grain yield obtained with a commercial field trial during the 2007/08 growing season where MKP was foliar applied at V8 at a 2% concentration. CV = 5.83%.

Due to a consistent tendency of MKP foliar applied at V8 at different concentrations to contribute towards enhancing maize yield, different plants were sprayed with a concentration range [2% (0.5x), 4% (1x), 8% (2x)] at the same growth stage while P and K content in leaves was analyzed as an indication of uptake by the leaves under glasshouse conditions (4.3.12).

4.3.12 P and K content in leaves 24 hours after foliar application of MKP at different concentrations and at the V8 growth stage

Foliar application of a 2% (0.5x) MKP solution contributed to a significant increase (30.8%) in leaf P content after 24 hours compared to leaves detached from control plants (Figure 4.15A). Thirteen percent less P accumulated in leaves when MKP was applied at a 4% concentration (1x; compared to the 0.5x treatment) while application of an 8% solution significantly reduced P accumulation by 42% compared to the control (Figure 4.15A).

In the case of K accumulation in leaves no significant differences between the control and neither the 2% (0.5x) nor the 4% (1x) foliar applied MKP solutions were observed (Figure 4.15B). However, foliar application of an 8% (2x) MKP solution contributed to significantly less (10%) K accumulating when compared to all other concentrations, including the control (Figure 4.15B).
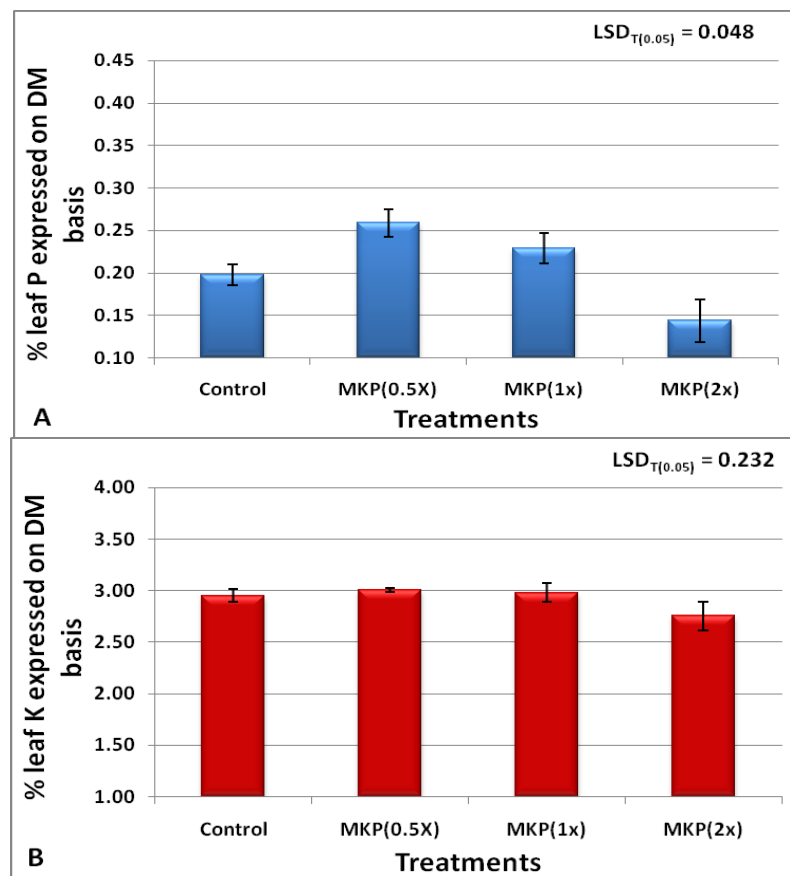
Figure 4.15: Percentage leaf A) P and B) K content expressed on a dry mass (DM) basis 24 hours after foliar application of a 2%, 4% and 8% MKP solution at the V8 crop growth stage. CV (A) = 8.82%; CV (B) = 3.04%.

According to Eichert and Burkhardt (2001) the uptake of fertilizer nutrients through leaves increases linearly with an increase in humidity, stomatal aperture and stomatal density. The authors concluded that penetration of nutrients into the leaf was mainly through stomatal pores and is strongly correlated with the number of stomata. The observed negative effect of P and K uptake by maize leaves following MKP application, at the highest (8%) concentration, prompted a qualitative investigation of the stomatal response to foliar application of MKP at 2, 4 and 8% concentrations.

4.3.13 The effect of MKP foliar applied at different concentrations on the appearance of stomata in the upper leaf 24 hours after application

Compared to stomata from water treated control leaves (Figure 4.16A), the guard cells of stomata from leaves treated with both the 2% (0.5x; Figure 4.16B) and 4% (1x; Figure 4.16C) MKP solutions maintained their appearance and turgor pressure relatively well and it was difficult to distinguish between the two. Nevertheless, MKP applied at both these concentrations contributed to a slight reduction in guard cell volume, especially at the site of attachment where the cellulose microfibrils and guard cells connect to the epidermal cell walls. However, the 8% (2x) MKP solution caused clearly visible dehydration and plasmolysis of guard cells (Figure 4.16D). In terms of guard cell size and stomatal aperture the stomatal structure was visibly impaired by the 8% MKP solution and this was concomitant with low P and K accumulation in these leaves (Figure 4.15).
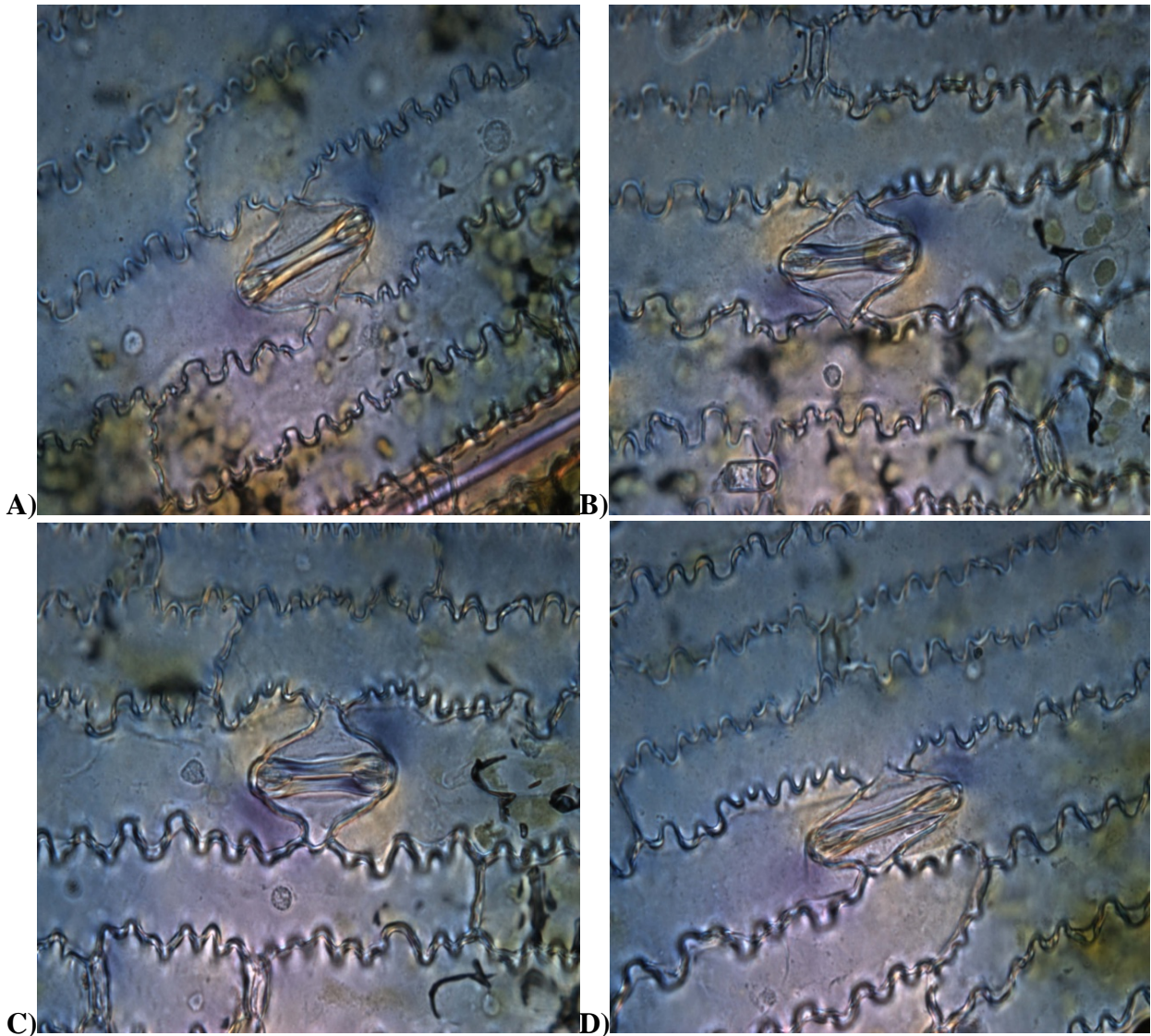
Figure 4.16: Micrograph of the adaxial epidermal cells and leaf stomatal complex for the last unfolded leaf 24 hours after foliar application of A) distilled water, B) a 2% MKP solution, C) a 4% MKP solution and D) an 8% MKP solution at the V8 crop growth stage.

