EFFECT OF LIQUID MAXIFLO (*AZOSPIRILLUM SPP*) AND TRYKOSIDE (*TRICHODERMA SPP*) CULTURES ON THE GROWTH AND YIELD OF SELECTED CROPS.

Submitted in fulfillment of the requirements for the degree

Magister Scientiae Agriculturae

By

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DECLARATION

I declare that the dissertation submitted by me for the Masters degree in Agriculture at the University of the Free State, South Africa is my own independent work and has not previously been submitted by me at another University. I further concede copyright of the dissertation in favour of the University of the Free State.

Signed in Bloemfontein, South Africa.

Signature: .....

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DEDICATION

This piece of work is dedicated to my father Nakampe Petrus Mashamba and my mother Mudadzho Johanna Mashamba. My parents sent me to school from the small income that they were getting from a subsistence farm and pension fund.

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CHAPTER 1

INTRODUCTION AND RATIONALE

Eight hundred to 850 million people in the developing world, of which 200 million are children, are chronically undernourished while an estimated 1 to 1.5 billion people, worldwide, do not receive sufficient quantities of nutrients that are needed on a daily basis (Monsanto, 2004). Considering the estimated growth in world population over the next two decades (Heidhues, 2001), it is clear that the challenge of providing nourishment to humans is significant.

In 2002 the World Food Summit recommitted itself to halve the number of hungry people by the year 2015 (Monsanto, 2004). How honourable this objective might be, the finding of a solution is not evident as it implies considering a number of factors including a) the economic status of individuals as determined by employment and minimum wages and b) ways and means to improve agricultural productivity. In both instances a considerable amount of research is inevitable. Further, population growth is a relative uncertain factor that has to be considered. The following quotation reflects the uneasiness that pertains in this regard:

"If current predictions of population growth prove accurate and patterns of human activity on the planet remain unchanged, science and technology may not be able to prevent either irreversible degradation of the environment or continued poverty for much of the world" (Joint statement by the U.S. National Academy of Sciences and the Royal Society of London, 1992).

It is predicted that population growth will occur, in large measure, in developing countries where poverty is rife. The challenge for science is to address the need for adequate food provision and a sustainable future for agriculture. The following quotation provides some hope, but also involves a warning:

"Disaster resulting from an insufficient capacity to supply food has been averted, at least for the present, through agronomic and genetic improvements. However, the price has been the uncertainty of our ability to continue such improvements" (Swaminathan, 1993).

The problem for the future seems to be related to the fact that a solution for increased food production can probably only be obtained in three possible ways, namely a) through expansion of arable land, b) by increasing irrigation practices or c) by increasing harvestable yields through the improvement of technology. However, according to Penning de Vries (2001) severe soil erosion, especially in Africa, is minimizing the number of acreage available for cultivation, leaving an almost impossible task of increasing the amount of arable land. Further, most of the irrigatable soil on the planet is probably already utilized and chances for expansion seem slim. This leaves the increase of crop yields on currently available land as the only and most likely alternative (Heidhues, 2001).

To obtain the latter goal of increasing crop yield , future agricultural research will have to focus on certain key areas. These include a) improved disease and pest control either through conventional breeding for resistance against specific diseases or by improving chemical control methodology and technology, e.g. by finding new effective but cheaper products for application by farmers in the developing world (Nelson *et al.* 2001) and b) by applying natural bio-stimulants from plants either as a seed treatment or a foliar spray or both (Roth *et al.*, 2000).

The development of natural products to achieve this goal has gained support in the recent past (Schnabl *et al.*, 2001). Previous studies showed significant increases in wheat yield when grown in mixed stands with corn cockle. A bio-stimulatory substance isolated from the corn cockle, agrostemin, increased grain yields when applied to both fertilized and unfertilized land areas used to grow wheat (Schnabl *et al.*, 2001). Chopped alfalfa also had a stimulatory effect on the growth of a number of vegetables and the active substance was later identified

as triacontanol (Putnam & Tang, 1986). Saponins isolated from crude mungbean extracts were found to increase germination and also enhance the vegetative growth of cultivated mungbeans (Chou *et al.*, 1995). The effective application of this knowledge can be instrumental in increasing crop yields and contributing towards food security in especially developing countries.

Underlying the need to develop new cheaper natural products is the fact that the lack of an efficient integrated disease-weed-pest management system has been identified as one of the main reasons for inadequate food production in Africa and other developing countries. Further, in developed countries increased resistance by consumers to purchase plant products grown from either genetically manipulated crops or crops treated with synthetic chemicals is currently experienced (Gorris & Smid, 1994).

Legislation restricting the use of many synthetic crop protectants in recent years as well as the banning of copper containing synthetic pesticides in Europe, has lead to increased organic farming practices (Rizvi & Rizvi, 1992). This means that indispensable tools used in crop production systems may be eliminated without existing alternatives. This prompted research activities towards developing natural products as alternative crop protectants in recent years and accelerated the search for natural chemicals from plants, also known as green chemicals (Gorris & Smid, 1994).

Isolation and purification of active compounds from plants, however, may place them in the same category as synthetic chemicals in terms of production costs and even their impact on the environment. Hence, the application of crude plant extracts may be a feasible alternative (Gorris & Smid, 1994) due to the general view that it is bio-degradable and environmentally safe compared to traditional synthetic agri-chemicals. However, the effective application of crude extracts in the agricultural practice has only been established in a few cases emphasizing the necessity for additional research.

The application of micro-organisms such as fungal *Trichoderma* and bacterial *Azospirillum spp* to obtain this goal is well known. Soil contains many microbes, including beneficial ones that are essential to good crop growth. Recently research has begun to show how to manage soil microflora to favour the microbes. One approach has been to add some of the best ones to fields in order to create a more favourable soil environment. Most of these introductions failed because the native microflora are more competitive than the introduced ones. Microbials are safe alternatives to the use of chemical pesticides. A number of products are available to control soil pathogens. Fungal products that suppress soil pathogens include *Gliocladium virens* (*SoilGard*®) and *Trichoderma harzianum* (Quaries, 1993a). The three decades that followed the pioneering work (Weindling, 1934 & 1937) on *Trichoderma* and *Gliocladium* were marked by blurred efforts to promote the idea that these two fungi have the potential to be effective agents for bio-control. In the last few years, there was a dramatic increase in research efforts, and several recent review articles (Papavizas & Lumsden, 1980; Schroth & Hancock, 1981) and books (Cook & Baker, 1983; Papavizas, 1981) considered the use of specific microorganisms for the bio-control of plant diseases. *Trichoderma* species are fungi that are present in substantial numbers in nearly all agricultural soils and in other environments such as decaying wood.

Azospirilla are free living N_2-fixing rhizobacteria that live in close association with plants and are capable of increasing the yield of important crops grown in various soils and climatic regions (Okon & Labandera-Gonzalez, 1994). Review data from field inoculation experiments with *Azospirillum spp* showed significant increases (5 to 30%) in yield of published reports.

The benefits observed from *Azospirillum* inoculation were mainly improved root development and enhanced water and mineral uptake. Available evidence indicates that secretion of plant-growth promoting substances by the bacteria is at least partly responsible for these effects (Okon & Itzigsohn, 1995). During

recent years, researchers have been focusing on the production of plant growth promoting substances by this bacterium (*Azospirillum*) as a possible mechanism for the observed plant growth promotion.

A recently established company, Axiom Bio-Products Pty Ltd, manufactured two products namely Maxiflo (*Azospirillum* based) and Trykoside (*Trichoderma* based) in liquid form. The rationale for this study was to investigate the possibility of increasing growth and yield in six economically important crops by treating with Maxiflo and Trykoside, as representative of bio-stimulatory agents in comperiring with two commercially available natural bio-stimulants, *ComCat*® and *Kelpak*®, to serve as positive controls. The objectives were to determine the effect of Maxiflo and Trykoside on the growth and yield of selected crops both separately and in combination.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Agriculture relies heavily on the use of synthetic chemical crop protectants for the control of insect pests and diseases and annual sales of these chemicals contribute greatly towards the economy of a country. It is estimated that the European agricultural industry utilizes about 350 million kilograms of active ingredients on pest control per annum, of which fungicides make up the largest proportion averaging about 2.2 kg/ha (Gorris & Smid, 1994). However, consumer resistance towards the application of synthetic pesticides in the agricultural industry is increasing. Recently, probably due to consumer resistance and the green peace organization, most of the copper containing synthetic pesticides have been banned in Europe and priority has been given to organic farming practices including the application of natural plant extracts in both the agricultural and health sectors (Rizvi & Rizvi, 1992).

In light of the emphasis on organic farming a renewed interest in the application of natural products, as alternatives to synthetic analogues, has been shown by the agricultural community. Claims that the application of micro-organisms such as fungal *Trichoderma* and bacterial *Azospirillum spp* to soil has the potential to contribute to achieving this goal, have been made in the past (Fallik *et al.,* 1994; Okon & Labandera-Gonzales, 1994).

The inoculation of plants with *Azospirillum* has also shown significant changes in various plant growth parameters that may affect crop yields (Fallik *et al.,* 1994). Based on worldwide field data accumulated over the past 30 years, a strong indication exists that *Azospirillum* is capable of promoting the yield of important crops in different soils and climatic regions. These data showed significant yield

increases in some published reports (Okon & Labandera-Gonzales, 1994). *Azospirillum* has shown a positive influence on plant growth, crop yield and nitrogen content of the plant under certain environmental and soil conditions (Okon, 1985; Wani, 1990).

2.2 The role of natural micro-organisms in soil from an agricultural perspective

Fertile soil is inhabited by the root systems of higher plants, by many animal forms and by numerous micro-organisms. Moreover, the vast differences in the composition of soils, together with differences in their physical characteristics and the agricultural practices by which they are cultivated, result in correspondinly large differences in the microbial populations both in total numbers and in kinds. The most important factors affecting soil micro-organism populations are 1) amount and type of nutrients, 2) available moisture, 3) degree of aeration, 4) temperature, 5) pH, 6) flooding and 6) cultural practices (Pelczar *et al.*, 1986).

Few environments on earth have as great a variety of micro-organisms as a fertile soil that includes bacteria, fungi, algae, protozoa and viruses may reach a total of billions of organisms per gram of soil. It is understandable that the great diversity of microbial flora makes it extremely difficult to determine accurately the total number of micro-organisms present. The bacterial population of the soil exceeds the population of all other groups of micro-organisms in both numbers and variety, and it includes aerobes and anaerobes, cellulose digesters, sulfur oxidizers, nitrogen fixers, protein digesters and other kinds of bacteria. Hundreds of different species of fungi inhabit the soil. They are most abundant near the surface, in both the mycelia and spore stage, where an aerobic condition is likely to prevail. Fungi are active in decomposing the major constituents of plant tissues and, in this way that contribute to soil structure that is important from an agricultural perspective (Pelczar *et al.*, 1986).

The micro-organisms that inhabit the soil exhibit many different types of associations or interactions that may be neutral, beneficial (e.g. mutualistic and commensalistic) or detrimental (antagonistic, competitive, parasitic or predatory). Probably the best known mutualistic relationship is between the roots of legumes and the nitrogen fixing bacteria *Rhizobium spp.* However, in this study the antagonistic characteristic of certain soil micro-organisms is of special practical importance since they often produce antibiotics or other inhibitory substances which affect the normal growth processes or survival of other organisms. For example, both *Staphylococcus aureaus* and *Pseudomonas aeruginosa* are antagonistic towards *Aspergillus terreus* by inhibiting germination of *Aspergillus* spores (Pelczar *et al.*, 1986). From an agricultural perspective the bacterium *Azospirillium* and the fungus *Trichoderma* naturally present in soil are of special importance due to their antagonistic function towards plant pathogens and hence their beneficial potential in preventing infection of agricultural crops by these pathogens and contributing to improved production in the agricultural and horticultural industries (Kapat *et al.*, 1998). This aspect will be elaborated upon in the following sections.

2.3 Existing technology to improve growth and yield of agricultural and horticultural crops

Before dealing with the role antagonistic soil micro-organisms can play in crop production systems, it is necessary to take note of existing technology to improve growth and yield of agricultural and horticultural crops.

Firstly, seeding rates and planting dates have always been the simplest measure to manipulate crop yields. However, a thorough knowledge of crop cultivars in terms of optimal planting dates as well as optimal environmental conditions necessary for optimal production is essential. Secondly, the use of fertilizers as a measure to manipulate crops has been practised for centuries in most countries where agriculture is well developed, but the essential role of fertilisers in modern

farming has become clear only during the last 50 years. In 1939 the world's farmers used 9 million tons of plant nutrients (mainly N, P and K) while in 1970 about seven times as much was used (Cooke, 1975). The application of plant nutrients was essentially to support the agricultural revolution which began in many temperate countries and had a great influence on the production of crops.

At present, bio-fertilization accounts for approximately 65% of the nitrogen supply to crops worldwide. Legumes were often used as green fertilizers in the past due to their nitrogen-fixing ability. The bacterial strains that are most efficient in this regard belong to the genera *Rhizobium, Sinorhizobium, Mesorhizobium, Bradyrhizobium, Azorhizobium* and *Allorhizobium* and are those strains that have been studied in most detail (Anonymous, 2005a).

One of the recent approaches to bio-fertilisation is to apply natural bio-stimulants such as *Seagro®, Kelpak®* and *ComCat®* together with normal fertilizers as a means to enhance plant growth and productivity on existing arable land. These products are normally applied as foliar sprays but they can also be applied as seed treatments. Similar objectives with foliar sprays of soil micro-organisms on agricultural crops, including *Trichoderma* and *Azospirillium*, have been set in recent research projects with the aim to test their application potential in agriculture. This approach prompted this study.

2.4 *Trichoderma* species and their application potential in agriculture

A *Trichoderma* species (fungus) forms the basis of the product Trykoside used in this study. Its taxonomic classification is as follows: Kingdom: Fungi; Phylum: *Ascomycota;* Class: *Euascomycetes*; Order: *Hypocreales;* Family: *Hypocreaceae* and Genus: *Trichoderma* (Samuel, 1996). Five species have been described namely, *T. harzianum; T. koningii; T. longibranchiatum; T. pseudokoningii* and *T. viride* that are widely distributed in the soil, plant material, decaying vegetation

and wood. What makes Trykoside different from previous products is that it is manufactured in liquid form.

Trichoderma species are generally found as dominant components of the micro-flora in most soil types including the forest humus layer as well as agricultural and orchard soils (Roiger *et al.*, 1991). It is rarely reported to grow on living plants and is not associated with plant diseases. However, there is one aggressive strain *(T. harzianum)* that has been found to cause a disease on the commercial mushroom (Seaby, 1998) and that has a significant effect on the industry. Despite its effect on mushroom, *T. harzianum* was selected as one beneficial organism that defends crop roots from antagonistic disease organism and improves the health of other crops.

The sexual stage of *Trichoderma* is unknown and it is believed to be mitotic and clonal. Most *Trichoderma* strains have no sexual stage, but instead produce only asexual spores. However, for a few strains, the sexual stage is known, but not among the strains that have usually been considered for bio-control purposes (Harman, 2000). Most of them are adapted to an asexual life cycle, in the absence of meiosis (chromosome plasticity is the norm), and have different numbers and sizes of chromosomes. There is a great diversity between the genotype and phenotype of wild strains, but they are all highly adapted and may be heterocaryotic (i.e. contain nuclei of dissimilar genotype within a single organism and, hence, are highly variable).

Trichoderma spp are parasitic because they attack and gain nutrition from other fungi. *Trichoderma spp* are used for food and textiles and are highly efficient producers of many extra cellular enzymes. They are used for the production of cellulases and other enzymes that degrade complex polysaccharides and are frequently used in the food and textile industries. For example, cellulases are used in "bio-stoning" of denim fabrics to give rise to the soft, whitened fabric-

stone-washed denim. The enzymes are also used in poultry feed to increase the digestibility of hemicelluloses from barley or other crops.