4.4 Discussion

The main aim of this study was to follow the physiological response of maize to foliar applied inorganic fertilizers under rain fed conditions, as outlined in chapter 3. Selected physiological parameters including Chl content and Chl *a* fluorescence as well as sugar and starch content in kernels at different development stages. These parameters were chosen due to the association between the carbohydrate producing process, photosynthesis, in the source (leaf) and the carbohydrate that are finally translocated to the harvestable sinks (kernels).

From an agricultural perspective photosynthesis is the initial and primary process involved in supplying energy rich intermediates (photosynthate), *inter alia* in the form of carbohydrate sugars that are utilized for growth while the remainder, that determines yield, is eventually translocated to harvestable parts. For this reason it was necessary to quantify the photosynthesis capacity in maize plants treated with different foliar fertilizers. Chl *a* fluorescence is a useful technique to quantify photosynthesis capacity. From Chl *a* fluorescence the JIP test measures several fluxes that, for example, quantifies the absorbance of photons by the reaction centre at PSII (ABS/RC), the trapping of excited electrons (TRo/RC) as well as the transfer of electrons through the photosynthetic electron transport chain (ETo/RC) to eventually produce an energy rich molecule, NADPH (Figure 4.17). The JIP test furthermore allow calculations such as the probability for an absorbed photon to move an electron into the electron transport chain (φ_{Eo}) and the overall performance of PSII based on absorption (PI_{abs}).
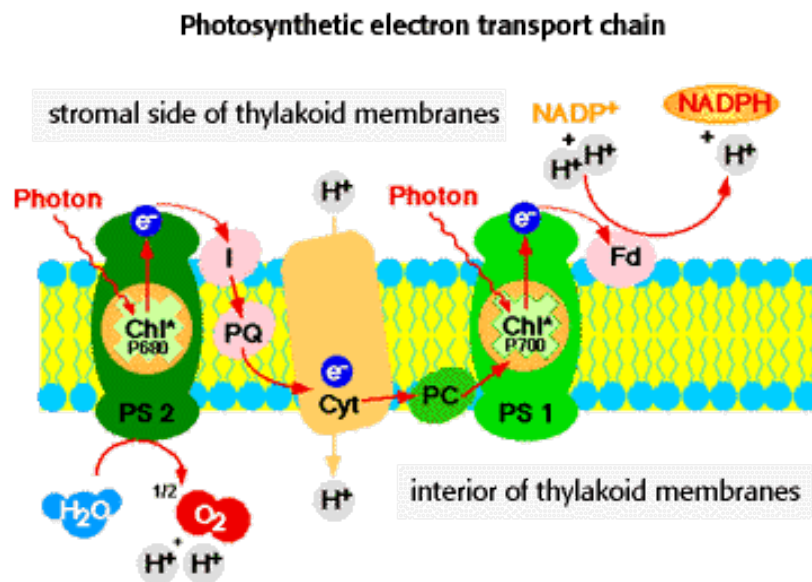
Figure 4.17: A schematic illustration of the photosynthetic electron transport chain (University of Arizona, 1996).

Chl *a* fluorescence was previously used by Earl and Tollenaar (1999) to distinguish between commercial cultivars in terms of their leaf photosynthetic rates under field conditions. The use of Chl *a* fluorescence to measure the effect of heavy metals on the efficacy of photosynthesis was studied by Jiang *et al.* in 2008. They investigated aluminium-induced effects on PSII photochemistry in

citrus leaves and found that treated leaves had a reduced φ_{Eo} and PI_{abs}, because of damage to all photo-chemical and non-photochemical redox reactions. More recently, Wang *et al*. (2008) reported that placement of N fertilizer in the seed furrow, followed by a top dressing, favourably enhanced specific Chl *a* fluorescence fluxes. In this study, the effect of macro nutrient foliar fertilizer applications at different growth stages and concentrations on ear leaf photosynthesis were measured during flowering. The time of measurement corresponded with the timeframe used by Earl and Tollenaar (1999), who reported differences in leaf photosynthetic rates when measurements were taken during the flowering stage.

Foliar applied KNO_3 tended to have the most consistent enhancement (seasons one and three) or had the least inhibitive effect (season two) on ABS/RC, TRo/RC as well as ETo/RC when applied at either growth stage V3 or V8 or either at single or double concentrations. More than two decades ago Suwanarit and Sestapukdee (1989) reported that Chl synthesis was stimulated in KNO_3 treated maize plants. From this the authors argued that KNO_3 could have contributed to increased light absorbance due to elevated Chl pigment content in leaves. In the underlying study, and in contrast, the total Chl content in leaves of plants foliar treated with KNO_3 at the single dosage rate and at V8 was consistently reduced in all three seasons, when compared to control plants. However, φ_{Eo} and PI_{abs} were neither significantly affected by KNO_3 applied at different application times nor at different concentrations, indicating that the reduction in Chl content by treatment with KNO_3 did not necessarily impair the photosynthesis capacity of the plants. This is in concert with the findings of Veberic *et al*. (2007) on apples, who reported that no significant negative effects on photosynthesis capacity (Chl *a* fluorescence) were detected after foliar application of P and K containing salts. Considering only Chl *a* fluorescence and total Chl data, it can be deduced that KNO_3 did not influence photosynthesis capacity to a large extent. However, despite of either the observation by Veberic *et al*. (2007) on apples or the one in this study on maize it cannot be assumed out of hand that no damage was done to the photosynthesis machinery in any way. This statement is based on the higher DIo/RC value when KNO_3 was applied to vulnerable small plants (V3) and at a higher (double) rate. This also corresponds with the findings of Jiang *et al.* (2008) that stressed plants protect their leaves from photo-oxidative damage by dissipating more light, particularly when chlorophyll levels are decreased. PI_{abs} being a summarized value for photosynthesis output was not affected in plants treated with KNO_3.

As it is difficult to verify the impact of a single Chl *a* fluorescence parameter on the total photosynthesis capacity in the broad sense of the word, all that remains is to measure it up to the outcome of the photosynthesis process namely sugar production. At this point it is important to note that, in this study, the production of sugars was not measured in the source (leaf) but only its accumulation in sink organs (kernels). The final sugar content in kernels, therefore, served as a means to determine the outcome or success of both sugar production and translocation simultaneously. Although the contents of sucrose as well as its two monasaccharide moieties, fructose and glucose, were measured separately, and due to fluctuating levels of these sugars as a result of, *inter alia*, conversion to starch in kernels at different development stages, only total sugar content data will be considered in this discussion for the purpose of simplifying matters.

KNO_3 foliar applied at either the V3 or V8 growth stages during the 2004/05 season contributed towards enhanced accumulation of total sugars, especially during the early grain filling stages when compared to the untreated control. Application at the double rate during the 2005/06 growth season, revealed more or less the same tendency as was observed during the first season for the single rate, but this was not repeated during the second and third growing seasons for the single rate treatment making it difficult to come to a foregone conclusion. Concomitantly, the accumulation of starch in kernels was also enhanced to a much greater extend during the earlier grain filling stages (especially during the milk and dough stages) in plants treated with KNO_3 either at V3 or V8 or at different concentration rates. Interestingly, kernels from plants treated with KNO_3 at V8 and the single rate constantly contained the highest starch content over all growing seasons compared to the rest of the treatments when measured at harvest, but this did not correspond with an increase in yield (chapter 3). The latter strongly indicates that yield differences could rather be attributed to differences in kernel number than kernel mass.

When considering the physiological response of maize plants to foliar applied MKP, it must be taken into account that this salt produces both K^+ and $H_2PO_4^-$ ions on dissociation. Again, independent of application growth stage or concentration rate, foliar applied MKP contributed towards the reduction in total Chl content (light harvesting complexes) during all growing seasons, as was the case where KNO_3 was applied. Due to the light energy binding function of Chl, a positive correlation exists between photosynthesis capacity and leaf Chl content while Chl concentration is one of the factors directly affecting photosynthesis rate (Rajcan *et al*., 1999). According to the authors, defoliation of maize plants starts between three and seven weeks after silking and this is normally associated with a

decline in Chl content in the ear leaf. For this reason, Chl readings were taken at the onset of the flowering stage in this study, in order to make sure that it was still detectable. A logical deduction would be that reduced Chl levels would decrease the photosynthesis rate, and a strong indication in favour came from results obtained in terms of specific photosynthetic fluxes.

Again irrespective of application growth stage or concentration and compared to the control, foliar applied MKP contributed to a relative consistent tendency towards either having no effect on the measured photosynthetic fluxes or in some cases significantly reducing them. This was especially the case during seasons two and three and was, to a large extent, in concert with the response of maize plants to treatment with KNO_3. From this it seems obvious to deduce that foliar applied MKP, in general, had a negative effect on the capacity of the photosynthetic reaction centre and hence the generation of energy (NADPH) needed for sugar synthesis during the Calvin cycle. However, in contrast, during season one MKP applied at the V8 growth stage and at the single rate enhanced the φ_{Eo} of treated plants by 10%, compared to control plants, indicating an increase in the probability for an absorbed photon to move an electron into the electron transport chain for the production of NADPH. PI_{abs} data not only confirmed the φ_{Eo} data obtained in leaves from MKP (V8) treated plants during the first season, but also revealed a tendency for MKP to have an enhance the overall photosynthetic performance output during all three seasons, independent of application growth stage and concentration.

Because of no constant differences between seasons regarding the MKP effect on photosynthesis capacity it was once again difficult to link Chl content and fluorescence data alone to an extrapolated possible final outcome in terms of photosynthate production. As a result sugar content data was once again used as a criterion.

Compared to all other treatments and the control, the separate measurement of sucrose, glucose and fructose content in kernels of the oldest ear revealed a tendency (in some cases significant) for MKP to have contributed to enhanced levels of either the monosaccharides glucose and fructose or the disaccharide sucrose during early grain filling stages. However, as expected, the same tendency for MKP to enhance the levels of individual sugars in kernels was also observed for the total sugar content. As sucrose is the form in which carbohydrate is translocated from the source to sinks in the vast majority of plants (Lemoine, 2000) due to its non-reducing nature (Arnold, 1968), it seemed

necessary at this point to elaborate somewhat on its translocation and the possible role the K and P containing salt, MKP, could have played in this regard.

For sucrose to eventually accumulate in harvestable parts (sinks), the transport of inorganic phosphorous (Pi), glycerate-3-phosphate and triose phosphates (Calvin cycle intermediates) across the chloroplast envelope must initially take place (Anderson and Beardall, 1991). According to the authors the availability of Pi in the cytosol controls the export of triose phosphate from chloroplasts, and therefore sucrose synthesis and indirectly the amount of sucrose loaded into the phloem to be transported to sink organs (Figure 4.18).
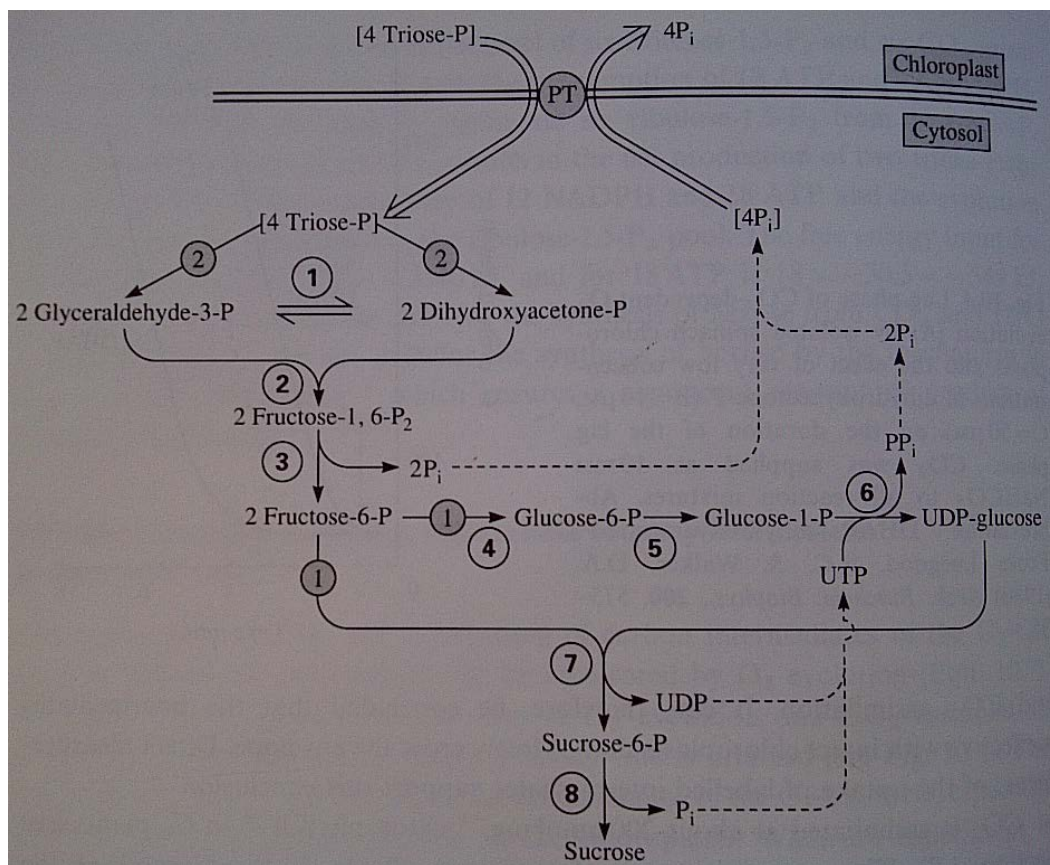
Figure 4.18: Pathway for the synthesis of sucrose from triose phosphate in the cytosol of photosynthetic cells. The reaction sequence four triose phosphate and one UTP with the net production of four Pi and one sucrose. Enzymes are: (1) triose-P isomerase; (2) aldolase; (3) fructose-1,6-P2 phosphatase; (4) hexose-P isomerase, (5) glucose-P mutase; (6) UDP-glucose pyrophosphorylase; (7) sucrose-P synthetase; (8)sucrose-P phosphatase. Triose phosphate is imported into the cytosol from the chloroplast via the phosphate translocator (PT) in exchange for an equimolar amount of Pi (Anderson and Beardall, 1991).

From the important role Pi plays in the transport of triose sugars over the chloroplast membrane (Anderson and Beardall, 1991) and the eventual conversion to sucrose in the cytosol, it can be argued

that the additional supply of Pi from MKP might have played a role in the amount of triose sugars reaching the cytosol in leaf tissue, the amount of sucrose produced and the amount of sucrose translocated to kernels during the productive phase. In kernels of maize plants foliar treated with MKP at V8 and at the single rate the amount of sucrose transported to kernels, especially during later grain filling stages (hard dough), was markedly higher compared to control plants. The conversion of sugars into starch and its accumulation in the kernels, as influenced by the application of MKP, revealed more or less the same tendency as for total sugar content, whereby MKP especially applied at the single rate and at V8, consistently contributed to a higher starch content in kernels at harvest, during all seasons, compared to the control plants. In contrast to the KNO_3 treatment, the latter coincided with a substantial increase in final yield (chapter 3).

During the third season MAP was foliar applied to maize plants in an attempt to verify the possible role of P in affecting selected physiological parameters as this salt lacked the presence of K. As was the case with KNO_3 and MKP, the MAP treatment also contributed to a marked reduction in leaf Chl levels which might have caused the light harvesting complexes to function sup-optimal compared to control plants. In contrast, Ling and Silberbush (2002) reported a positive response to foliar applied N, P and K (0.12 g N L^{-1}, 0.08 g P L^{-1}, and 0.06 g K L^{-1}) fertilizers in terms of Chl content, while no significant differences were observed between different salt forms. Moreover, no explanation could be found in literature for the fact that foliar applied nutrients in this study tended to reduce Chl content rather than promote it. It can, however, be speculated that the application of salts might have created a micro-climate imitating drought conditions on the top surface of the leaves, due to a lower osmotic potential ($\Psi\pi$), which might explain the typical Chl reducing reaction as was also observed by Efeoglu *et al*. (2009) under drought conditions. At this point it is important to note that environmental drought conditions prevailed during the third season when MAP was applied.

Surprisingly, despite the reducing effect of foliar applied MAP on Chl content, it had the opposite effect to that of MKP on photosynthetic fluxes inasmuch as it had a marked elevating influence on all of the measured photosynthetic fluxes, of which differences were significant in some cases. However, the MAP treatment contributed to a marked, but not significant, reduction in both φ_{Eo} and PI_{abs}. Therefore, although the photosynthetic fluxes were enhanced in plants treated with MAP, the probability that an absorbed photon will move an electron into the electron transport chain (φ_{Eo}) as well as the overall performance output (PI_{abs}) was markedly reduced. In general Efeoglu *et al.* (2009)

found that drought (as was experienced during the 2006/07 season) caused a significant decrease in several photosynthetic flux ratios. This was, however, not observed in this study during the third season, compared to the first two seasons.