Interestingly, *Trichoderma spp* are also used as bio-control agents and used, with or without legal registration, for the control of plant diseases and plant growth promoters in the agricultural industry. From an agricultural perspective the biological control of soil-borne plant pathogens with *Trichoderma spp* has been well documented (Papavizas & Samuel, 1985; Chet, 1987; Chet, 1990; Kloepper, 1991; Whipps & Lumsden, 1991; Wilson *et al.,* 1991; Quaries, 1993a, b; Kloepper, 1994). *T. harzianum* has been extensively used as a bio-control agent because it apparently is capable of controlling a large variety of phytopathogenic fungi that are responsible for major crop diseases (Elad & Chet, 1995).

Trichoderma species have provided varied levels of biological control of a number of important soil-borne pathogens, including *Phytophthora cactum* (Smith *et al.,* 1990), *Pythium spp.* (Sivan *et al.,* 1984) and *Verticillium dahliae* (Marois *et al.,* 1982). Isolates of *T. harzianum* have been reported as antagonists of mycelia or sclerotia of these soil borne pathogens (Steadman, 1979; Lewis & Papavizas, 1987). *T. harzianum* formulated in alginate pellets (Lewis & Papavizas, 1987) colonized sclerotia of *Sclerotium sclerotiorum* under laboratory and field conditions (Knudsen *et al.,* 1991).

Specific strains in the genus *Trichoderma* colonise and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which in the end leads to induced sytemic resistance (ISR) in the entire plant (Yedidia *et al.,* 1999). Tronsmo (1989) reported that *T. harzianum* is sensitive to temperature but grows optimally at 30^{o}C while some strains can still be effective at temperatures close to 0^{o}C. The author also observed that a cold tolerant strain of *Trichoderma* was able to significantly reduce the diseases caused by *Botrytis cinerea, Sclerotinia sclerotionum* and *Rhizoctonia carotae* during the long-term storage of

carrots. Importantly, even though *T. harzianum* generally is a soil living fungus, it has been shown to be able to control diseases in the phyllosphere.

Tronsmo (1991) also reported that a fungicide resistant strain of *T. harzianum* was found to control dry eye rot on apple caused by *B. cinerea* under natural field conditions on the western coast of Norway. The fungicide resistance of the strain allowed for its usage both in biological control experiments and in an integrated control experiment while reducing the dosages of the fungicide. According to Samuel (1996), *T. harzianum* was effective in the control of other diseases, some of which are caused by the pathogen *Rhizoctonia solani, Sclerotinia minor, Fusarium oxysporum, Sclerotium rolfsii* and some *Pythium* and *Phytophthora species*. Alone or in combination with other *Trichoderma species,* it is regarded as the active component of several products inhibiting the growth of fungal plant pathogens (Chet, 1987).

Trichoderma virens DAR 74290 provided some protection in terms of seedling survival, whereas both *T. virens* and Trichodex reduced the severity of diseases as compared to controls inoculated with the pathogen alone. Dix (1964) reported that *Trichoderma spp* are considered to be inhabitants of root surfaces and it may be used, and would give better protection if applied to the tuber surface in the presence of the pathogen.

The antagonistic activities of *Trichoderma* and *Gliocladium* species against plant pathogens have been studied extensively (Burgess & Hepworth, 1996; Chet, 1987; Elad *et al.*, 1980). Elad (1994) reported that *T. harzianum* isolate T39 (which is the active ingredient of Trichodex) control *Botrytis* grey mould on a range of crops. However, *T. harzianum* T39 failed to protect chickpea seed from *Botrytis cinerea*, and this was perhaps due to low temperatures that prevailed during the experiments (Burgess & Keane, 1997).

Metcalf & Wilson (2001) described the colonization of onion roots, infected with *Sclerotium cepivorum*, by *T. koningii* (Tr5). The authors ascribed this bio-control phenomenon to production of endo- and exo-chitinases by *T. koningii*. Baek *et al.* (1999) disrupted or over-expressed the gene coding for chitinase(cht42) in *T. virens* (Gv29-8) and the transformants leading to reduced enzyme activity while over-expression of the enzyme significantly decreased or enhanced bio-control activity, respectively, against *R. solani*-incited cotton seedling disease.

Batta (1999) used *Trichoderma sp.* (strain CI306) to control *B. cinerea* on strawberry while Harman *et al.* (1996) used *Trichoderma spp.* against *Botrytis* bunch rot on grape. *Trichoderma* is considered an effective antagonistic fungus to many other plant pathogenic fungi including *B. cinerea, Crinipellis perniciosa,* and soil borne fungi such as *Rhizoctonia, Sclerotinia, Pythium* and *Fusarium* (Bastos, 1996; Conney & Lauren, 1998; Fravel, 1998; Batta, 1999).

According to Zhang *et al.* (1996), strains of *Trichoderma (Gliocladium) virens* are effective biological control agents against Fusarium wilt and seedling diseases of cotton (*Gossypium hirsutum L.)* caused by *Pythium ultimum* Trow and *Rhizoctonia solani* Kuhn (Howell, 1982). They also reported that the severity of Fusarium wilt in cotton was reduced through application of *T. virens* a treatment, but the mechanism has not been investigated.

Weindling (1932) described in detail the mycoparasitism of *Rhizoctonia solani* hyphae by the hyphae of bio-control agents, including coiling around pathogen hyphae, penetration and subsequent dissolution of the host cytoplasm. He also considered the possibility that, under certain circumstances, *T. lignorum* might act as a competitor for nutrients with *R. solani* and favored mycoparasitism as the principal mechanism for bio-control. Two years later, Weindling (1934) reported that a strain of *T. lignorum* produced a "lethal principle" that was excreted into the surrounding medium, allowing parasitic activity by the bio-control agent. He characterized the "lethal principle" as toxic to both *R. solani*

and *Sclerotinia americana* and named it gliotoxin. However, Lifshitz *et al.* (1986) showed that control of *Pythium* species on peas by *T. harzianum* (T-12) and *T. koningii* (T-8) was not due to either mycoparasitism or competition.

The bio-control of *Pythium ultimum* has also been correlated with antibiotic production (Howell & Stipanovic, 1983; Wilhite *et al.*, 1994). Howell & Stipanovic (1995) reported that mutants that lack antibiotic production are still effective bio-control agents against *Rhizoctonia solani*. Howell *et al.* (2000) had shown that strains of *T. virens* are effective in controlling *R. solani* by inducing the production of resistance-related compounds, such as terpenoids, that can lead to increased peroxidase activity in cotton roots. Peroxidase is one of the known pathogenesis related (PR) proteins involved with systemic acquired resistance (SAR) in crops (De Meyer *et al.,* 1998).

Further, Woo *et al.* (1999) disrupted chitinase (ech42) activity in *T. harzianum* (p1) and showed reduction in its bio-control activity against *Botrytis cinerea* on bean leaves. Chitinase is another enzyme known to be part of the systemic acquired resistance (SAR) mechanism in crops that show natural resistance towards fungal infection. The possible role of chitinolytic enzymes in the bio-control of fungal pathogens was further supported by the work of Lorito *et al.* (1998), who transferred the gene encoding endochitinase from *T. harzianum* (p1) into tobacco and potato and demonstrated a high level and broad spectrum of resistance against a number of plant pathogens. The involvement of *Trichoderma* species in systemic induced resistance (SIR) in crops such as cotton (Azimkhodzbayeva & Ramasanova, 1990; Keinath *et al.*, 1990) has also been reported. Systemic induced resistance implies that treatment of crops enhances the resistance of crops towards fungal infection by activating the natural mechanisms within the plants.

Protease production by *T. harzianum* has also been associated with bio-control of the root-not nematode *Meloidogyne javanica* on tomato plants. Sharon *et al.*

(2001) showed that tomato plants treated with the bio-control agent (T-203) and grown in nematode infested soil exhibited a drastic reduction in root galling compared to the control. According to Migheli *et al.* (1998) transformants of *T. longibrachiatum* (CECT2606) contributed to the over-expressing of the gene encoding β-1, 4-endoglucanase and were slightly more effective in the bio-control of *P. ultimum* on cucumber than the wild type. Yedidia *et al.* (2001) reported that inoculation of cucumber roots with *T. harzianum* (T-203) induced an array of PR-proteins, including a number of hydrolytic enzymes.

Elad & Kapat (1999) and Kapat *et al.* (1998) reported that bio-control of *B. cinerea* by *T. harzianum* (T39) might be due, in part, to the actions of *T. harzianum* producing proteases that inactivate the hydrolytic enzymes produced by *B. cinerea* on bean leaves. The authors showed that protease solutions produced by bio-control fungi partially deactivated hydrolytic enzymes and reduced disease severity by 56 to 100% when the solutions were used to treat leaves infected with the pathogen.

Importantly, *Trichoderma* species are often able to suppress the growth of endogenous fungi on agar medium and therefore mask their presence. As a result, according to Baker (1983), the routine use of bio-control agents for controlling plant diseases in agriculture has not been realized. There is one feature that could make such agents more attractive and that is the possibility of enhanced crop growth in addition to disease control. Baker *et al.* (1984) reported that such enhancement has been achieved with *T. harzianum.* Recently Guo Jing *et al.* (2001) showed that *Trichoderma* application has alleviated pathogen infection resulting in promoting plant growth, the root-colonizing ability, yield and quality of lettuce.

Pink rot in potato is principally caused by *Phytophthora erythroseptica* (Carroll & Sasser, 1974). The authors reported that pink rot was most severe in waterlogged soil and developed rapidly at 20-30^{o}C. Goodwin and McGrath

(1995) observed insensitivity to metalaxyl among isolates of *P. erythroseptica that* also caused root and stem rot of tomato. Similarly, Grisham *et al.* (1983) reported that *P. erythroseptica* isolated from potato in North and South America caused the disease when tomato fruits were inoculated, whereas Gillings & Letham (1989) reported that *P. erythroseptica* isolated from tomato did not cause pink rot of wound-inoculated potato tubers. This information emphasizes the need for bio-control agents in the event that commercial synthetic fungicides are unable to solve the problem.

Finally, Harman (2000) observed that *Trichoderma spp.* are favoured by the presence of a high level of plant roots, which they colonize. Some *Trichoderma* strains are highly competent in the rhizosphere where they colonize and grow on roots while contributing to root development. Thus, if applied as a seed treatment, the best strain will colonize root surfaces even when the roots are one meter or more below the soil surface and can be useful up to 18 months after application in enhancing plant and root growth (Howell *et al.* 1997).

2.5 *Azospirillum* species and their application potential in agriculture

Azospirillum (bacterium), on the other hand, forms the basis of the product Maxiflo and belongs to the Azotobacteraceae family. It is an aerobic bacterium meaning that it requires oxygen to play its role in the soil. These microorganisms are characterized by their high nitrogen-fixing ability (diazotrophs) and are found in abundant numbers in the rhizosphere as well as in the intracellular spaces of the roots of certain cereals and other plants (Dobereiner *et al.*, 1976; Bashan & Holguin, 1997a). It lives in close proximity to plant roots (i.e. in the rhizosphere or within plants). As is the case for Trykoside, Maxiflo is also manufactured in liquid form. *Azospirillum* living in association with roots of cereal grain has been reported to stimulate growth. This relationship is viewed as associative symbiosis in which bacteria receive non-specific photosynthate carbon from the plant and, in turn, provide the plant with fixed nitrogen, hormones, signal molecules,

vitamins, iron, etc (Bashan & Holguin, 1997b; Zuberer, 1998). Gaskins & Hubbel (1981) confirmed an increased growth rate in *Pennisetum americanum* cv. Gahi after inoculation with *A. brasilense,* strain 3t, as compared to treatment with kinetin and GA used as positive controls. Venkateswarlu & Rao (1983) reported that the inoculation of pearl millet with *A. brasilense*, strain S14, has resulted in significant increases in growth and dry matter production under both sterile and nonsterile conditions. The beneficial effects of *Azospirillum* species on plant development are attributed to the production of phyto hormones (Okon, 1985; Vande Broek & Vanderleyden, 1995; Bashan & Holguin, 1997a). Inoculation of rice with *Azospirillum* has even been suggested as an alternative to chemical fertilization.

The plant stimulatory effect exerted by *Azospirillum* has been attributed to several mechanisms, including biological nitrogen fixation and production of plant growth promoting substances (Tien *et al.,* 1979; Umalia-Garcia *et al.,* 1980; Okon & Itzigsohn, 1995; Gadagi, 1999; James, 2000). Environmental factors such as oxygen partial pressure (Volpon *et al.* 1981; Nur *et al.* 1982) and mineral nitrogen concentration (Hartmann *et al.* 1986; Fritzsche *et al.* 1990) have been reported to influence the process of nitrogen fixation in *Azospirillum.* Actually, agricultural applications of *Azospirillum spp.* are commonly limited by low concentrations of assimilative carbon in the field (Klucas, 1991). However, the use of *Azospirillum spp.* and other free living nitrogen fixing bacteria represents an enormous opportunity for agriculture as plant-growth promoting rhizobacteria (Dobereiner *et al.* 1976; Glick 1995; Bashan & Holguin, 1997a, b).

The beneficial impact of bacterial N_2-fixation on plant growth appears to be less significant than that of the rhizobia-legume symbiosis (Okon, 1985; Okon & Vanderleyden, 1997; Okon & Labandela-Gonzalez, 1994; Bashan & Holguin, 1997a; Holguin *et al.,* 1999). However, N_2-fixation remains important for bacterial survival in N-poor soils and possibly in the root environment. Improved nitrogen fixation resulted in an increased bacterial population on roots and consequently

increased plant growth. Further, inoculation of crop plants or the seeds of crop plants with *Azospirillum* increased the number of lateral roots and root hairs (Salomone *et al.,* 1997), thus enhancing the uptake of nutrients through increased root surface.

According to Bashan *et al.* (1989) *Azospirillum brasilense* increased the growth and yield of tomato plants. Recently Kenny (2001) confirmed that an *Azospirillum* spp. significantly reduced the occurrence of diseases in tomatoes and green peppers and simultaneously increased both plant size and yield under field conditions

Most studies of the *Azospirillum* plant association have been conducted on cereals and other grasses (Tyler *et al.,* 1979; Okon, 1985; Wani *et al.,* 1988; Alagawadi & Krishnaray, 1998; Wani *et al.,* 1988) but only a few plant families have been investigated so far (Bashan *et at,* 1989; Crossman and Hill, 1987; Kolb & Martin, 1985, Saha *et al.,* 1985). However, a few field studies on flowers (El-Nagger & Mahamoud, 1994; Gadagi, 1999), oats (Tanwar *et al.,* 1985), sorghum (Desale & Konde, 1984; Okon *et al.,* 1981) and other crops (Steenhoudt & Vanderleyden, 2000) under appropriate growth conditions confirmed increases in plant dry mass and yield due to *Azospirillum* inoculation.

From an agricultural perspective, the confirmation of growth improvement and yield increases in crop plants due to *Azospirillium* inoculation have been well documented. Hegazi *et al.,* (1981) reported that the inoculation of wheat with *A. brasilense* increased the rhizosphere population of *Azospirillum* and increased plant height, dry weight, tillering, nitrogenase activity and grain and straw yields. In a field experiment in India, nitrogen fertilizer applications (up to 120 kg N/ha) and inoculation of seed with *A. brasilense* and *Azotobacter chroococcum* showed a significant increase in tillering, dry matter production, grain yield and grain protein content of wheat (Zambre *et al.* 1984). Similar results have been published on sorghum (Pacovsky *et al.,* 1985; Sarig *et al.,* 1988) and maize (Lin

et al., 1983). However, cognizance has to be taken of the work of Subba-Rao *et al.,* (1985) who observed that soil inoculated with *Glomus mosseae* or *Glomus fasciculatum* together with seed inoculation with *Azospirillum brasilense* produced significantly greater dry matter and grain yields than inoculation with either the mycorrhizal or bacterial component alone.