The fact that Chl content, φ_{Eo} and PI_{abs} were suppressed in the leaves (source) of foliar MAP treated plants did not seem to prevent the transport and eventual accumulation of sugars in the kernels (sinks). As was the case with the MKP and KNO_3 treatments, kernels from plants treated with MAP contained higher sucrose and total sugar levels except at the soft dough stage of grain filling. Interestingly, kernels from plants treated with MAP contained elevated total sugar levels at both the hard dough stage and at harvest, but low starch content at corresponding stages. From this it is deduced that the conversion from sugars to starch in these kernels were either delayed or impaired. It can be speculated that this may have been the result of the drier climatic conditions that prevailed during the 2006/07 season. The process of starch formation in amyloplasts is an energy (ATP) consuming process (Figure 4.19) and the possibility exists that plants were under a form of energy stress during the third season, an aspect which is supported by morphological and yield data (chapter 3).
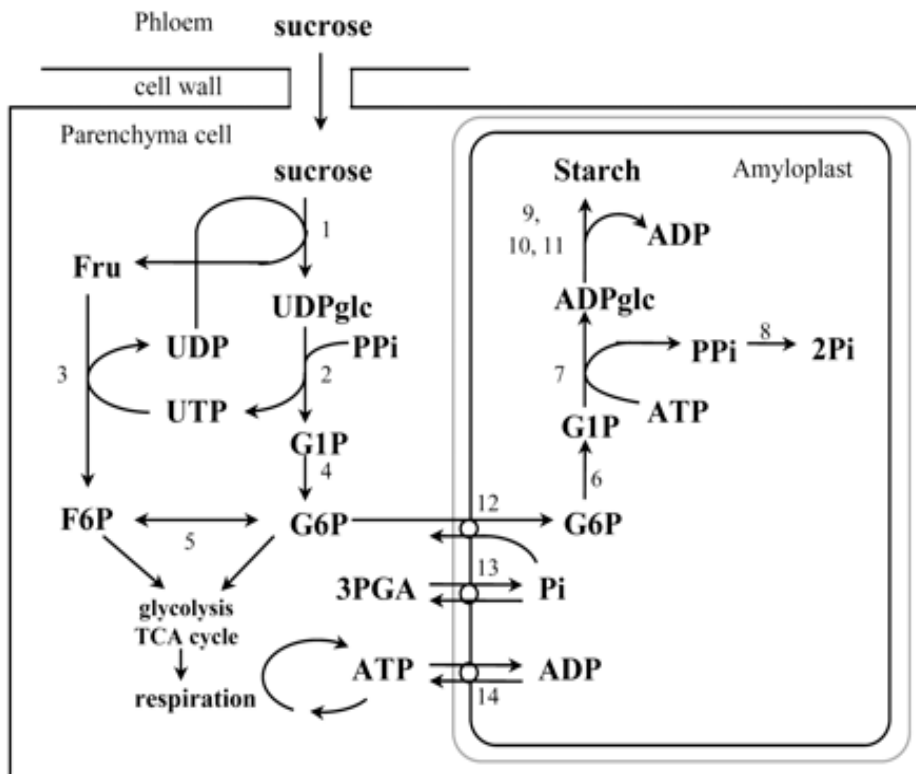

Figure 4.19: Assimilate transport, breakdown and formation of starch in the non-pigmented starch storage organelle called the amyloplast (Anonymous, 2010).

In this regard, Ankorion (1995) reported that sugar and starch levels were increased in the harvestable parts of a variety of crops following MKP application via the soil, but the authors did not attempt to explain the phenomenon on the basis of photosynthesis involvement. Moreover, no references in literature could be traced dealing with foliar applied KNO_3, MKP or MAP in terms of the possible influence of these salts on sugar and starch content in kernels at different development stages during the grain filling period. A further complicating factor is that sugar production is only one aspect that needs to be considered while the eventual translocation of sugars to harvestable parts is probably equally critical.

In this study, understandably, sucrose was the most abundant sugar in kernels during the early grain filling stages as it is the form in which carbohydrate is translocated. The application of both KNO_3 and MKP contributed to elevated sucrose levels in kernels, especially during the milk and soft dough stages of kernel development, indicating that both treatments must have had a promoting effect on sucrose translocation into the kernels. The fact that both salts had no significant effect on promoting photosynthesis capacity, supports the notion that sugar translocation and not sugar production is probably primarily responsible for the observed differences in kernel sugar content. Although the total sugar content decreased while the starch content in kernels increased steadily from the milk stage up to harvest, as could be expected due to conversion, the sucrose content tended to remain highest where the two salts were applied, compared to the control plants. In light of the fact that sugar and starch contents were measured in maize kernels (sinks) and not leaves (source) in this study, a discussion on the translocation of sucrose from the source to the sinks, as well as the conversion of sugars to starch, follows.

In terms of K involvement in sugar translocation in plants, Bush and Li (1990) reported that high concentrations of K stimulated the rate of sucrose transport four fold in isolated plasma membrane vesicles. The authors explained that an electrogenic (charged) process caused by K^+ protons, through which the co-transport of sucrose and protons mediated a positive membrane potential, were responsible for sucrose transport across membranes. Results obtained in this study confirmed previous reports on the ability of K containing salts to enhance the transport of sugars into kernels (Buckhout, 1989; Bush and Li, 1990).

As far as the role of P in sucrose translocation and accumulation in sinks are concerned, data obtained in this study revealed the potential to manipulate the before mentioned processes in plants

by treatment with Pi containing salts, MKP and MAP. Besides the role of Pi in the transport of triose sugars from the chloroplast to the cytosol (Anderson and Beardall, 1991), information on its role in the translocation of sucrose from source to sinks within the plant is rather scarce, an aspect that will be dealt with in chapter 5. However, once sucrose reaches the sink organ, the enzyme sucrose synthase (E.C. 2.4.1.13) catalyzes the conversion of sucrose and UDP to fructose and UDP-glucose, while invertase (EC 3.2.1.26) catalyzes the hydrolysis of sucrose to glucose and fructose (Doehlert and Chourey, 1990). According to the authors, both glucose and fructose are uncompetitive inhibitors of sucrose degradation due to the latter sugars binding to the enzyme-UDP complex. It is also known from previous studies (Thomas *et al.,* 1987) that maize kernel endosperm can directly acquire sucrose during phloem unloading and it is not a prerequisite for sucrose to be hydrolyzed by invertase to hexose sugars for uptake by sink organs.

According to Doehlert (1990) one molecule of pyrophosphate (PPi) is required for the conversion of sucrose into hexose (glucose and fructose) phosphates via the sucrose synthase pathway (Figure 4.20), and this is a possible metabolic reaction where inorganic phosphates (P) (supplied by MKP and MAP) could have influenced the sugar/starch pathway. The enzyme ADP-glucose pyrophosphorylase (AGPase) subsequently converts ADP-glucose to glucose-1-phosphate (G-1-P) and then to glucose-6-phosphate (G-6-P), which is transported into the amyloplast in exchange for Pi (Figure 4.19, reaction number 12). This is another possible metabolic reaction, requiring the presence of Pi, which might also have had an influence on the sugar/starch pathway.
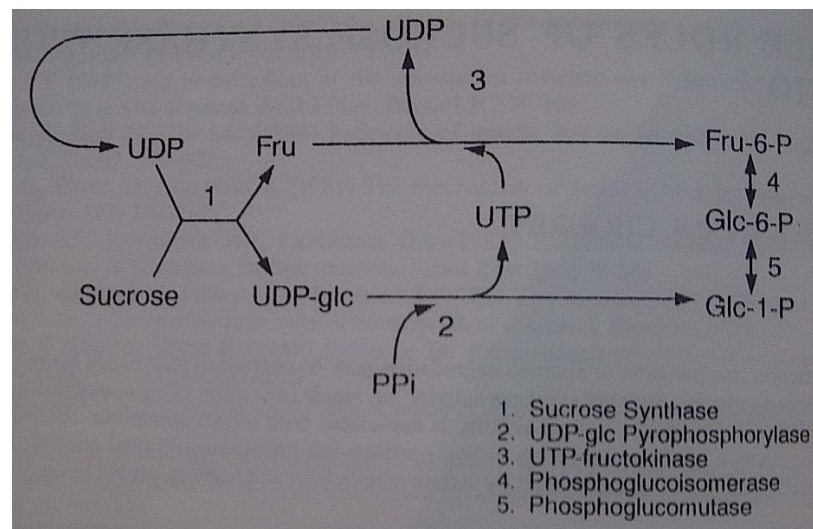

Figure 4.20: A diagrammatic representation of sucrose synthase pathway involving the breakdown of sucrose to fructose and UDP-glucose (glc) (Doehlert and Chourey, 1990).

AGPase is regulated by 3-phosphoglycerate and inorganic P (Pi; Stark *et al.*, 1992) and this is generally regarded as the rate-limiting step in starch biosynthesis. Based on the importance of a rate limiting step and the role Pi can play, additional Pi supplied in the form of either MKP or MAP or both might have contributed towards enhanced starch formation. Although a relative erratic tendency was observed for especially MKP in terms of starch accumulation during the early grain filling stages, kernels from plants foliar treated with MKP specifically at V8 and at the single (4% solution) rate contained on average (over three seasons) about 8.6% more starch at harvest, compared to kernels from control plants. Evidence do exist that sink biosynthetic activity is affected by the quantity and composition of nutrients supplied by the phloem to the sink tissues (Singletary and Below, 1989).

In considering the N moiety of KNO_3, Singletary and Below (1989) reported that the *in vitro* supply of N to maize kernels under laboratory conditions stimulated sucrose synthase activity several fold in 20 day post pollination endosperm as well as starch accumulation measured in the mature kernels. In this study, interestingly, mature kernels from plants foliar treated with KNO_3 at V8 and at the single (3% solution) rate, contained on average 11% more starch (over three seasons) harvest compared to kernels from control plants. Starch values calculated in this study at harvest corresponded with that reported by Szyszkowska *et al.* (2007) for mature maize kernels, which ranged from 536 to 573 g kg^{-1} on a DM basis.

Slight differences in the response of maize plants to the different concentrations at which salts were applied in this study as well as the thumb rule recommendation that the concentration of salts applied to leaves should not exceed 2%, initiated the question whether either of the two concentrations could have had an effect on the stomatum structure. Further, Neumann *et al.* (1981) reported that minor differences in the chemical structure of applied foliar fertilizers to intact leaves had large effects on the threshold concentration above which damage was induced. The authors demonstrated that the penetration of K_2HPO_4 and KH_2PO_4 through the intact cuticle of secondary leaves occurred at similar rates and that uptake was dependent on salt concentration. They concluded that foliar damage can also occur when fertilizer is foliar applied at relatively low concentrations and the possible cause is a pH-related toxic effect or simply plasmolysis. In this regard Gamble and Emino (1987) foliar applied urea to maize plants at a concentration of 120 g N L^{-1} with the aim to record leaf burning. A 4 µL drop of the latter solution was applied to the adaxial leaf surface and damage was observed under the dissecting microscope after 2 h. The damage consisted of a darkening in the epidermal

cells. After eight hours they also observed that the epidermis appeared desiccated, sunken and discoloured while a lesion formed on the leaf surface. The authors concluded that events associated with visual damage appeared to be related to water loss resulting in a drop in turgor pressure, since epidermal and mesophyll cells became desiccated.

In order to verify the possibilities outlined above, maize leaves were treated with MKP at 2% (½x), 4% (1x) and 8% (2x) concentrations while the effect on especially the stomatal complex was followed qualitatively under a light microscope. Micrographs of the adaxial epidermal cells and the leaf stomatal complex for the last unfolded leaf 24 h after application of MKP at different concentrations showed a tendency similar to that observed by Neumann *et al.* (1981). These included a concentration dependant response in terms of the amount of, especially, P taken up by the leaves and the appearance of the stomatal complex. The 2% and 4% MKP solution had no visual effect on the appearance of stomata while the 8% solution clearly contributed to disfigurement of guard cells probably due to extensive water loss and consequent plasmolysis. However, this double rate did not seem to have a significant negative effect on either the morphological (chapter 3) or physiological response of maize plants. From this it is concluded that the visual damage to stomata by the 8% (2x) MKP treatment could have been either overcome or reversed and hence no permanent impairment of essential processes leading to the final yield occurred. In concert, a field trial conducted by Harder *et al.* (1982) on maize foliar treated with N, P, K and S containing salts at various stages of development had no effect on stomatum structure, but revealed a depression of leaf photosynthesis by up to 17% one day after application, while a complete recovery was observed by the second day.

In conclusion, except for starch content in kernels, a rather consistent relationship was observed between the influence of P contained in both MKP and MAP on Chl content in leaves as well as sugar content in kernels. On the other hand, the physiological response of maize to foliar treatment with KNO_3 did not entirely differ from the response to the two P-containing salts in such a way that the influence of K stood out above the influence of P. Compared to the other salts, MKP applied at V8 and at 4% (1x), showed a consistent tendency to enhance the photosynthetic quantum yield (φ_{Eo}) during the first season, the performance index (PI_{abs}) and the transport of sugars to kernels as well as the conversion of sugars to starch over three seasons. Further, MKP at the single rate (4%; 1x) had no effect on stomatum structure and, while the double (8%) rate contributed to a distortion of guard cells, the latter neither significantly affected physiological processes related to final yield outcome.

In an attempt to integrate the morphological, yield and physiological responses of maize plants to foliar treatment with different salts, a general discussion follows in chapter 5.

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CHAPTER 5

General Discussion

Nutrient deficiencies in plants are often recognized during the vegetative growth and development phase despite the fact that good soil fertilization practices were followed during the growing season. Moreover, the formation of insoluble compounds due to soil chemical reactions sometimes limits the availability of specific nutrients to plants. For example, soil phosphorous (P) is known to be utilized very inefficiently by plants (Barber, 1984) and according to Fageria (2009) plants absorb less than 20% of the total P fertilizer applied during a growing season due to immobilization. Therefore, appropriate management of fertilizer application is not only essential, but should also be a major matter of concern for farmers and scientists alike. In light of the pressure on, *inter alia*, crop producers to take responsibility for food security in the world and to satisfy the needs of an ever growing world population in terms of the sustainable supply of food, the efficient use of nutrients is becoming increasingly important (Kaeppler *et al.*, 1998). Further, there are also economic and environmental concerns that emanate from the abundant use of soil fertilizers which more or less compels the research community to identify possible alternatives, e.g. foliar fertilization, that has the potential to decrease the amount of fertilizer used and subsequently possible pollution of the environment.

With regard to foliar fertilization as a practice and the extent to which it should be promoted, there is currently no consensus amongst decision makers, including scientists and fertilizer agents. For example, Lewis and Kettlewell (1993) concluded that in maize and other cereals the foliar application of P salts is not recommendable while others (Ankorion, 1995; Ling and Silberbush, 2002; Li *et al.*, 2009) endorsed foliar fertilization as a viable economical method to supplement the plant's nutritional status and to promote more efficient fertilizer usage. However, some fertilizer agents consider foliar fertilization as a practise to merely initiate specific reactions in plants at specific time intervals, such as tuber initiation in potatoes, or to assist crops to overcome stress periods rather than to increase the plant's nutritional level ([1]personal communication; Dr C.J.J. Schmidt, 2011). In general, very little is currently known about the advantages or disadvantages of foliar nutrition, especially in terms of the application of

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macro nutrients on row crops such as maize cultivated under rain fed conditions. In light of this rather confusing and non-satisfying situation pertaining to the formation of a scientifically sound viewpoint on foliar fertilization as a viable practice, this study was undertaken. The principle aim was to quantify the growth, yield and physiological responses of *Zea mays* L., cv.DKC 78-15B, to three inorganic salts that are frequently used as foliar fertilizers in South Africa, namely MKP, MAP technical and KNO$_3$. Field trials were conducted using standard agronomic practices on the same land over three seasons in order to gather information regarding **i)** the optimal application stage (2004/05 season), **ii)** the concentration rate (2005/06 season) and **iii)** the contribution of either the P or K moiety (or both) in foliar applied salts towards responses measured in maize (2006/07 season). In this general discussion all of the vegetative growth, yield and physiological aspects quantified in this study for each growing season were integrated in an attempt to acquire answers to the main objectives (problem statements **i**, **ii** and **iii**) set for each season.

During the 2004/05 season, when an attempt was made to indentify the optimum growth stage **(i)** for foliar application of KNO$_3$ and MKP, a strong indication supporting the later (V8) application of both salts, as opposed to the earlier (V3) application came from the morphological parameter plant height. However, the only significant interaction between growth stage and salt in terms of plant height was for the MKP (V8) treatment that contributed to an increase of 11.5% above the control, when measured at the end of the vegetative period (V19). However, contradictory to the findings of Guatum *et al*. (1999), no correlation was observed between plant height and yield (results not shown) as influenced by any of the foliar applied salts. Subsequently, and based on a recent report by Kapanigowda *et al.* (2010) that plants which produce less tillers generate significantly higher grain yields, this aspect was evaluated more closely.