The optimization of the *in vitro* production of potatoes in South Africa: cytokinin and growth regulators are believed to have strong promotive effects on tuberization and constitute the tuberization stimulus, either alone or in combination with other substances (Palmer & Smith, 1970; Forsline & Langille, 1976; Pelacho & Mingo-Casel, 1991; Leclerc *et al.,* 1994). However, according to Harmley & Clinch (1966); Leclerc *et al.*, (1994), growth regulators failed to induce tuberization when sucrose supply was inadequate. It has been stated that the use of growth retardants rather than bio-stimulants have improved the micro-tuber formation of potato (Forti *et al.*, 1991; Harvey *et al.*, 1991; Leclerc *et al.*, 1994). According to Forti, *et al.* (1991); Leclerc, *et al.* (1994) the effect of growth regulators on tuberization in potatoes depends on genotype.

More recently, reports that *Azospirillum* has the ability to protect plants against various stresses, e.g. drought stress through adjusting the turgor in cells, have become available (Barassi *et al.,* 1996). This aspect needs more consideration from an agricultural perspective.

2.6 Natural bio-stimulants, *ComCat*® and *Kelpak*®

ComCat® is a commercial bio-stimulant that is based upon naturally derived plant materials (Agraforum, 2003). It is a finely ground wettable powder specially blended with a carrier to permit conventional application on seeds and growing plants. The "active ingredient" is a complex combination of natural biological substances including amino acids, plant proteins, mixed phytosterols (including brassinosteroids) and flavonoids.

ComCat® products are a diverse blend of plant materials which have been selected from specific European plant species known for their history of positive growth effects on beneficial plants (Agraforum, 2003). These selected plants are grown under controlled environments, harvested, dried and naturally processed to produce a concentration of natural bio-stimulants which can be controlled and monitored for uniform quality and returned to nature to nurture and enhance the health of vegetables, flowers and agricultural crops.

According to the manufacturers (Agraforum, 2003), *ComCat*® activates natural defense mechanisms in plants towards abiotic and biotic stress factors. The activation of the target plant by the biochemical within *ComCat*® stimulates biosynthesis which is generally expressed by a greater production of sugars, which are building blocks for cellulose and fruiting bodies. These natural biochemicals are the transmitters of molecular signals which trigger the defense mechanisms within the plant that increase resistance to stress factors. Further claims made by the manufacturers are that treatment of crop plants with *ComCat*® promotes root development, leading to efficient nutrient uptake and yield increases.

Kelpak® is a commercial bio-stimulant manufactured from seaweed. Similar claims as for *ComCat*® are made by the manufacturers about *Kelpak*®. These include promotion of root development, increased resistance to abiotic stress factors and yield increases in treated crops.

2.7 Economic important crops investigated in this study

2.7.1 Cabbage

Cabbage (*Brassica oleracea* var. *capitata*) is a member of the Cruciferae family, the same family as broccoli, brussel sprouts, cauliflower, kale, green mustard and collards. Collectively, these crops are referred to as cole crops or crucifers. Cabbage is well adapted for growth in cool climates. Cabbage is a popular vegetable worldwide because of its adaptability to a wide range of climate and soil, its ease of production and storage, and its food value (FAO, 1984 & USDA, 1986)

Cabbage is a cool-season biennial crop that is grown as an annual vegetable requiring 60 to 100 days from sowing until market maturity depending on the cultivar. The ideal monthly temperature for optimal growth of cabbage ranges from 15 to 18^{o}C. Temperature greater than 24^{o}C induces bolting in cabbage, but cultivars differ in their susceptibility. Cabbage has been used as a food crop since antiquity (Simmonds, 1976). Cabbage does well in a relatively cool, moist climate. For this reason cabbage is cultivated in the Transvaal mainly in the autumn, winter and spring. The optimum temperature for growth and development on average is approximately 18^{0}C, with an average maximum of 24^{0}C and an average minimum of 4.5^{0}C (Olivier, 1995). It is also fairly resistant to frost, and readily survives minimum temperatures of as low as -3^{0}C without noticeable damage. Optimum temperature and humidity are seldom encountered, but cabbage fortunately has a wide adaptability.

It can consequently be cultivated in most areas throughout the year although quality and yield are usually poor during the summer months because of high infestation of pests. Cole crops do best in well drained, fertile loam, but they can be successfully grown on a wide range of soils, provided that drainage and fertility are good. For fall, summer, and early winter planting, cabbage does best on the heavier loams, while the spring crop does best on a sandier loam

(Anonymous, 2005b). As these vegetables respond readily to organic fertilisers it is recommended that adequate organic material be incorporated in the soil (Jackson, 1998). Cole crops do best in soil with a pH value between 6.0 and 6.5. If the value is below 5.8 it is advisable to apply lime and the type and amount of lime will be indicated by soil analysis.

2.7.2 Lettuce

Lettuce (*Lactuca sativa*) belongs to the Compositae (sunflower or daisy) family. It has a small cylindrical root system, with an effective root width of 25cm , which implies that the plants should be closely spaced (both in and between rows). It is an annual plant native to the Mediterranean area cultivated as early as 4500 BC, initially for the edible oil extracted from its seed. Salad lettuce became popular with the ancient Greeks and Romans (Ryder, 1979).

Lettuce is a cool-season crop and grows best within a tepmperature range of 12°-20°C. It is an annual plant closely related to the common wild or prickly lettuce weed (Robinson *et al.*, 1983 and Ryder, 1986). It is so sensitive to low temperature provided there is high elevations during summer. Lettuce grows well on a wide variety of soils, provided climatic requirements are met.

Cultivated lettuce was derived from the wild or prickly lettuce *Lactuca sioriola.* There are five types of lettuce namely crisp head, butter head, cos or romaine, loose leaf or bunching and stem lettuce (Ryder, 1986). Lettuce is currently an economically important crop grown in large quantities all over the world. The leaf colour of commercial lettuce cultivars varies from yellow-green to dark red. Head lettuce grows best at temperatures between 15 to 18°C. Warm sandy soils are preferred for the early harvestable types while loam to clay loam or peat are suited for lettuce produced later in he season (Ryder, 1986).

The part that contains the highest nutritional value is the dark green outer leaves despite the fact that in calories it is low. Each head contains only 65 to 70 kilocalories (Jansen, 1994). The use of lettuce includes extraction of oil from its seed and its use in salad and relish.

2.7.3 Peas

Peas (*Pisum sativum*) is a cool-season annual crop, adapted to semi-arid climates and belongs to leguminosae family. Peas have the capacity to fix atmospheric nitrogen into the soil so that it can be available for utilization by plants and are generally considered low fertility crop that do well on fertile soil (Cutcliffe, 1978). Peas require a cool, relatively humid climate and are grown at higher altitudes in tropics with temperatures from 7 to 30^{o}C (Duke, 1981 & Davies *et al.,* 1985). The optimum temperature levels for vegetative and reproductive periods of peas were reported to be 21 and 16^{o}C, and 16 and 10^{o}C (day and night), respectively (Slinkard *et at.,* 1994). The optimal planting dates for peas ranges from mid-April when soil temperatures are above 40°F to mid-May. Peas are very sensitive to drought and grow best in regions of moderate rainfall or with irrigation.

Peas can also be grown successfully during mid Summer and early fall in those areas with relatively low temperatures and a good rainfall, or where irrigation is practiced. For very early crops, a sandy loam is preferred; for large yields where earliness is not a factor, a well-drained clay loam, sandy loam or silt loam is preferred (Duke, 1981). It also requires the pH of 6.5 or higher for maximum yields. Peas can also be grown in a no-till or conventional tillage cropping system and it requires high amount of moisture for germination than cereal grains (Anonymous, 2002). Pea growing season varies from 80-100 days in semi-arid regions and it can reach up to 150 days in humid and temperate areas (Davies *et al.*, 1985).

Fresh market peas continues to decrease in part because of the high labour demand for hand harvesting and shelling. However,harvest mechanization allows for a very large production of peas for processing into canned or frozen product (Valenzuela, 1983).

2.7.4 Wheat

All wheats, whether wild or cultivated, belong to the genus *Triticum.* Wheat is a cereal grain crop classified under the Gramineae or grass family. Its complete botanical classification is as follows: genus: *Triticum*, species: *aestivum* and *turgidium*. The species are categorized into groups, *aestivum* for bread wheat; *compactium* for common wheat; *spelta* for spelt wheat and *turgidium*, *poulard* (branched) wheat and *durum* for hard wheat. Wheat is the most important world crop today judging from the land area under production. (Cornell & Hoveling, 1998).

Wheat has a relatively broad adaptation, is very well adapted to harsh climates, and will grow well where rice and maize cannot. Generally the winter climate of a particular area determines whether winter or spring types are grown. Wheat is grown on a wide range of soils in temperate climates where annual rainfall ranges between 30 and 90 cm. Such areas constitute most of the grasslands of the world's temperate regions. Many of these soils are deep, well-drained, dark-colored, fertile, and high in organic matter, and they represent some of the world's best soils. Loam to sand loamy soils are ideal for planting of wheat crops (Metcalfe & Elkins, 1980).

2.7.5 Potato

Potato (*Solanum tuberosum*) is a herbaceous plant belonging to the Solanaceae family. Other well-known crops belonging to the same family are tomato (*Lycopersicon esculentum*), the eggplant (*S. melongena*), various species of chili

peppers (*Capsicum*) and tobacco (*Nicotiana tabacum*) (Hawkes, 1990). Potato is also classified under the hemispherical type of root systems. Potatoes are growing in temperate climates or the mountains of tropical areas. Amongst all tuber crops potato top the list in terms of hectares under cultivation followed by cassava and sweet potatoes. Potato tubers give an exceptionally high yield per hectare, many times that of any grain crop (Burton, 1969) and are used as processed food and livestock feed (Feustel, 1987; Talburt, 1987).

The potato may be classified as a dicotyledonous annual, although it can persist in the field vegetatively (as tubers) from one season to the next (Horton, 1987). Potato is a cool-season crop, slightly tolerant of frost, but easily damaged by freezing wheather near maturity. Today potato encircles the globe, they are grown on every continent (FAO, 1984). Potato can also be planted as soon as soil temperature reaches about 5°C, the emergence is more rapid at 20 to 22°C. Soil temperatures of 15 to 18°C appear to be the most favorable for common potato varieties. For example, in varieties of the tuberosum subspecies, short days and moderate temperature, particularly low night temperatures, stimulate tuber initiation, however, mature late under short days (Horton, 1987). Maximum yields of high quality tubers are produced when the mean temperature is between 15°C and 18°C during the growing season. Tuberisation (tuber formation) is also favored by long days of high light intensity. An optimum temperature for tuber development is about 18°C. Tuberisation is progressively reduced when night temperatures rise above 20°C and totally inhibited at 30°C (Ewing, 1978).

The potato crop develops best on deep, friable soils that have good water retention. Because it has a relatively weak root system, impermeable layers in the soil limit rooting depth, which in turn, restricts availability of water to the plant in dry period (Horton, 1987).

2.7.6 Tomato

Tomato (*Lycopersicon esculentum*) also belongs to the plant family Solanaceae, is a native of tropical America and is also classified as an annual season under the plant group with a hemispherical type of root system (McCollum & Ware, 1975).

Tomato is a warm-season plant which requires three to four months of sunshine from the time of seeding up to production of the first ripe fruit (McCollum & Ware, 1975). It thrives best when weather is clear and rather dry and temperatures are uniformly moderate (18 to 29^{o}C). Tomato can be cultivated on nearly all types of soils although light, well-drained and fertile soil is best suited for producing early fruit of high quality. Loams and clay loams have a greater water holding capacity and are well suited for producing tomatoes at a pH ranging from 5.5 to 7.0.

World production of tomato has increased to approximately 10% since 1985, reflecting a substantial increase in dietary use of the crop. Nutritionally, tomato is a significant dietary source of vitamin A and C. Further, recent studies have shown the importance of lycopene, a major component of red tomatoes with strong antioxidant properties, which reduces the incidence of several cancer types (Anonymous, 1996).

2.8 Scope of this study

This study focused on monitoring the effect of bio-products on the growth and yield of one grain crop (wheat), one legume (peas) and four vegetable crops (cabbage, lettuce, potato and tomato). The main aims were to determine the effects of Maxiflo and Trykoside, bacterium and fungus based bio-product respectively, on the vegetative growth and total yield of all the selected crops while *ComCat*® and in some instances *Kelpak*®, both commercially available bio-stimulants, were used as positive controls. Although neither of these two

products used as positive controls are micro-organism based, they were used to measure the capability of Maxiflo and Trykoside to increase crop yields by comparison.

CHAPTER 3

EFFECT OF LIQUID MAXIFLO AND TRYKOSIDE ON THE GROWTH AND YIELD OF TWO LEAF VEGETABLES

3.1 INTRODUCTION

According to the National Department of Agriculture in South Africa Directorate: Agricultural Statistics (2001), the production of cabbage and lettuce in the country were 195 000 and 28 000 tons respectively, during the 2003 growing season. These yields were obtained by applying standard fertilisation practices. The question to be answered is whether the yields of cabbage and lettuce can be increased by using organic products with micro-organisms such as *Azospirillum* and *Trichoderma* as active components. Bhagavantagoudra & Rokhade (2001), reported that the application of *Azospirillum* through soil plus seedling dipping recorded the highest cabbage yield (41,61 t/ha), which was 33,67% more than that of the untreated control. Treatment with *Azospirillum* through soil plus seedling dipping recorded the highest values for plant spread (46.22 cm), plant height (26.44 cm), number of outer leaves (22.70), leaf area (315.02 cm^2), head diameter (13.33 cm), head surface area (577.31 cm^2), number of inner leaves per head (41.92) and head weight (687.98 g).

Agwah & Shahaby (1993) reported that *Azospirillum* inoculation of chinese cabbage significantly increased leaf nitrogen content and dry mass but had no effect on fresh weight, leaf length and yield. According to these authors, *Azospirillum brasilense* Sp7 also increased the vitamin C content in cabbage at all N rates applied. None of the treatments affected the leaf chlorophyll content. Three years later Gunasekaran and Sivakumar (1996) confirmed that inoculation of chinese cabbage with *Azospirillum* significantly enhanced the plant biomass, nitrogen content and assimilatory enzyme activities.

In the case of lettuce, Coley-Smith *et al.* (1991) reported evidence for the enhancement of growth and marketable yield of lettuce cultivated in polythene tunnels by inoculation with *Trichoderma viride* (IMI 298375). In a series of repeated trials, six strains of *T. harzianum* and *T. viride,* applied as a dried powder from liquid culture on a molasses/yeast medium, consistenly promoted the growth of lettuce seedlings grown in peat sand potting medium in the glasshouse (Ousley *et al.*, 1994).

Trichoderma has been known for many years to produce a wide range of antibiotic substances (Sivasithamparam & Ghisalborti, 1998) and that they parasitize other fungi. They can also compete with other micro-organisms. For example, they compete for key exudates from seeds that stimulate the germination of propagules of plant pathogenic fungi in the soil (Howel, 2002) while also competing with soil micro-organisms for nutrients. Further, they inhibit or degrade pectinases and other enzymes that are essential for plant pathogenic fungi, such as *Botrytis cinerea*, to penetrate leaf surface (Zimand *et al.,* 1996).

Root colonisation by specific non-pathogenic micro-organisms such as plant growth promoting rhizobacteria (Tuzun & Kloepper, 1994) and fungi (Meera *et al.*, 1994) can also induce a systemic increase in resistance to. A *Trichoderma harzianum* T39 soil application seven days before *B. cinerea* innoculation significantly reduced grey mould severity in tomato, lettuce and pepper although the biocontrol agent was not detected on the leaves of the plants. The latter was confirmed by Ryals *et al.* (1996) in pepper who reported that a *T. harzianum* T39 soil treatment reduced *B. cinerea* stem infections significantly. Kloepper *et al.* (1997) confirmed the control of *B. cinerea* leaf infections in tomato, lettuce, pepper, tobacco and bean by means of *T. harzianum* T39 innoculation.