The number of tillers per plant, counted at V19 during season one, showed a tendency to be increased by both KNO$_3$ (+23%) and MKP (+53%) when foliar applied at V3 as opposed to the later application at V8 with an increase of 8% and decrease of 3% for KNO$_3$ and MKP, respectively. Further, the total length (growth) of tillers per plant expressed as a function of the number of tillers, indicated that the application of both KNO$_3$ (+45%) and MKP (+21%) at V3, stimulated not only the number of tillers produced, but also their length when measured at V19. From this it seems that application of the salts at an early growth stage not only contributed to tiller initiation, but also its subsequent growth. Based purely on the much lower number of tillers formed and the higher yield obtained from plants treated with MKP at V8 during the first two seasons, compared to all the other treatments as well as MKP application at V3, the results obtained in this study corresponded with the correlation reported by Kapanigowda *et al.* (2010).

The higher yield (+12%) obtained with MKP applied at V8 during season one, compared to treatment at V3, corresponded with an increase in grain filling of the second ear (+16%), total ear mass (+20%), kernel mass (+20%) and number of kernels (+16%) as well as higher total sugar and starch that accumulated in kernels, indicating strongly that the later application of MKP (at V8) is preferred to the earlier application (at V3). As no significant differences in leaf chlorophyll (Chl) *a* fluorescence fluxes between plants treated with MKP at V3 or V8 were observed, the higher sugar and starch accumulation in kernels of V8 treated plants could rather be ascribed to increased sucrose translocation from the source (leaves) to the sink (kernels) than to an increase in photosynthate production due to improved photosynthesis capacity, as was found in apples by Veberic *et al*. (2007). Based upon the supporting data discussed above, the decision was made to apply MKP only at the V8 growth stage in the seasons that followed. As maize plants are larger at V8 than V3 this decision was also in concert with the recommendation of Ling and Silberbush (2002) that foliar application of fertilizer to crops should proceed later in the season as a larger leaf surface area contributes to higher absorption of compounds.

In considering the other salt, KNO_3, almost the exact opposite tendency was found for yield and most of the vegetative and reproductive parameters measured as was found for the MKP treatment during the first season. This implied that treatment of maize plants at the V3 growth stage with KNO_3 proved to be optimal in terms of growth and yield. However, as the final yield was neither markedly nor significantly different from that of control plants when applied at either V3 or V8, it was decided to also apply KNO_3 at V8 during future trials in order to compare with the MKP treatment.

During the 2005/06 season the main objective **(ii)** was to identify the optimum concentration for KNO_3 and MKP as applied at the V8 growth stage. In both instances the salts were applied at single (1x; 3% for KNO_3 and 4% for MKP) and double (2x) rates. In terms of all the vegetative growth and yield parameters measured, no significant differences between the single and double rates for both salts applied at V8 were observed. Interesting, however, was the fact that the double rate of both fertilizers, applied at V8, tended to reduce plant height when measured at V13. A similar reducing tendency applied for aerial part fresh (FM) and dry mass (DM) as well as for the two yield parameters namely ear length and kernel filling (both ears) at V13. Nevertheless, although non-significant, KNO_3 applied at both the single and double rates contributed to a slight yield increase of 7%, while application of MKP at the single and double rates contributed to marked increases of 12% and 14% respectively. Further, the commercial field trial (2008/09) conducted with MKP at half (0.5x) the rate (2% solution) resulted in a 10% (+600 kg ha^{-1}) yield increase. This is in concert with the findings of Sarakhsi *et al*. (2010), who reported

that the lower concentration rate of foliar applied fertilizer resulted in the highest yield response from maize, while higher concentrations induced the opposite response. Kulczycki *et al.* (2008) concluded that the concentration at which foliar fertilizer is applied is critical in order to attain an economical increase in crop yield. Interestingly, leaf analysis for P and K uptake in this study showed that the highest level of P was from the half rate (0.5x) MKP application, followed by the single rate, while much less P-uptake occurred with the application of MKP at the double rate. A possible explanation might be the movement of water out of the guard cells due to a low water potential (π) created on the leaf surface by the higher salt concentration, leading to stomata closure that prevented P uptake (Eichert and Burkhardt, 2001). No differences in K-uptake were observed between the three different concentrations of MKP applied foliar.

From a physiological perspective, neither of the two concentration rates at which KNO_3 and MKP were applied during the 2005/06 season had a significant influence on either Chl content or photosynthesis efficacy (Chl *a* fluorescence). This is in concert with the findings of Richards (2000) who reported yield increases in maize despite the fact that no significant change in the rate of photosynthesis was apparent. Despite the latter, higher sugar levels were detected in kernels at the milk and soft dough stages as well as at harvest of plants treated with both salts at the double rates. This once again indicates strongly that the probable cause was either increased sugar transport from the source to the sinks or increased sink biosynthetic activity (Singletary and Below, 1989), rather than increased sugar production. The roles of both P (Anderson and Beardall, 1991) and K (Bush and Li, 1990) in sugar transport were confirmed previously. However, the rate at which sugars were converted to starch in kernels from plants treated with double concentrations of KNO_3 and MKP was slower compared to treatment with the single rates, indicating that sucrose was possibly imported into the kernels at a faster rate than its conversion to starch, excluding biosynthetic activity (Singletary and Below, 1989) as a major factor. In this regard, despite the report by Doehlert and Chourey (1990) that glucose and fructose are inhibitors of sucrose degradation in sinks, it was shown in maize that kernels can directly acquire sucrose during phloem unloading (Figure 5.1) while regulation by *invertase*, the enzyme involved in sucrose hydrolysis, is not a prerequisite for sucrose uptake by sink organs (Thomas *et al.* (1987), independent of starch formation and subsequent osmotic gradients.
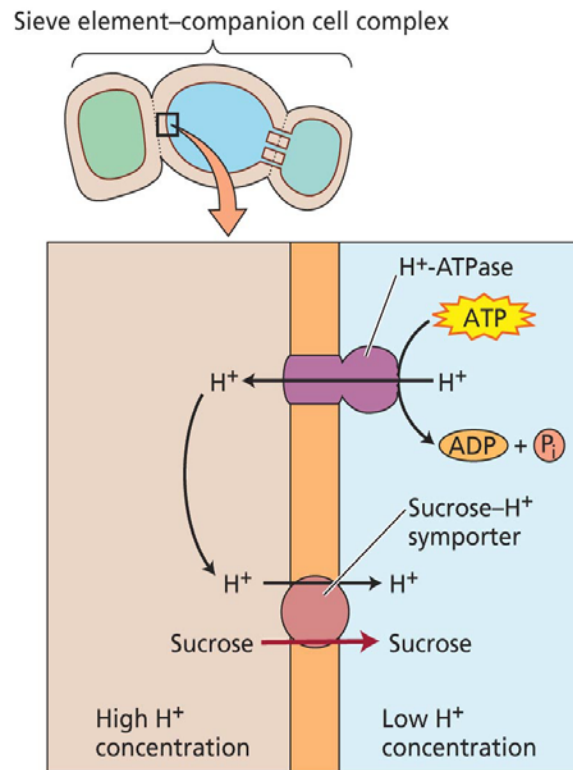
Figure 5.1: Phloem unloading of sucrose into sink cells (Anonymous, 2011).

In plants, sucrose is transported over long distance in solution in the phloem sap while the flow of sap occurs in a specialized network of cells, called the sieve elements (Lemoine, 2000). Movement of sucrose during phloem unloading is either apoplastic or symplastic. The apoplastic pathway requires active transport against a chemical potential gradient. When sucrose is unloaded into the apoplastic space, it can then be taken up as sucrose into the sink cells or cleaved by an *invertase* to hexoses that are transported by specific carriers (Büttner and Sauer, 2000). The symplastic pathway involves movement of sucrose from cell to cell over membranes and the energy for this transport is generated via a proton (H^+) gradient established by a H^+/ATPase symporter (Figure 5.1) located in the plasma membrane (Lemoine, 2000). The energy dissipated by protons moving back into the cell is coupled to the uptake of sucrose. Interestingly, an energy mediated co-transport process, driven by protons generated by a plasma membrane H^+/ATPase symporter has also been proposed for P-uptake (Pi) in suspension cultured *Catharantus roseus* cells (Sakano *et al*., 1992).

In this study foliar treatment of maize, especially with MKP, resulted in higher sugar accumulation in kernels as well a marked increase in final yield during the first two seasons. Due to the latter, and in the event that the same ATP consuming mechanism might be responsible for the movement of both sucrose and Pi over membranes in maize, it is postulated that either P alone or the synergistic effect between P and K, as it is contained in MKP, could have influenced the

movement of sucrose over membranes. The involvement of Pi in ATP production (ADP + Pi = ATP) and consumption (ATP = ADP + Pi) is well documented and ATP involvement in sucrose transport over membranes is widely accepted (Panigrahy *et al*., 2009). In further support of the postulate Ciereszko *et al*. (2005) demonstrated that sucrose and starch accumulated significantly higher in Pi-accumulating than Pi-deficient *Arabidopsis thaliana* strains, while Sicher and Kremer (1988) more than two decades ago already demonstrated that low Pi content inhibited carbon export (translocation) from the primary leaf of barley. Results obtained in this study suggest that additional Pi applied foliar can have an effect on the rate of loading and unloading of sucrose into phloem and/or sink cells.

In their recent review Panigrahy *et al*. (2009) reported that plants have developed highly specialized morphological, physiological and biochemical adaptations to acquire and utilize Pi from the environment, especially in response to low levels of available Pi. According to the authors these adaptations include many features of which the gene controlled activation of high affinity transporters and the induction of a tonoplast H^+-pumping pyrophosphatase (Wu, *et al*., 2003) relate closely to the postulate formulated higher up. Well planned and sophisticated methodology is needed in future research to verify this postulate. This should also include considering the possible role of additionally applied P to enhance movement of triose phosphates (Calvin cycle intermediates) across the chloroplast envelope to the cytosol of leaf cells in exchange for Pi (Anderson and Beardall, 1991) where it is converted to sucrose and transported to sink organs (e.g. kernels).

The set objective during the 2006/07 season **(iii)** was to verify whether P or K moieties contained in the different salts were singly or collectively responsible for morphological, physiological and/or yield effects observed during the previous two seasons. For this purpose, mono ammonium phosphate (MAP) was additionally used during the third season in order to compare the response of maize to a N and K-containing (KNO_3), K and P containing (MKP) and a N and P-containing (MAP) salt, all applied at V8 and at single (1x) dosage rates. The K-containing salt (KNO_3) caused a significant reduction in plant height when measured at V19, and a similar tendency already at V13, while both MKP and MAP had the opposite effect. This can partly be explained on grounds of the role P plays in cell division (FSSA, 2003) while K is not associated with vegetative growth *per se*. Further, MAP also contains NH_4^+ known to stimulate vegetative growth in plants (Poorter and Evans, 1998, as cited by Fageria, 2009). However, in contrast the MAP treatment tended to reduce not only tiller length growth, when expressed as a function of the number of tillers per plant, but also FM (significantly) and DM (markedly) at the end of the vegetative period (V19). Unfortunately the third season was significantly drier compared to the

first two seasons and this might have had a more severe effect on growth than any of the applied salts. Contrasting vegetative growth results obtained during the third season, most probably due to the pertaining drought conditions, hindered the separation of the K and P-containing salts in terms of their possible roles on grounds of vegetative growth alone.

Subsequent quantification of yield and physiological parameters and final yield during the rather dry third season, by including MAP as a third treatment, also did not contribute towards using this P-containing salt as a measure to distinguish between K and P involvement in the observed responses of maize during the first two seasons. All three fertilizer treatments contributed to marked decreases in kernel number, kernel mass and final yield, most probably due to the drought condition, unfortunately making it impossible to come to a foregone conclusion regarding P or K involvement. Clear signs confirming the restriction that the prevailing drought condition probably placed on the physiological processes of maize during the last season included decreases in Chl content, photosynthetic capacity and total sugar content in kernels of treated plants, especially those treated with the P-containing salts. This was in concert with previous findings of Horton *et al*. (1996) in maize under drought conditions and contrary to findings by Suwanarit and Sestapukdee (1989), who reported higher leaf Chl content where foliar fertilizer was applied under optimal conditions. Due to the unfortunate drought condition during the third season, no rationale was provided for further speculation on K or P involvement in growth and yield of maize foliar treated with different K and P-containing salts.

Despite the latter, MKP applied foliar at the V8 growth stage of maize and at a concentration of 4% was regarded as the optimum treatment in this study, as shown over the first two seasons. Whether P or K or synergism between the two elements was accountable for the responses of maize to foliar treatment with MKP measured in this study remains debatable. However, the marked yield increases obtained in statistical trials during two of the three seasons as well as in a commercial trial with this specific maize cultivar confirmed the potential of foliar applied MKP to be considered as additional nutrition at relatively low cost to the farmer.

Further support for this consideration is to be found in P itself. P is an essential element required to sustain plant life (Panigrahy *et al*., 2009) and is the most important inorganic nutrient after nitrogen (N) for plant growth while it often limits primary productivity in natural and cropping systems unless supplied as fertilizer (Vance *et al.*, 2003). It is the key substrate in energy metabolism in the form of ATP, is one of the major constituents of nucleic acids, is needed in the formation of bio-membranes and cell division and is also essential in the transformation of starch and sugar (Panigrahy *et al*., 2009). Further, the high P-fixing ability capacity of soils often results in very low P-uptake by plants (Gerke *et al*., 1994) supporting foliar application of phosphate

salts as a possible alternative to improve the phosphate status of crops. As was shown in this study, especially foliar applied MKP has the potential to do just that. However, some controversy still surrounds the efficacy of foliar fertilization, especially as it relates to foliar application of macro nutrients and its uptake by leaves. Foliar uptake of nutrients is a function of many variables including **(1)** solubility and mobility of salts, **(2)** use of wetting agents, **(3)** type of salt, **(4)** concentration, **(5)** crop growth stage when applied and **(6)** synergism or antagonism between cations and/or anions for uptake.

The most important probably being the relative solubility and mobility **(1)** of the salt within the transport-limiting barrier of the leaf cuticle. Inorganic mineral salts, being strong electrolytes, penetrate cuticles by diffusing in aqueous pores (0.45 nm in size) of molecular dimensions (Tagliavini *et al.*, 2002). The uptake of foliar inorganic nutrients by leaves can be enhanced by the use of, for example, alkyl-polyglucoside type wetting agents **(2)** which decreases the half time of nutrient penetration into the leaf. Schönherr (2001) reported that the penetration of $CaCl_2$ (0.2 g L^{-1} solution) into leaves decreased from 204 hours to 17 hours by using an alkyl-polyglucoside wetting agent. Under South African agricultural conditions, MAP technical, KNO_3 and MKP are generally not applied with any wetting agents. The latter might be due to confusion amongst agronomists following published results showing that certain surfactants, such as ethoxylated type surfactants, strongly bind Ca^{2+} and other divalent cations, forming complexes that do not penetrate the cuticles of leaves (Uhlig and Wissemeier, 2000). Nevertheless, Li *et al*. (2009) recently concluded that surfactants are necessary to enhance the absorption of foliar applied fertilizers. It is, however, important to note that most of the commercially available surfactants have been developed for use with pesticides and might not be suitable for use with fertilizers due to poor solubility.

The type of salt **(3)** applied foliar as well as the combined effect of climate conditions, especially humidity (affected by daytime), influences the uptake of nutrients by leaves (Schönherr and Luber, 2001). Penetration requires that the salt is dissolved and this is determined by the point of deliquescence (POD). Negative yield results obtained with all three fertilizer salts during the rather dry 2006/07 season might have been due to low relative humidity values (55% to 60%) measured during the time of application. The relative high POD values for MKP (95%) and KNO_3 (95%), might have caused applied salts to accumulate on the leaf surface (Tagliavini *et al.,* 2002). A nutrient that accumulates on the leaf surface and is not absorbed may become phytotoxic to plants and is referred to as "burnout".

According to [2]H.S. Vrey (personal communication) inorganic salts such as MAP technical is sometimes applied at rates **(4)** as high as 4% (w/v) in practice without observing any visual leaf burn symptoms. In this study this aspect was investigated qualitatively using a light microscope to observe the effect of different MKP concentrations (2%, 4% and 8%), applied at the V8 development stage, on the epidermal surface of maize leaves including the appearance of stomata. Although no lesions were observed on the epidermis as a result of leaf burning, the stomatal guard cells showed signs of plasmolysis with increasing concentration when compared to a water control. Of these the stomata from leaves treated with 8% MKP was severely affected. Stomata from leaves treated with 2% and 4% MKP appeared slightly different from the water control and, although slight plasmolysis set in, the changes were probably elastic indicating that stomata could recover after the salt was either absorbed by the leaf or washed off by rain. Interestingly, significantly less P was absorbed by leaves 24 hours after application of the high MKP concentration (8%) rate compared to the lowest (2%) one indicating that closure of the stomata following plasmolysis of the guard cells prevented the uptake of P by leaves. This confirmed the findings of Eichert and Burkhardt (2001) who showed that penetration of nutrients into the leaf was mainly through stomatal pores. From this it is clear that the concentration at which MKP is applied foliar to plants should not exceed 4%.