The recently discovered new generation phytohormones known as brassosteroids (BRs), are one of the active substances of the commercial product *ComCat*® used in this study as a positive control. It seems BRs are

widely distributed in the plant kingdom, are natural growth promoting substances, are also involved in the translocation of phytosynthate in plants and the build up of photosynthate in seeds as well as involved in the induction of root growth and flower bud formation (Schnabl *et al.*, 2001). Claims have also been made that BRs induce the natural resistance of crop plants to abiotic and biotic stress conditions (Zurek & Clouse, 1994; Takatsuto *et al.,* 1996).

The active substances in *Kelpak*®, used as a second positive control in this study, are natural auxins and cytokinins. The manufactureres claim that *Kelpak*® improve plant perfomance through increased root growth, more efficient use and uptake of applied nutrients and enhanced flower formation in vegetables and ornamental plants (Qwemico; Personal communication).

The objective of these experiments was to determine the effect of bioproducts (Maxiflo & Trykoside) on the vegetative growth and yield components of cabbage and lettuce under field conditions.

3.2 MATERIALS AND METHODS

3.2.1 Trial site

Two field trials on cabbage and lettuce were conducted at the experimental site (West Campus) of the Department of Soil, Crop and Climate Sciences at the University of the Free State in Bloemfontein.

3.2.2 Experimental design

A randomized complete block design (RCBD) was used for both field trails conducted in this study. Six different treatments were randomly applied to the plots and each treatment was replicated four times. The soil was analyzed beforehand with the following results:

Table 3.1: Physical and chemical properties of the topsoil used in the field trial

Property	
Soil type	Clay loam
pH (H_2O)	6.88
EC (Ohm)	1600
Nutrients	**ppm**
N ($NaHCO_3$)	530.4
P ($NaHCO_3$)	16.88
K (NH_4OA_c)	80.0
Ca (NH_4OA_c)	1825.8
Mg (NH_4OA_c)	397.5
Na (NH_4OA_c)	67.5
Zn (HCl)	2.2

Determined with standard procedures (The Non-Affiliated Soil Analysis Working Committee, 1990)

Fertilizer was applied according to the withdrawal amounts and an expected yield of 25 ton ha^{-1} and 60 ton ha^{-1} for lettuce and cabbage, respectively. In the case of cabbage, 36 kg P ha^{-1}, 102 kg N ha^{-1} and 216 kg K ha^{-1} was applied before planting and incorporated with a rotavator while for lettuce the rates were 8.5 kg P ha^{-1}, 34 kg N ha^{-1} and 121.5 kg K ha^{-1}. A top dressing was applied broadcast two weeks after planting for both cabbage and lettuce at rates of 102 kg N ha^{-1} and 34 kg N ha^{-1}, respectively. Irrigation commenced immediately after applying the fertilizer.

Cabbage seedlings (cv. Drumhead), were transplanted in moist soil within-row spacing of 35 cm and between-row spacing of 60 cm which represents a plant population of approximately 47 600 plants ha^{-1}. Lettuce (cv. Winter Crisp),

seedlings were transplanted with an in row spacing of 25 cm and between row spacing of 40 cm which represents a plant population of approximately 100 000 plants ha^{-1}. The plot size for cabbage and lettuce was 1.68 m^2 and 0.8 m^2, respectively.

3.2.3 Treatments

ComCat® and *Kelpak*®, two commercial bio-stimulants from plant origin were used as positive controls in these two field trials (Table 3.2). The manufacturers of both claim that the products promote plant growth and development, physiologically assists and strengthens the plants' natural resistance to pathogen attacks and encourages root growth (Agraforum, Germany).

Trichoderma (fungus) forms the basis of the product Trykoside and *Azospirillum* (bacterium) that of the product. Maxiflo and Trykoside were generously supplied by AXIOM Bio-products Pty Ltd, Isando, South Africa. The effects of Trykoside and Maxiflo was tested both separately and in combination on the growth and yield of cabbage and lettuce (Table 3.2).

Table 3.2: Summary of the treatments applied on both lettuce and cabbage

Treatment	Concentration	Spray information
Control		
Maxiflo	1L ha^{-1}	Spray at planting and every 2 weeks thereafter
Trykoside	1L ha^{-1}	Spray at planting and every 2 weeks thereafter
Maxiflo + Trykoside	1L + 1L ha^{-1}	Spray at planting and every 2 weeks thereafter
ComCat®	100g ha^{-1}	Spray at planting and three sprays every 4 weeks thereafter
Kelpak®	33.75L ha^{-1}	Spray at planting and every 3 weeks thereafter

Suspensions of Maxiflo and Trykoside were applied at a rate of 600 L ha^{-1} while that of *ComCat*® and *Kelpak*® were applied at 1200 L ha^{-1} for cabbage as foliar sprays. For lettuce the same volume rates per hectare were applied except in the case of *ComCat*® and *Kelpak*® that were also applied as a foliar spray at 600 L ha^{-1}. Foliar sprays were applied using a knapsack spray.

3.2.4 Irrigation

A micro irrigation system was used. Irrigation started immediately after planting, up to one week before harvest.

3.2.5 Weed, insect and disease control

The plots where kept free of weeds by hand-weeding every two weeks throughout the growing season. Cutworm, aphids and American bollworm were controlled with Chlorpyrifos on both crops at a rate of 2 ml L^{-1} according to the instructions on the manufacturer. Three applications during the trial period were sufficient for total control of the insect pests.

Septoria leaf spot was identified on lettuce on the fourth week after planting. Treatment with copper oxide chloride contained in Captan (2 g L^{-1}) and Viripot (4 g L^{-1}) commenced the same day and repeated every 3 weeks. Dithane M45 was sprayed every two weeks at a rate of 4 g L^{-1} of water to control other fungal diseases.

3.2.6 Harvesting

Cabbage was harvested 19 weeks and lettuce 14 weeks after planting when head size was at the maturity stage and of market related quality. Harvesting was done by lifting whole plants, together with roots, from the soil using a pitch fork.

The bulk of the root system was cut off in the laboratory for further measurements taking into consideration that some of the fine roots remained in the soil.

3.2.7 Parameters measured

3.2.7.1 Growth parameters

3.2.7.1.1 Plant diameter

Plant diameter was measured every second week up to eight weeks after planting using a common ruler. The top canopy of both lettuce and cabbage plants were measured in two perpendicular 90° angles and the mean of the two values taken to represent the average diameter.

3.2.7.1.2 Plant height

Plant height was measured from the soil surface up to the most apical leaf using a ruler. This was done up to eight weeks after planting with two week intervals.

3.2.7.1.3 Stem diameter

The stem diameter was measured at a position 10 mm above the soil surface of each plant using a digital caliper. Data was collected every second week up to eight weeks after planting.

3.2.7.1.4 Root fresh mass

After severing roots from above soil parts after harvesting, excess soil was washed from the roots with tap water, blotted dry on tissue paper and then weighed.

3.2.7.2 Yield parameters

3.2.7.2.1 Leaf and head mass

Heads of both cabbage and lettuce were harvested when firm and before they split or burst. The heads were separated from the outer leaves and the fresh mass determined separately.

3.2.8 Statistical analysis

Analysis of variance (ANOVA) was performed on the data, using the NCSS 2000 statistical program to determine the significance of differences between means of treatments while Tukey's least significant difference (LSD) procedure was applied to separate means ($P< 0.05$).

3.3. RESULTS

3.3.1 Cabbage

3.3.1.1 Plant diameter

Statistically significant differences in plant diameter were observed only during the first four weeks of vegatative growth and only in terms of the *Kelpak*® treatment (Figure 3.1). However, during the same growth stages both the Trykoside and *ComCat*® treatments contributed to increased plant diameter although statistically non-significant. The Maxiflo treatment tended to have a slight but non-significant decreasing effect on plant diameter during the whole vegetative growth cycle. Six and eight weeks after planting neither of the treatments influenced the plant diameter significantly but all treated plants tended to be smaller than the control plants.

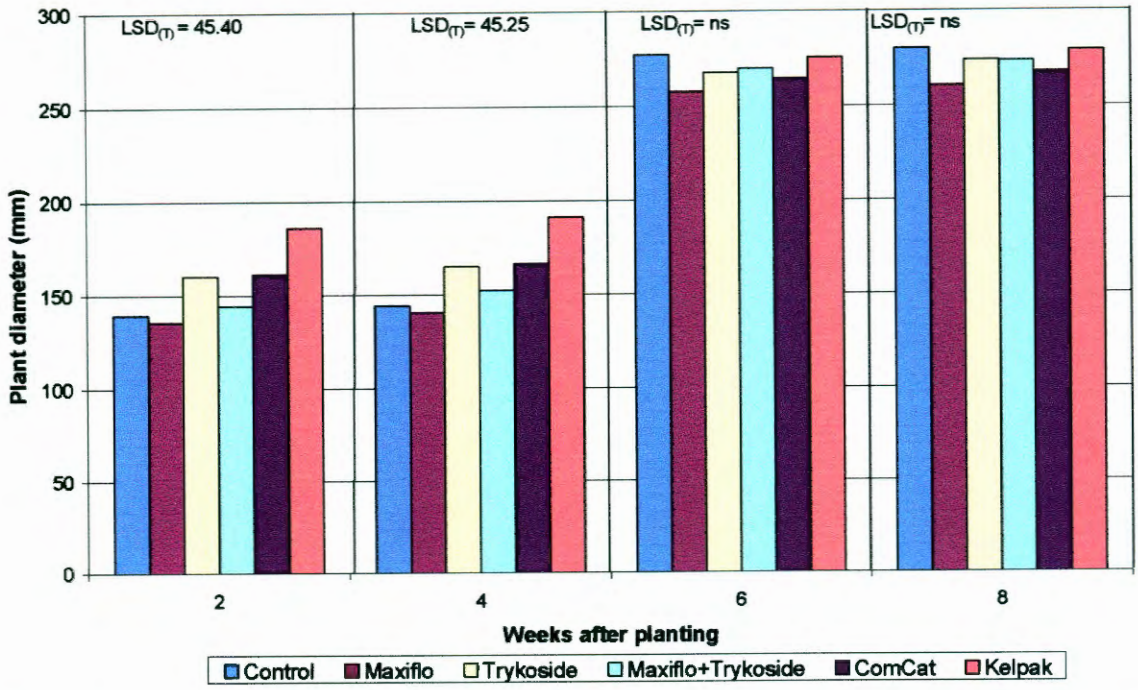

Figure 3.1: The effect of Maxiflo and Trykoside applied both separately and in combination on the plant diameter (mm) of cabbage.

3.3.1.2 Plant height

After the first two weeks, significant differences in plant height were observed in cabbage where treatment with Trykoside, both separately and in combination with Maxiflo, as well as the two commercial bio-stimulants *ComCat*® and *Kelpak*®, tended to increase plant height compared to the Maxiflo treatment and untreated control (Figure 3.2). Already at this early growth stage the *Kelpak*® treatment clearly had the most significant enhancing effect on plant height followed by the *ComCat*® and Maxiflo/Trykoside combination treatments. This tendency prevailed over the eight week data collection period.

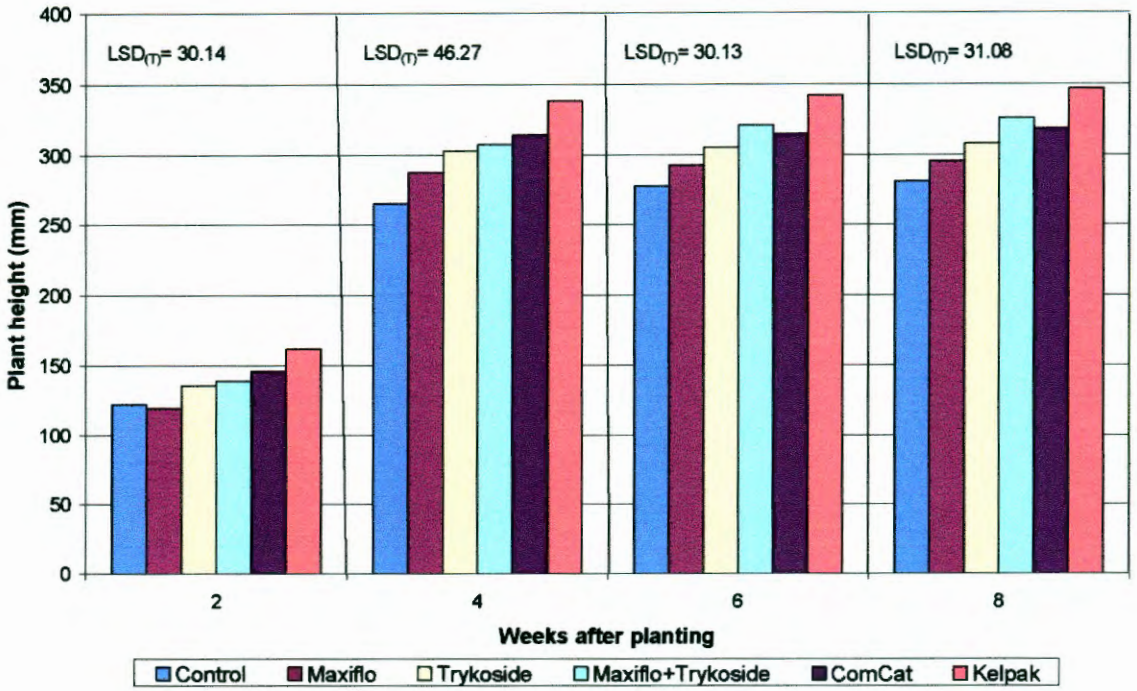

Figure 3.2: The effect of Maxiflo and Trykoside applied both separately and in combination on the plant height (mm) of cabbage.

3.3.1.3 Stem diameter

The stem diameter of cabbage plants was significantly increased by the two bio-stimulants *ComCat®* and *Kelpak®* (positive controls) during the first four weeks after planting (Figure 3.3). Although the increase in stem diameter by these two bio-stimulants was also observed six and eight weeks after planting, the differences were not significant compared to the untreated control. Neither of the Maxiflo, Trykoside or Maxiflo/Trykoside combination treatments had a significant effect on stem diameter during the vegetative growth stage as measured over the first eight weeks after planting.

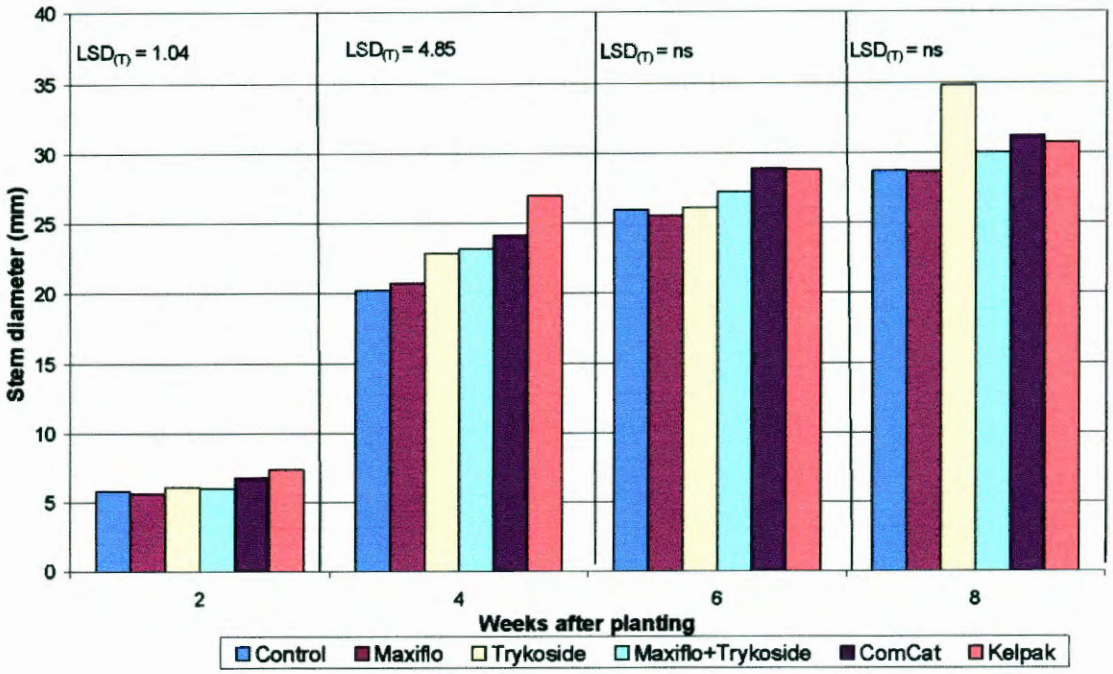

Figure 3.3: The effect of Maxiflo and Trykoside applied both separately and in combination on stem diameter (mm) of cabbage.

3.3.1.4 Head and leaf fresh mass

The *ComCat*® treatment significantly increased both the head (Figure 3.4A) and leaf (Figure 3.4B) fresh mass of cabbage compared to all other treatments except the Trykoside treatment. The latter treatment also increased the head mass but not significantly compared to the untreated control. Neither Maxiflo nor the Trykoside/Maxiflo combination treatment had a significant effect on either head or leaf mass. *Kelpak*®, on the other hand, had a significant inhibitory effect on head mass, compared with other treatments and especially the untreated control, but this was not the case for leaf mass.

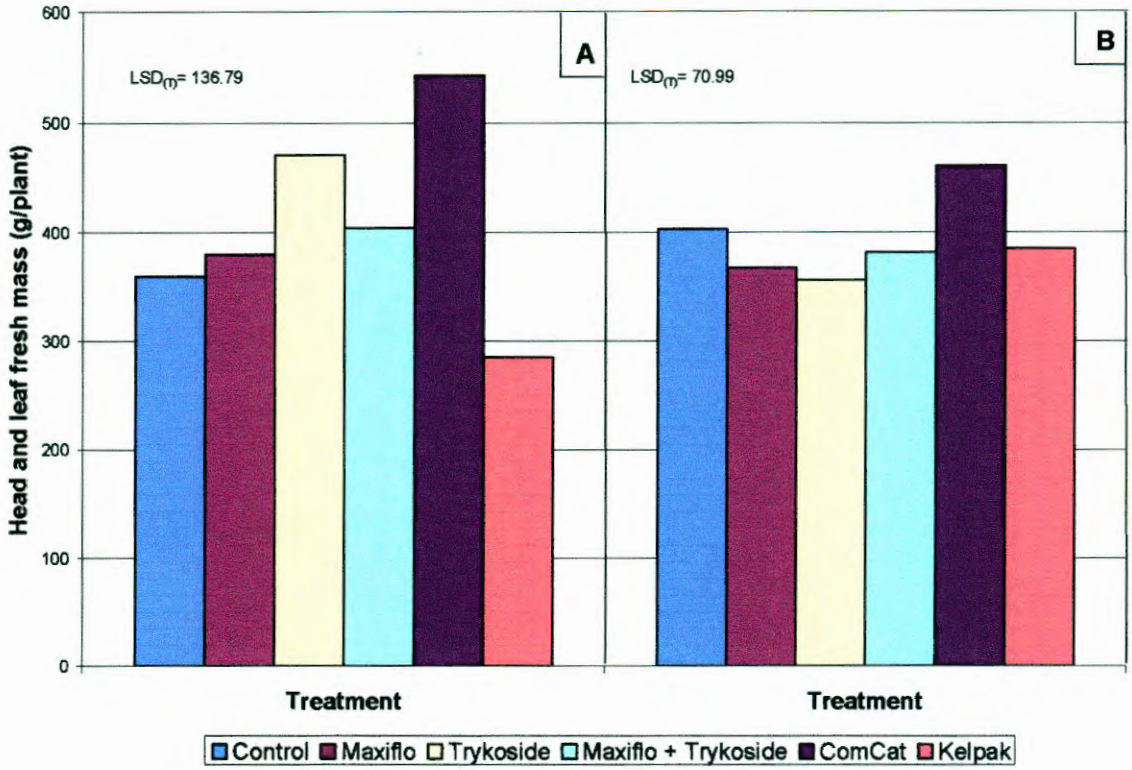

Figure 3.4: The effect of Maxiflo and Trykoside applied both separately and in combination on A) head and B) leaf fresh mass (g/plant) of cabbage.

3.3.1.5 Root fresh mass

Both Maxiflo and Trykoside applied separately had a statistically significant decreasing effect on root fresh mass compared to the untreated control (Figure 3.5). However, although non-significantly, the opposite was observed when these two products were applied in combination. All other treatments had no significant effect on root mass.

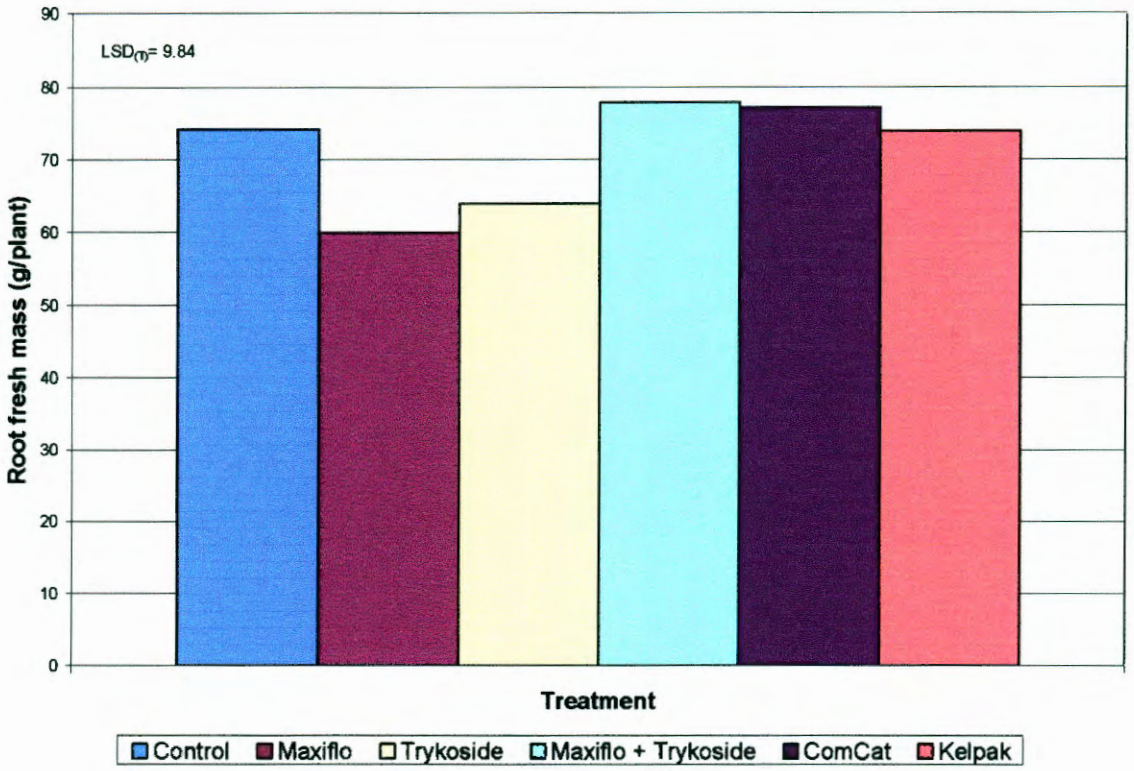

Figure 3.5: The effect of Maxiflo and Trykoside applied both separately and in combination on the root fresh mass (g/plant) of cabbage.

3.3.2 Lettuce

3.3.2.1 Plant height

In lettuce, compared to the untreated control, the same tendency of all treatments to reduce plant height was observed at two week intervals over the first eight weeks of vegetative growth (Figure 3.6). However, this reduction was statistically significant only for the Trykoside/Maxiflo combination treatment while, overall, the Maxiflo treatment had the least reducing effect followed by the two positive controls.

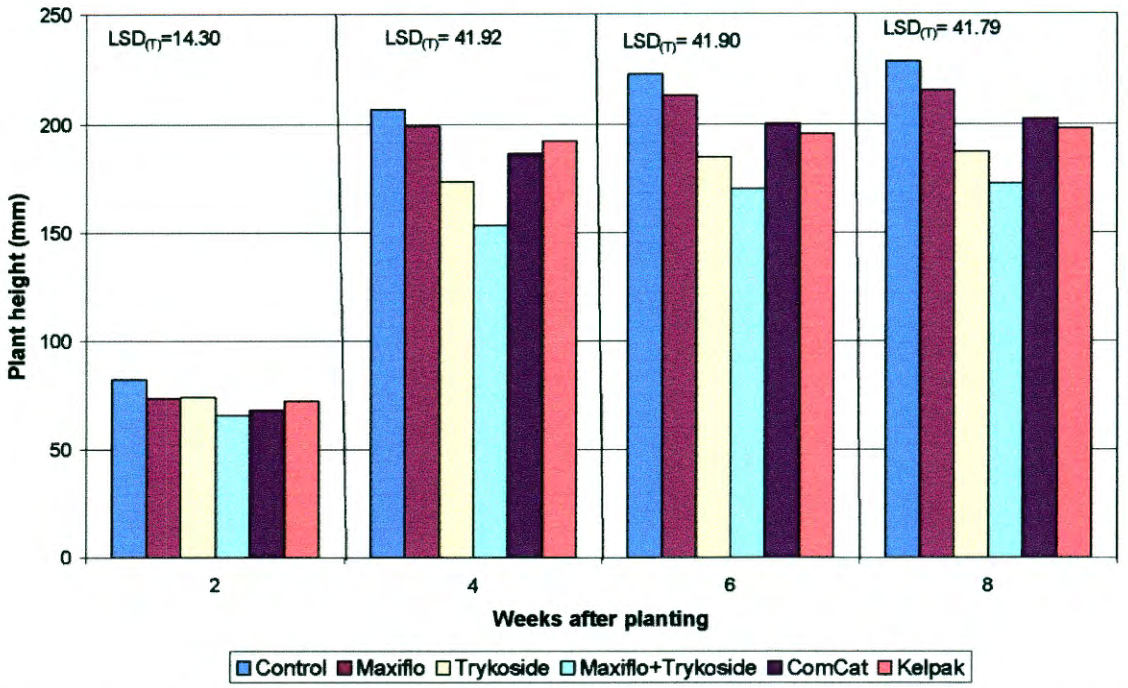

Figure 3.6: The effect of Maxiflo and Trykoside applied both separately and in combination on plant height (mm) of lettuce.

3.3.2.2 Plant diameter

Except for the Trykoside treatment two weeks after planting, exactly the same tendency of all treatments to reduce plant diameter as was observed for plant height, prevailed (Figure 3.7). Once again the Trykoside/Maxiflo combination treatment had the most severe reducing effect and this was also statistically significant compared to the untreated control and most of the other treatments at least up to six weeks after planting.

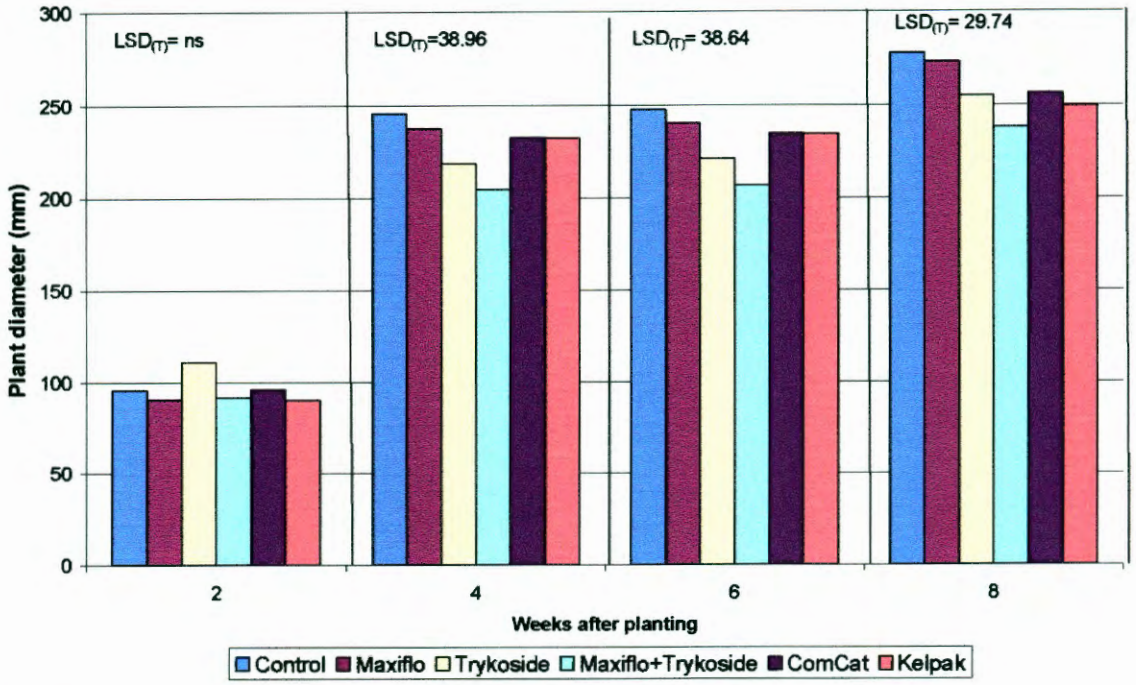

Figure 3.7: The effect of Maxiflo and Trykoside applied both separately and in combination on plant diameter (mm) of lettuce.

3.3.2.3 Head and leaf fresh mass

Statistically, no significant differences between treatments in terms of either head or leaf fresh mass were observed for lettuce. However, except for the Trykoside/Maxiflo combination treatment, all other treatments tended to have a decreasing effect on head fresh mass compared to the untreated control (Figure 3.8). Albeit non-significant, the opposite tendency was observed for the combined Maxiflo/Trykoside treatment in terms of leaf mass. *ComCat*® also tended to decrease the leaf mass as compared to the control. All other treatments had no significant effect although the same tendency to decrease leaf fresh mass, as was observed for head mass, did not repeat itself again .