Another important aspect that could influence the efficacy of foliar applied fertilizer is the plant growth stage **(5)**. Amanullah *et al.* (2010) reported that urea applied foliar to maize at growth stage V12 and at a 6% (w/v) concentration increased plant height, number of kernels per ear as well as grain yield while grain yield and yield components were decreased when the same concentration was applied at V9 or R1. The latter might be explained on grounds of the fact that nutrients have specific effects on plants, ranging from the expression of genes to the activation of certain metabolic processes, depending on the growth stage and, subsequently, the metabolic activities at that stage. In this regard Sarakhsi *et al.* (2010) concluded that the application of foliar fertilizers must be synchronized with the specific plant response that is required, e.g. early in the vegetative growth cycle when growth stimulation is required to, for instance, improve seedling establishment or later in the season (in the case of maize close to tasseling) when yield increase is the objective. In this study it was shown that the later application of MKP at V8 resulted in a marked yield increase whereas treatment at V3 had no effect on yield.

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Lastly, on cellular level synergism or antagonism **(6)** between elements applied foliar is a possibility that needs consideration. Serrano (1989) explained that when ATP is hydrolyzed in the cell membrane by the enzyme ATP phosphohydrolase (ATPase), it can combine with a proton and assist its movement across the membrane. Importantly, ATPase requires K^+ for its activity, although it does not transfer K^+ across the membrane. ATPase activity usually contributes towards increasing the pH of the cytosol to about 7 while anions and sugars are absorbed into the cell via a pH gradient between the cell wall (pH = 5) and the cytosol (pH = 7). This emphasises possible synergism where the K^+-moiety in MKP might have played a role in the uptake of P and sugars into cells, especially sinks. Further, Tagliavini *et al.* (2002) demonstrated that equal amounts of cations and anions penetrate the leaf when foliar applied. The authors postulated that this is due to electrical neutrality that has to be maintained within the leaf so that homeostasis is not disturbed. From the latter it seems that competition for uptake between nutrient elements, when applied foliar, is of minor concern compared to soil applied fertilizer.

In conclusion, considering all parameters measured, the 4% MKP solution (1x) applied during the eight leaf stage was most successful in improving plant height and contributing to the highest accumulation of DM during the vegetative growth stage. This was also the only treatment that consistently improved total ear length, ear kernel length, kernel number, kernel mass of both ears and final yield for the first two seasons. From a physiological perspective the MKP treatment also showed a consistent tendency to enhance the photosynthetic capacity in terms of performance index (PI_{abs}) as well as total sugar and starch content in kernels over all three seasons. From an economic perspective an average yield increase of 600 kg ha^{-1} over two seasons, which tended towards significance, is a strong indication that MKP has the potential to become a manipulation instrument in the hands of a farmer, which can contribute to the sustainable production of maize under rain fed conditions.

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SUMMARY

Regardless of past research, great uncertainty still exists amongst the research community, fertilizer agents and farmers alike with regard to the benefits of foliar inorganic fertilizer application for crop production. This supplied a rationale for investigating foliar nutrition as an agricultural practice, with the main aim of increasing maize kernel yield, under standard farm management practices. Fertilizer salts used in this study included potassium nitrate (KNO_3), mono potassium phosphate (MKP) and mono ammonium phosphate (MAP). Three objectives were set in order to obtain information about **i)** the optimal application growth stage (2004/05 season), **ii)** the optimum concentration rate (2005/06 season) and **iii)** the contribution of either the P or K moiety (or both) contained in foliar applied salts towards growth, physiological and yield responses measured in maize (2006/07 season).

During the 2004/05 season a 4% solution of MKP and a 3% solution of KNO_3 were applied at growth stages V3 and V8. MKP applied at V8 was the best treatment as it increased plant height significantly (11%) as well as plant fresh and dry mass markedly, compared to the control, when measured at the end of the vegetative growth period at V19. The higher yield (+23%) obtained with MKP applied at V8 during season one, compared to treatment at V3, corresponded with an increase in grain filling of the second ear (+16%), total ear mass (+20%), kernel mass (+20%) and number of kernels (+16%) as well as higher total sugar and starch that accumulated in kernels.

During the 2005/06 season, vegetative growth and yield parameters showed no significant differences between the single and double rates for both salts applied at V8. However, MKP applied at both the single and double rates contributed to marked yield increases of 12% and 14% respectively. Although neither KNO_3 nor MKP significantly influenced chlorophyll content or photosynthesis efficacy (chlorophyll *a* fluorescence), higher sugar levels were detected in kernels at the milk and soft dough stages as well as starch at harvest of plants treated with both salts and at two different concentrations. This strongly indicated that improvement of sucrose translocation from the leaves to kernels was rather causative of higher carbohydrate content measured in harvestable parts than sugar production *per se*.

During the 2006/07 season MAP had either no significant effect or suppressed both growth and yield eliminating its purpose to verify P or K involvement. Further, the third season was significantly drier compared to the first two seasons further complicating the use of third season data to distinguish between P and K in terms of its involvement in causing the vegetative and yield responses measured in maize. Overall, it was only MKP applied at 4% and at V8 that tended to increase kernel yield over the first two seasons.

OPSOMMING

Ten spyte van vorige navorsing bestaan daar nog groot onsekerheid tussen navorsers, bemestingsagente en boere ten opsigte van die potensiële voordele, al dan nie, wat blaartoegediende bemestingstowwe vir gewasproduksie inhou. Dit het 'n rasionaal verskaf vir onderhawige ondersoek na blaarbemesting as 'n landboupraktyk met die hoofdoel om die opbrengs van mielies onder standaard plaasbestuurspraktyke te verhoog. Bemestingsoute wat in die studie gebruik is het kaliumnitraat (KNO_3), mono kaliumfosfaat (MKP) en mono ammonium fosfaat (MAP) tegnies ingesluit. Drie doelwitte is geformuleer ten einde inligting te bekom betreffende **i)** die optimum toedieningstyd (2004/05 seisoen), **ii)** die optimum konsentrasie (2005/06 seisoen) en **iii)** die moontlike bydra van P of K of albei om 'n groei-, fisiologiese- en/of opbrengsrespons by mielies te ontlok (2006/07 seisoen).

Tydens die 2004/05 seisoen is 'n 4% MKP en 'n 3% KNO_3 oplossing apart toegedien op groeistadia V3 en V8. MKP, op V8 toegedien, was die beste behandeling in die sin dat dit planthoogte betekenisvol (11%) sowel as plant vars- en droë massa merkbaar verhoog het teen die einde van die groeiseisoen op stadium V19, in vergelyking met die onbehandelde kontrole. Verder het die hoër opbrengs (+23%) wat met die MKP behandeling op V8 tydens die eerste seisoen bekom is ooreengestem met 'n verhoging in graanvulling van die tweede kop (+16%), totale kopmassa (+20%), pitmassa (+20%) en aantal pitte per kop (+16%) asook hoër suiker- en stysel akkumulasie in graan in vergelyking met behandeling op die V3 groeistadium.

In die 2005/06 seisoen is geen verskille ten opsigte van vegetatiewe groei en opbrengs tussen die enkel en dubbel konsentrasie bespuitings van beide soute wat op V8 toegedien is waargeneem nie. Maar, MKP teen beide konsentrasies toegedien het bygedra tot merkbare opbrengsverhogings van 12% en 14% respektiewelik. Alhoewel nie KNO_3 of MKP die chlorofilinhoud of fotosintese kapasiteit (chlorofil *a* fluoressensie) van blare betekenisvol beïnvloed het nie, het beide soute teen beide konsentrasie bygedra tot hoër suikervlakke in pitte tydens die melk- en sagtedeeg ontwikkelingstadia asook styselvlakke tydens oes. Laasgenoemde het sterk daarop gedui dat verhoogde sukrose translokasie vanaf blare na pitte eerder verantwoordelik was vir hoër koolhidraatvlakke in oesbare gedeeltes as suikerproduksie.

Tydens die 2006/07 seisoen het MAP geen betekenisvolle effek op groei en oesopbrengs gehad nie, of beide komponente verlaag, wat die doelwit wat met die MAP toediening gestel was, naamlik om tusssen P en K betrokkenheid te onderskei, genullifiseer het. Die derde seisoen was verder aansienlik droeër as die eerste twee seisoene wat die gebruik van derde seisoen data, om die moontlike rolle van P of K om die gemete response in mielies te verklaar, verder bemoeilik het. Oorhoofs was dit slegs die MKP behandeling teen 4% en op V8 wat geneig het om oesopbrengs te verhoog oor die eerste twee seisoene.

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The Lord is my shepherd, I lack nothing.
[2] He makes me lie down in green pastures,
He leads me beside quiet waters,
[3] He refreshes my soul.
He guides me along the right paths
for His name's sake.