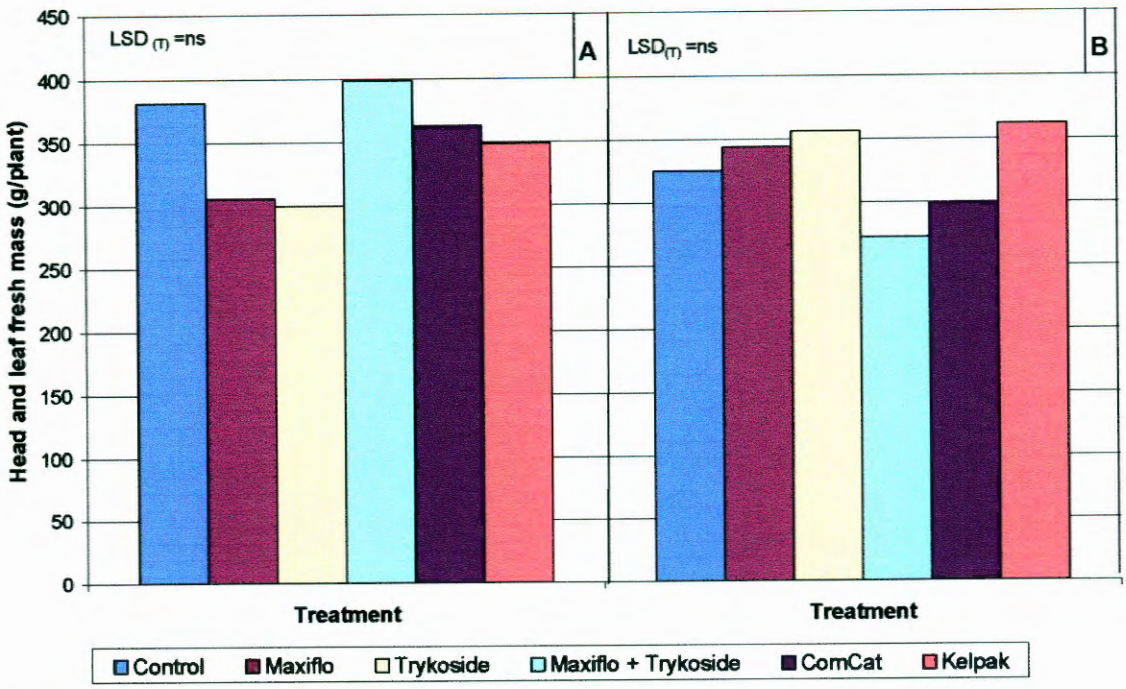

Figure 3.8: The effect of Maxiflo and Trykoside applied both separately and in combination on A) head and B) leaf fresh mass (g/plant) of lettuce.

3.3.2.4 Root fresh mass

Although the Maxiflo and *Kelpak*® treatments had no effect on root development, all other treatments significantly reduced the root fresh mass of lettuce (Figure 3.9).

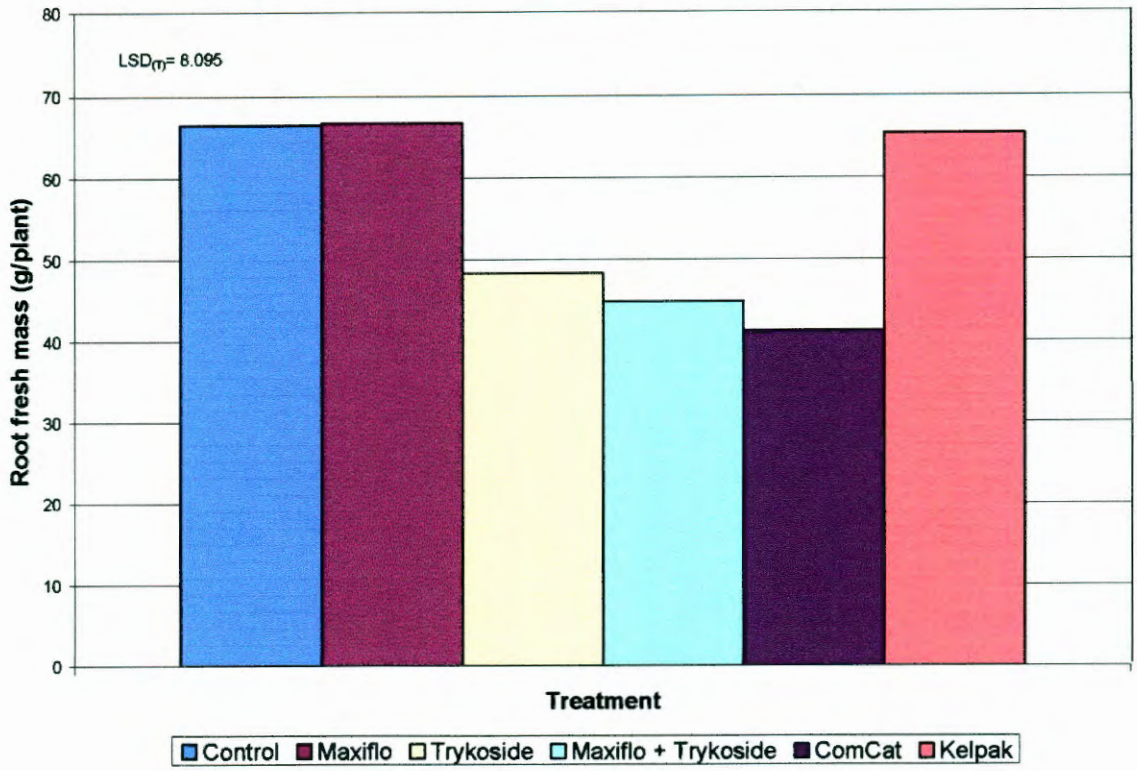

Figure 3.9: The effect of Maxiflo and Trykoside applied both separately and in combination on root fresh mass (g/plant) of lettuce.

3.4 DISCUSSION

When applied separately both products significantly reduced the root growth in cabbage and this was somewhat unexpected as the manufacturers claimed root growth stimulation in the past. As this was not the case when Maxiflo and Trykoside were applied in combination, it is difficult to make a conclusion about the inability of the products to stimulate root growth. A possibility is that *Azospirillum* and *Trichoderma* applied together generate a synergistic effect in terms of root growth stimulation that is not present when the products are applied separately. In light of the fact that both *Azospirillum* and *Trichoderma* are mainly considered as being beneficial to crops by eliminating harmful micro-organisms in the vicinity where crops are grown (Agwah & Shahaby, 1993; Bastos, 1996). Another possibility is that the beneficial claims made for these products in the

past are not as accentuated when harmful micro-organisms are not abundant. It is suggested that future research be undertaken to monitor the population dynamics of known harmful soil micro-organisms where Maxiflo and Trykoside is applied in order to verify the impact of the two products.

Further, neither Maxiflo nor Trykoside had any significant effect on the vegetative growth of aerial parts of cabbage in terms of all the parameters measured when applied either separately or together and when compared to the untreated control. In seeking an explanation, the same reasoning as for the absence of its effect on root development could apply here. There is also the possibility that different crops react differently to treatments with bio-products and it remains to be seen how other crops react before a final conclusion could be made.

However, although not significantly, both products tended to increase the final head yield of cabbage compared to the untreated control. Interestingly, in this case both contributed to a simultaneous reduction in the foliage fresh mass of cabbage. However, this was not the case where *ComCat*® was applied as the product increased both the head mass and the foliage significantly in cabbage. From this it is difficult to assume that an increase in head mass predisposes a reduction in foliage growth. On the other hand, the application of the second positive control, *Kelpak*®, significantly reduced cabbage head mass while foliage growth was also lower than that of the untreated control. From this it rather seems that head mass and foliage growth are positively correlated indicating that lush foliage growth may lead to elevated head formation and fresh mass. More trials are needed to verify this assumption.

Statistically significant differences in plant diameter and plant height were observed in cabbage only in terms of the *Kelpak*® treatment while for stem thickness there were no significant differences measured in cabbage between treatments. The *ComCat*® treatment significantly increased both the head and

leaf fresh mass of cabbage compared to all other treatments. *Kelpak®*, on the other hand, had a significant inhibitory effect on head mass, compared with other treatments and especially the untreated control, but this was not the case for leaf mass. Results obtained with the positive controls were again too erratic to arrive at a general conclusion in terms of the relationship between foliage growth and head mass.

In the case of lettuce, differences among treatments were observed for the vegetative growth of aerial parts of lettuce in all parameters measured, when Maxiflo and Trykoside were applied either separately or together and when compared to the untreated control. However, no significant differences were detected in head mass and leaf mass when Maxiflo or Trykoside was applied either separately or together. The application of both products separately, again significantly reduced the root growth as was the case in cabbage.

Statistically, significant differences in plant diameter and plant height were observed in lettuce in terms of only the combination of Maxiflo and Trykoside treatments while for head mass and leaf mass there were no significant differences between treatments. Maxiflo + Trykoside, on the other hand, had a significant inhibitory effect on plant height and plant diameter, compared to other treatments and especially the untreated control, but this was not the case for head and leaf mass. No literature on the effect of these products on either cabbage or lettuce, in order to verify these findings, could be found. As mentioned earlier, different crops might react differently to treatment with Maxiflo and Trykoside and the effect on other crops need to be considered first before a final conclusion could be reached in terms of the potential of these products to increase yields of agricultural or horticultural crops in organic farming systems.

CHAPTER 4

EFFECT OF LIQUID MAXIFLO AND TRYKOSIDE ON ROOT GROWTH AND YIELD OF WHEAT AND PEAS

4.1 INTRODUCTION

The usefulness of associative diazotrophs like *Azospirillum* has been based on its ability to enhance either root biomass or nitrogen fixation or both in a number of crops (Okon, 1985; Panwar *et al.*, 1990). Nodule like structures have been induced on a large number of non-leguminuos plants using different plant homones like 2,4-D, NAA, BAP and Zeatin (Tchan & Kennedy, 1989; Tchan *et al.*, 1991; Kennedy & Tchan, 1992; Ridge *et al.*, 1992, 1993; Panwar & Elanchezhian, 1995). Chinese researchers have reported that *Azospirillum* inoculation has further induced the effect of 2,4-D on root nodules in wheat (Tchan & Kennedy, 1989; Nie *et al.*, 1992; Yu *et al.*, 1993). Liu *et al.* (1993) studied the stimulating, inhibiting and nodulating effect of 2,4-D on wheat seedlings and found that 30% of nodules were infected after inoculation with *Azorhizobium caulinodans*. The nodulation *per se* has also been reported to be a physiological process independent of bacterial action (Francisco & Akao, 1993; Ridge *et al.*, 1993).

According to Inbal & Feldman (1982) the inoculation of wheat seedlings with *Azospirillum* increased the grain yield by 91%. It was suggested that hormonal action rather than N fixation may have accounted for the increased yield. Barbieri *et al.,* (1988) reported that inoculation of wheat seedlings with wild types strains of *Azospirillum brasilense* significantly increased the number and length of lateral roots.

Many studies have shown that the inoculation of wheat, and other cereal seed, with bacteria of the genus *Azospirillum* resulted in an increase in both volume and number of roots (Bashana & Levanony, 1990; Didonet & Magalhaes, 1993). An increase in cereal production by inoculation with *Azospirillum* was observed in many field experiments (Sarig *et al.*, 1984; Baldani *et al.*, 1986, 1987; Boddey *et al.*, 1986; Kapulnik *et al.*, 1987a).

Shivanna *et al.,* (1994) reported that wheat treated with *Trichoderma spp* showed significant increases in shoot length and dry matter as well as spike length and seed number per spike. According to Duffy *et al.* (1996) *Trichoderma koningii* applied to the seed furrow under field conditions increased the yield of spring wheat by 65% and reduced crown root infection by *Gacumannomyces graminis* var. *tritic* on winter wheat by 40%. No information on the effect of *Trichoderma* on legume crops could be traced in the literature.

The objective of this study was to determine the effect of the bio-products Maxiflo and Trykoside on the vegetative growth and yield of wheat and pea crops under field conditions.

4.2 MATERIALS AND METHODS

4.2.1 Trial site

Field experiments on peas and wheat were conducted at the experimental farm of the Department of Soil, Crop and Climate Sciences of the University of the Free State, near Kenilworth in the Bloemfontein district (29°01'00"S, 26°08'50"E) during the 2003 growing season.

4.2.2 Experimental design and treatments

A randomized complete block design (RCBD) was used for both field trails conducted in this study. Six different treatments (Conrol, Maxiflo, Trykoside, Maxiflo + Trykoside, *ComCat*® and *ComCat*® + Maxiflo + Trykoside) were applied randomly to the plots and each treatment was replicated five times.

The type of soil where the experiments were conducted was a sandy loam soil. Fertilizer was applied according to the withdrawal amounts and expected yield of 60 ton ha^{-1} and 50 ton ha^{-1} for wheat and pea respectively. In the pea trial fertilizer was applied at the rate of 142.84 kg N ha^{-1}, 56.89 kg P ha^{-1} and 56.8 kg K ha^{-1} before planting and incorporated with a rotavator. For wheat the rates were 81.73 kg N ha^{-1}, 40.86 kg P ha^{-1} and 20.43 kg K ha^{-1}. Irrigation was applied immediately after applying the fertilizer.

Pea (cultivar Solara) seeds were planted in moist soil. The planting density for peas was estimated at 35 556 plants per hectare with 4 cm inter-row spacing and 75 cm intra-row spacing in plots. The wheat cultivar planted was SST 363. The planting density per hectare for wheat was unknown but standard inter-row and intra-row spacing was applied. Plot size for peas was 5.625 m^2 and for wheat it was 24 m^2.

ComCat®, a commercial bio-stimulant of plant origin, was used as a positive control in these two field trials (Table 1). The manufacturers claim that the product promotes plant growth and development, physiologically assists and strengthens the plants' natural resistance mechanisms to pathogen attacks and encourages root growth (Agraforum, Germany).

Trichoderma (fungus) forms the basis of the product Trykoside and *Azospirillum* (bacterium) that of the product Maxiflo and was generously donated by AXIOM

Bio-products Pty Ltd, Isando, South Africa. The effect of Trykoside and Maxiflo was tested both separately and in combination on the growth and yield of peas and wheat (Table 4.1).

Table 4.1: Summary of the treatments applied on both peas and wheat

Treatment	Concentration	Spray information
Control		
Maxiflo	1L ha^{-1}	Spray at planting and three sprays every 2 weeks thereafter (peas), Single spray at the 3-leaf growth stage (wheat)
Trykoside	1L ha^{-1}	Spray at planting and three sprays every 2 weeks thereafter (peas), Single spray at the 3-leaf growth stage (wheat)
Maxiflo + Trykoside	1L + 1L ha^{-1}	Spray at planting and three sprays every 2 weeks thereafter (peas), Single spray at the 3-leaf growth stage (wheat)
ComCat®	100g ha^{-1} peas (10:90 ratio) & wheat (4:96 ratio)	Spray at planting and three sprays every 4 weeks thereafter (peas), Single spray at the 3-leaf growth stage (wheat)
ComCat® + Maxiflo + Trykoside	100g+1L+1L ha^{-1}	Spray at planting and three sprays every 4 weeks thereafter (peas), single spray at the 3-leaf growth stage (wheat)

Suspensions of Maxiflo and Trykoside as well as *ComCat*® were applied at the rate of 890 L ha^{-1} for peas. For wheat the suspensions were applied at a rate of 833 L ha^{-1}. Foliar sprays were applied using a knapsack spray.

4.2.3 Irrigation

A micro irrigation system was used. Irrigation started immediately after planting, up to one week before harvest.

4.2.4 Insect and weed control

Cutworm and aphids were controlled with Chloropyrifos at 15 ml L^{-1} according to the instructions on the manufacturer. Three treatments during the trial period were sufficient for total control of these insects. The plots were kept free of weeds by hand-weeding every two weeks throughout the growing season.

4.2.5 Harvesting methods and dates

Peas

Pods were harvested four times by hand, 13, 14, 15 and 16 weeks after planting during the 2003 growing season.

Wheat

Wheat was harvested 15 weeks after planting during the 2003 growing season using a Nursery Master Hydrostatic combine harvester.

4.2.6 Parameters used to quantify growth and yield data

4.2.6.1 Growth parameters

4.2.6.1.1 Root fresh mass of wheat

Root fresh mass was measured for wheat in the laborotary after the root system was severed from the plant, the soil washed from it and the roots blotted dry on tissue paper.

4.2.6.1.2 Root volume of wheat

Root volume was measured for wheat in ml by using a measuring cylinder. A known volume of water was transferred to the measuring cylinder and recorded. Subsequently, the roots were severed from the plant and after the soil was washed from it, the roots were blotted dry on kimwipe tissue paper, submerged in the water and the difference in volume readings taken as the root volume. Care was taken to remove all air bubbles before the readings were recorded.

4.2.6.1.3 Root dry mass of wheat

After fresh mass was measured the roots were placed in a drying oven at 70^{o}C for one week and the dry mass was measured in the laboratory.

4.2.6.2 Yield parameters

4.2.6.2.1 Pea pod number

The number of pea pods per plot was counted 13, 14, 15 and 16 weeks after planting, respectively. This was added to caclculate the total number of pods per hectare.

4.2.5.2.2 Pea pod and seed fresh mass

The fresh mass of pods containing seeds and seed fresh mass separately were measured per plot at harvest on the 13, 14, 15 and 16 weeks after planting.

4.2.6.2.3 Wheat ear and kernel dry mass as well as kernel number per ear

Dry ears from 20 plants in each replicate were randomly harvested at the end of the drying cycle, the mass determined and the average weight calculated per plot. Subsequently, kernels were removed from the ears, counted by means of

an electronic seed counter and the dry mass determined per ear. Finally, 15 m^2 (5 x 3 m) of the plots were harvested in areas where the ear samples have not been taken and the yield expressed in ton ha^{-1}.

4.2.6.3 Statistical analysis

Analysis of variance was done on all parameters used to quantify the vegetative growth and yield components to determine the significance of differences between means using the NCSS 2000 program. Tukey's least significant difference (LSD) procedure was employed to separate means at the 5% ($P<0.05$) level.

4.3 Results

4.3.1 Peas

4.3.1.1 Pod number

Maxiflo and Trykoside applied separately as well as together had a significant reducing effect on the pod number compared to the untreated control (Figure 4.1). On the other hand, although *ComCat*® applied separately had only a slight enhancing effect on pod number, it contributed to a significant increase (excess of 20 000 pods/ha) in pod number compared with when applied in combination with Maxiflo and Trykoside.

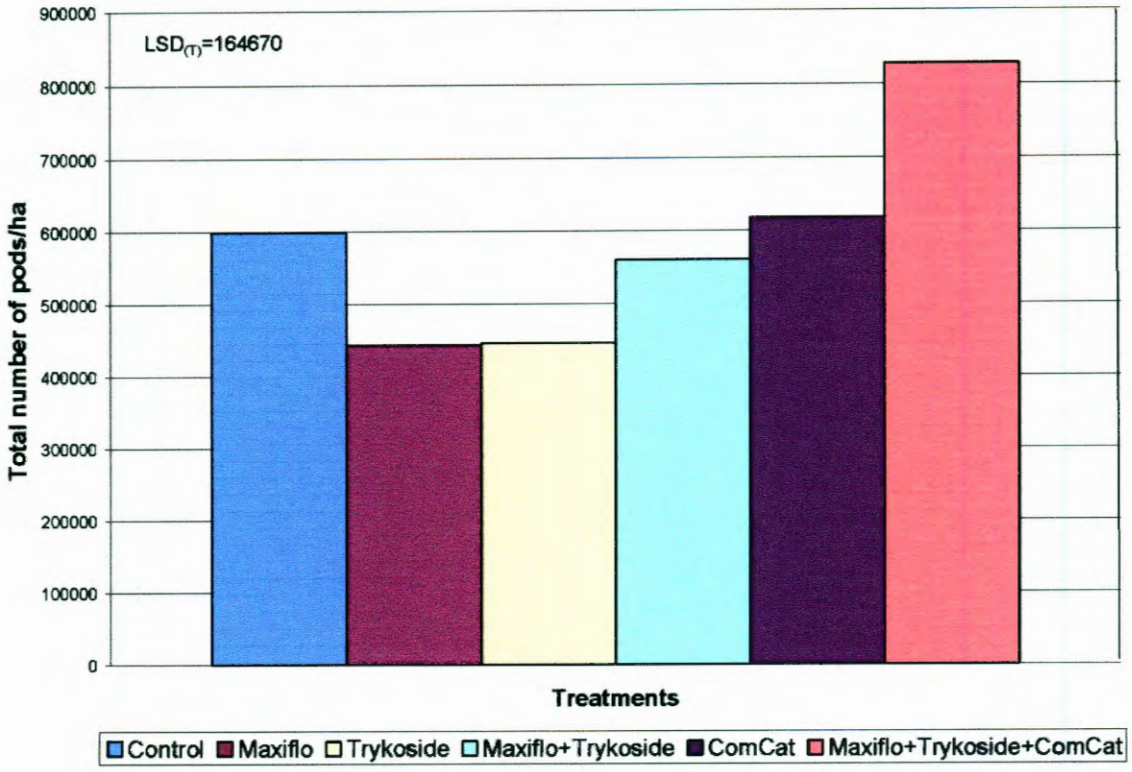

Figure 4.1: The effect of Maxiflo and Trykoside both separately and in combination on the total number of pea (*Pisum sativum*) pods per hectare.

4.3.1.2 Pod and seed fresh mass

Interestingly, although Maxiflo applied separately had a reducing effect on total number of pods (Figure 4.1) it contributed to a significant increase in both pod fresh weight (Figure 4.2A) and seed fresh weight (Figure 4.2B). Trykoside applied separately showed the same tendency to reduce both pod and seed fresh weight as was the case for pod number. Maxiflo and Trykoside in combination as well as *ComCat*® applied separately showed the same tendency towards reducing pod fresh weight but increased the seed fresh weight. This was significant in the case of *ComCat*® compared to the untreated control. However, when Maxiflo, Trykoside and *ComCat*® were applied in combination a sharp and

statistically significant increase in seed fresh mass,as was observed for pod number, prevailed.

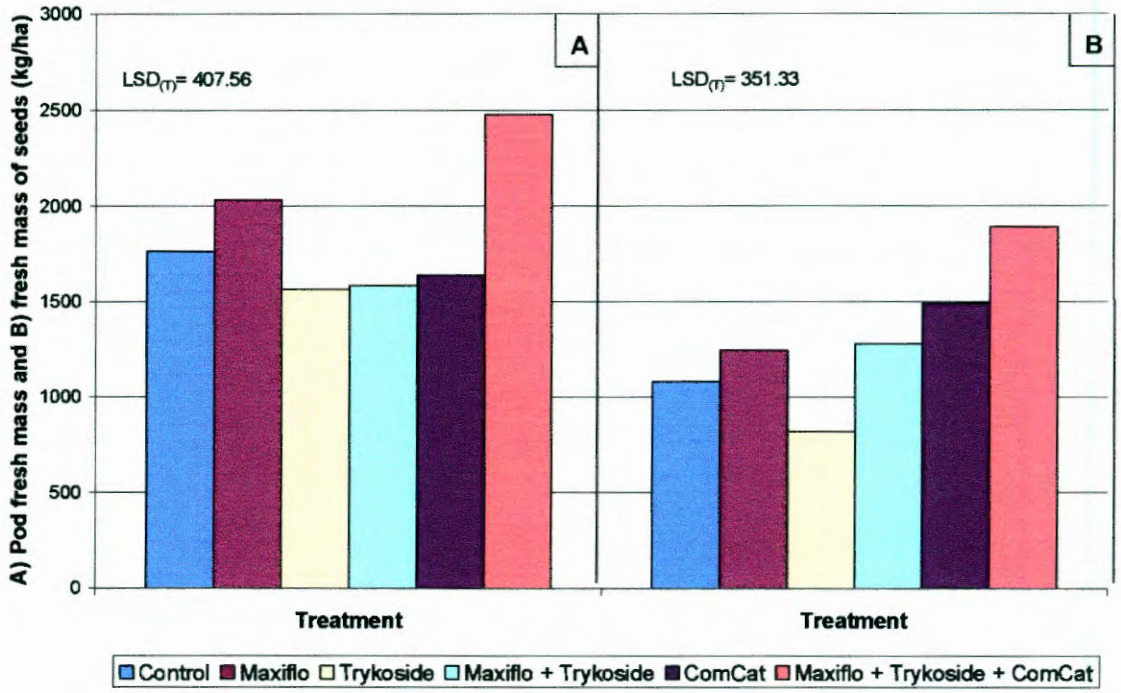

Figure 4.2: The effect of Maxiflo and Trykoside both separately and in combination on the A) pod fresh mass and B) seed fresh mass of pea (*Pisum sativum*).

4.3.2 WHEAT

4.3.2.1 Root volume of wheat

Maxiflo had no significant effect on root volume while Trykoside applied separately and in combination with Maxiflo slightly, but non-significantly, increased the root volume of wheat (Figure 4.3). *ComCat*® showed the same tendency to increase root volume slightly but, interestingly, when applied in combination with both Maxiflo and Trykoside it caused a rather sharp decrease in root volume. The latter was, however, still non-significant.

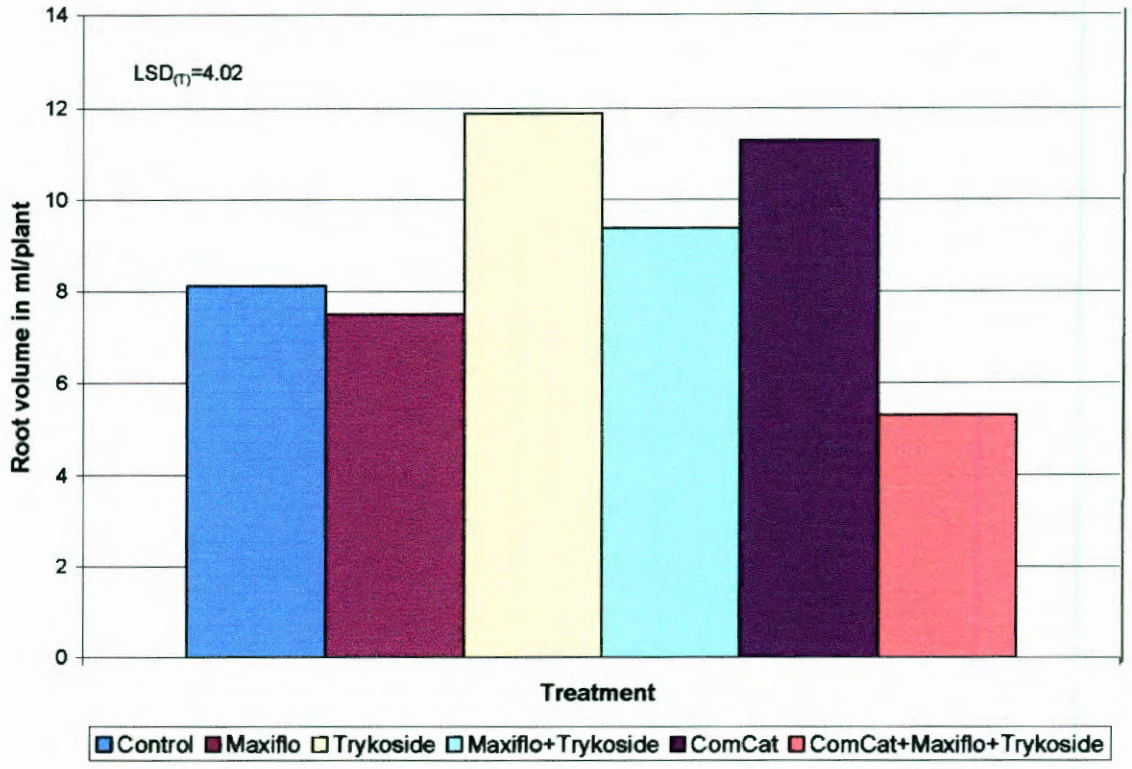

Figure 4.3: The effect of Maxiflo and Trykoside both separately and in combination on the root volume (ml/plant) of wheat.

4.3.2.2 Wheat root fresh mass and dry mass

No statistically significant differences between treatments were observed in root fresh mass compared to the untreated control (Figure 4.4A) but, there was a significant difference detected in root dry mass (Figure 4.4B). Both Maxiflo and Trykoside applied separately significantly increased the root dry mass but when applied in combination had no effect. Both *ComCat*® applied separately and the Maxiflo/Trykoside/ *ComCat*® combination reduced the root dry weight, albeit not significantly, compared to the untreated control as well as all other treatments.

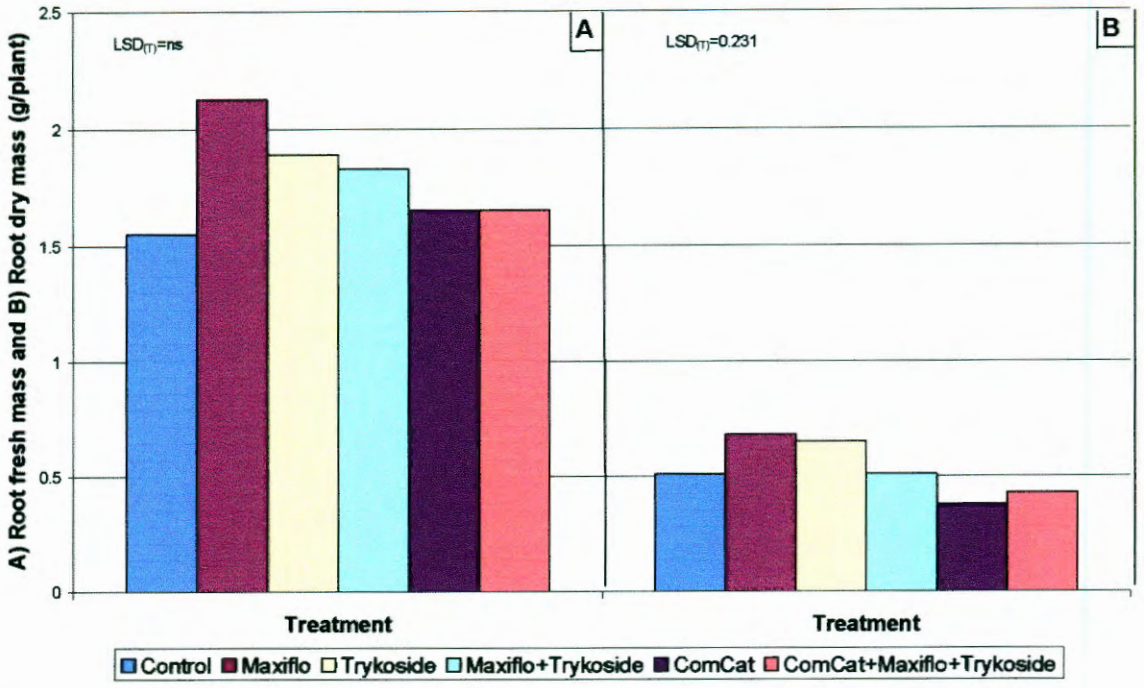

Figure 4.4: The effect of Maxiflo and Trykoside both separately and in combination on the A) root fresh mass and B) root dry mass of wheat.

4.3.2.3 Kernel number per ear

Twenty ears were selected randomly in plots, and replicated four times, to calculate an acceptable average number of kernels per ear. All treatments contributed to an increase in the number of wheat kernels per ear (Figure 4.5). Of these the Trykoside treatment was the only one that increased the kernel number significantly.

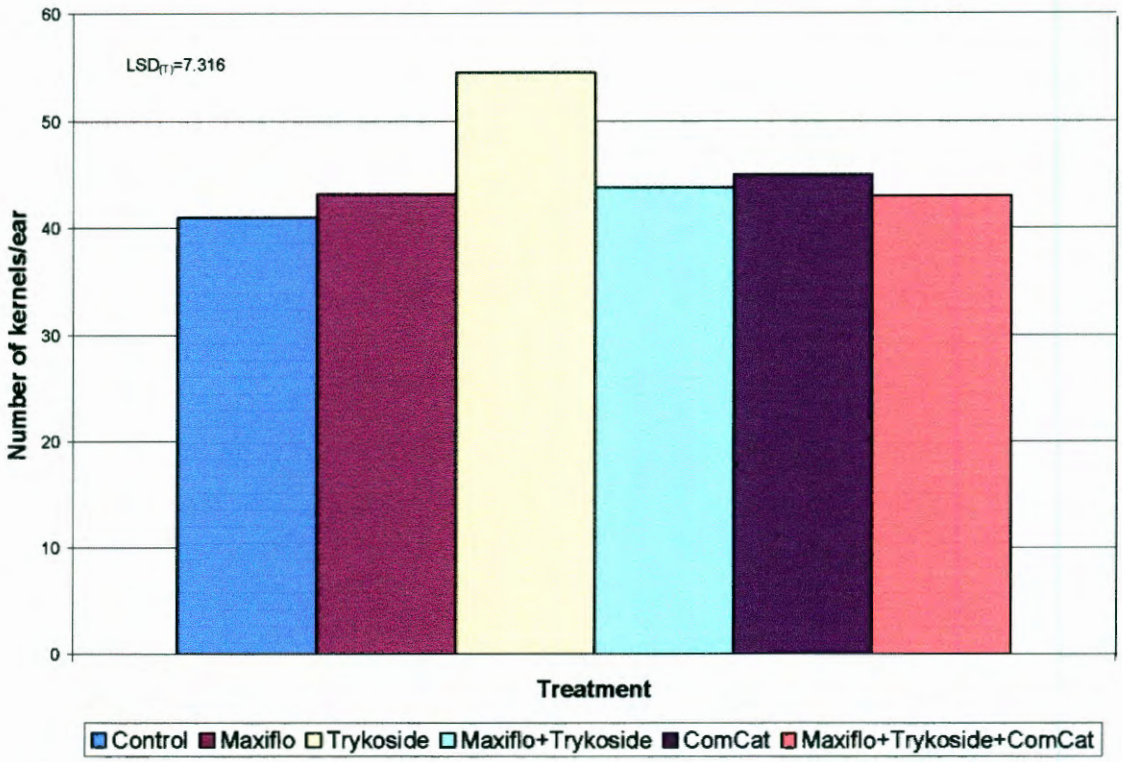

Figure 4.5: The effect of Maxiflo and Trykoside both separately and in combination on the number of wheat kernels per ear.

4.3.2.4 Kernel dry mass per ear

Although all treatments contributed to an increase in the kernel weight per ear compared to the untreated control, this was more marked when Maxiflo and Trykoside were applied separately (Figure 4.6). However, none of these differences were statistically significant. Where *ComCat®* was added to both Maxiflo and Trykoside and tested in combination, the dry mass increase of kernels was more marked, but not significant, than where Maxiflo and Trykoside was tested in a combination.

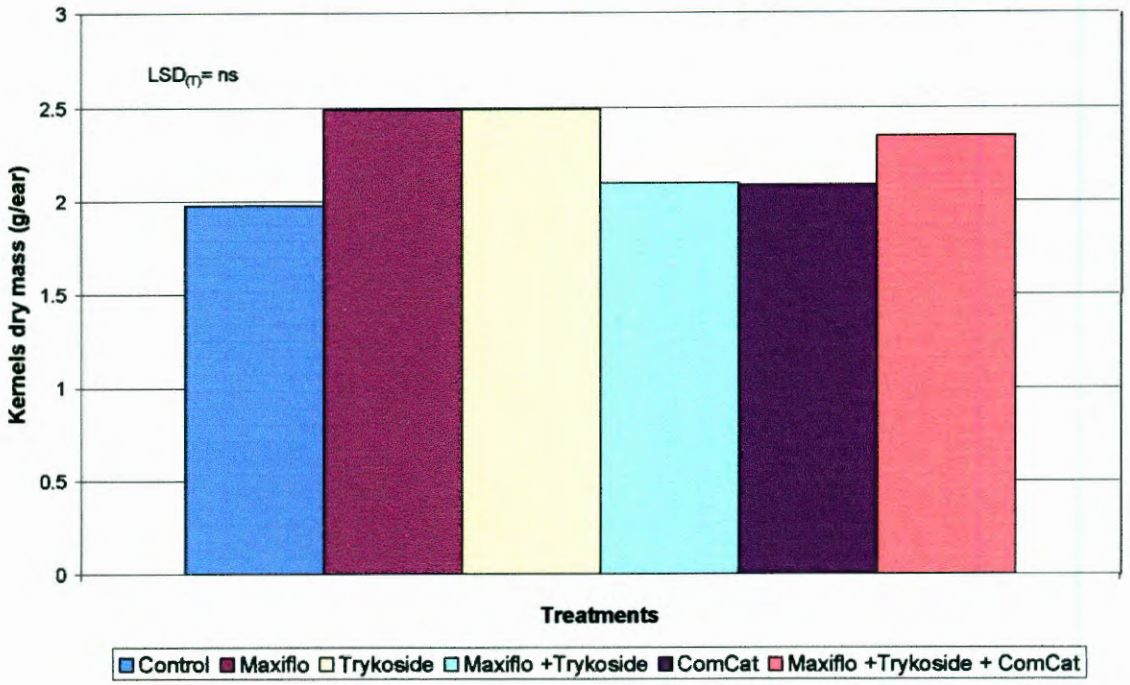

Figure 4.6: The effect of Maxiflo and Trykoside both separately and in combination on the wheat kernel mass per ear. Note: one replicate was discarded during data analysis due to poor stand establishment.

4.3.2.5 Total dry kernel yield per plot

Maxiflo and *ComCat*® applied separately as well as the Maxiflo-Trykoside-*ComCat*® combination treatment tended to increase the total dry kernel yield slightly but non-significantly (Figure 4.7). Of these the increase by *ComCat*® was most marked. Interestingly, Trykoside on its own contributed to a reduction in kernel yield of almost 50%. Probably due to large standard deviations between replicates, this was again regarded non-significant by the statistical procedure.

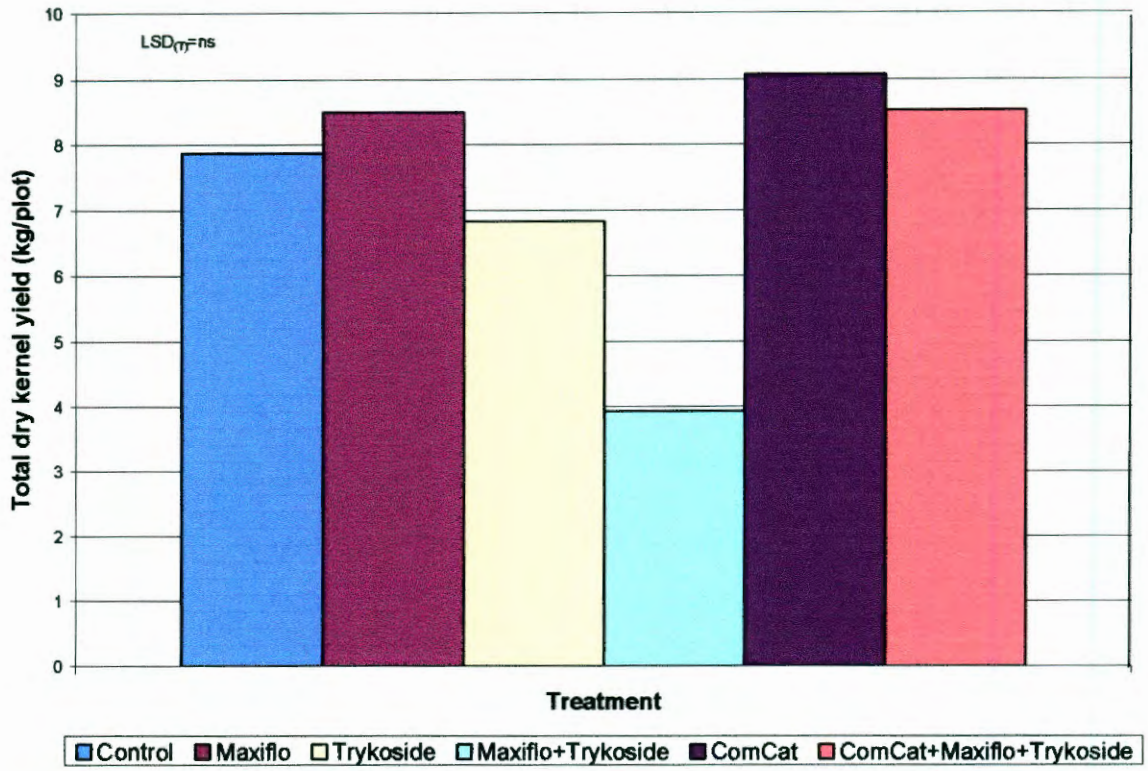

Figure 4.7: The effect of Maxiflo and Trykoside both separately and in combination on the total dry kernel yield (kg/plot) of wheat. Note: one replicate was discarded during data analysis due to poor stand establishment.

4.4 DISCUSSION

In peas the three way Maxiflo + Trykoside + *ComCat*® combination treatment significantly increased pod number as well as pod and seed fresh mass while *ComCat*® applied separately and Maxiflo + Trykoside applied as a two way combination treatment only resulted in increased seed mass. It was expected that an increase in pod number would result in an increase in seed number and mass. It is speculated that the three way combination treatment must have had an enhancing effect on flower formation and subsequently pod number to achieve this increase. Interestingly, *ComCat*® alone as well as the Maxiflo + Trykoside two way combination treatment did not have the same effect on pod

number making it difficult to ascertain which of these products had an effect on flower formation and pod yield increases.

It has been reported that the brassinosteroids (BRs) contained in *ComCat*® act as the active substance (Agraforum, 2003) but also that BRs stimulate flower formation in a number of crops (Schnabl *et al.*, 2001). However, as *ComCat*® applied separately did not have a significant effect on pod number in peas. It seemed that the effect on flower formation reported on other crops is not applicable to a legume crop such as pea. This might indicate that, depending on the foliar application of these products either separately or in combination, a different metabolic related mechanism of action is triggered in the plants. Furthermore, combination of treatments might indicate a synergistic effect between products in the case where the combination treatments showed a tendency to enhance the pea yield or an antagonistic effect in the case where combination treatments resulted in the opposite. By means of a metabolic approach this aspect needs further investigation.

On the other hand, compared to the untreated control, Maxiflo and Trykoside supplied separately rather inhibited the total number of pods counted over four harvests while the addition of *ComCat*® in a three way combination increased the pod number. From this it seems reasonable to postulate that the commercial bio-stimulant might have prevented the abscission of pods, a phenomenon that is well known in legume crops such as pea and beans (Burdman *et al.,* 1998), leading to the increased pod number observed in this study. This is in agreement with the report of Molahlehi (2000) on the reducing effect of *ComCat*® on pod abscission in dry beans under glasshouse conditions.

Interestingly, not withstanding the effect of the products under scrutiny on pod number, Maxiflo and *ComCat*® supplied separately as well as the Maxiflo + Trykoside two way and Maxiflo + Trykoside + *ComCat*® three way combination

treatments had a significant enhancing effect on the seed fresh mass in peas. It can be speculated that the increase in seed mass was achieved via enhanced carbohydrate photosynthate translocation from the storage organ (source) to the developing seed (sink) during the grain filling stage leading to increased protein levels via conversion from carbohydrates. It has been reported that brassinosteroids, the active compound of *ComCat*®, has a membrane energizing effect enhancing sucrose translocation over membranes (Arteca, 1995) as well as the source: sink relationship. However, for Maxiflo and Trykoside this mechanism of action is unknown.

In fact, only a few reports on the effect of the *Azospirillum* (bacterium) based product, Maxiflo, on the yield of legumes are available in the literature and none on the *Trichoderma* (fungus) based product Trykoside. Field inoculation of garden peas and chickpeas with *A. brasilense* produced a significant increase in seed yield, but did not affect the dry matter yield (Sarig *et al.*, 1986). The author suggested that the increase in yield was the result of increased nitrogen fixation after inoculation with *Azospirillum*. Besides its nitrogen fixing ability, *Azospirillum spp.* secretes phytohormones such as auxins, cytokinins and gibberellins (Steenhoudt & Vanderleyden, 2000). Of these auxin is quantitatively the most abundant phytohormone secreted by *Azospirillum,* indicating that auxin production, rather than nitrogen fixation, might be the major factor responsible for the stimulation of rooting and, hence, enhanced plant growth and yield. The inoculation of legumes with *Azospirillum* alone in the case of naturally-nodulated legumes, were shown to benefit plant growth under both greenhouse and field conditions (Burdman *et al.,* 1998).

In this study treatment of peas with Trykoside only rather inhibited seed fresh mass at final harvest indicating that its mechanism of action was different from that of Maxiflo. However, it has also been shown that *Trichoderma* is capable of synergistically interacting with Maxiflo and *ComCat*® in promoting the growth and yield of peas. It is well established that *T harzianum* protects crop roots against

pathogenic fungi and improves the health of crops (Harman (2000). This has also been argued for the bacterium *Azospirillum* (Maxiflo) against bacterial pathogens. In itself this mode of action will probably only have an indirect effect on crop growth and yield. The possibility that one or other unknown chemical X-factor or X-factors, that are responsible for a more direct effect on crop metabolism, might be secreted by these two beneficial organisms can not be ignored.

Bashan & Holguin (1997a, b) reported on the growth regulating properties of *Azospirillum* where the X-factor mentioned earlier might be related to the phytohormones secreted by the bacterium. However, *Trichoderma* has only been described as a parasitic organism in the past (Papavizas and Samuel, 1985) and no mention in literature has been made of a possible secreted chemical or hormone that might meet the description of an X-factor. According to Lifshitz *et al.* (1986) the control of *Pythium* species on peas by *Trichoderma harzianum* as well as *T. koningii* was not due to either mycoparasitism or competition but rather due to the suppression or inhibition of *Pythium* growth masking its presence. Whether this inhibition of pathogen growth is related to the inhibitory effect Trykoside had on the seed yield in the legume under investigation is not clear and needs further investigation.

In wheat the same tendency of Maxiflo, Trykoside and *ComCat*® to increase the dry kernel yield at final harvest as was seen in peas was not observed indicating that different crops react differently to treatment with these bio-products. What is especially confusing is the fact that Maxiflo had no effect on the root growth of wheat in terms of root volume while *Azospirilium*, on which the product is based, is known for secreting growth hormones. On the other hand *Trichoderma* on which the product Trykoside is based, not known for secreting growth promoting substances, significantly enhanced the root volume of wheat. Although not significant, the same tendency was observed in terms of marked increases in root fresh weight. This phenomenon is difficult to explain and needs further

investigation on a mechanistic level (i.e. to elucidate the mechanism of action of these products).

The wheat results obtained in this study contradicts previous findings reported in the literature. According to Saubidet & Barneix (1998) inoculation of wheat with *Azospirillum brasilense* resulted in significant root and shoot growth stimulation although the degree of stimulation was different for different *Azospirillum* strains. The authors suggested that *A. brasilense* has the potential to supply substantial amounts of N to wheat plants, although an adequate strain is still to be identified. Most of the information available in the literature on yield increases in cereals and grasses is due to *Azospirillum* inoculation (Sarig *et al.,* 1984; Okon, 1985; Baldani *et al.,* 1986; Boddey *et al.,* 1986; Kapulnik *et al.,* 1987b; Wani, *et al.,* 1988). However, Zimmer *et al.* (1984) reported that it was difficult to detect statistical differences in grain yield increases due to high (10%) standard deviations between replicates. In the present study high standard deviations between replicates were also encountered.

Shivanna *et al.* (1994) reported that treatment of wheat with *Trichoderma* resulted in a significant increase in seed number. The results in this study were in agreement although non-significant. However, the staggering dry kernel yield increase of 65% in wheat treated with *Trichoderma koningii* reported by Duffy *et al.* (1996) could not be repeated in this study. More research is necessary to reach a foregone conclusion.

A number of plant growth regulating products applied in agriculture to manipulate plants either by means of hormones or other chemical mechanisms in order to enhance crop yield and quality, are currently available on the market. These include bio-stimulatory products such as *ComCat*® (Agraforum, Germany) and Kelpak® (Qwemico, South Africa). Current research at the University of the Free State revealed the presence of triglycerides in seeds of specific plants that show similar bio-stimulatory activities (Van der Watt, personal communication).

Enhancement of both yield and quality in different crops is claimed by the producers of *ComCat*® and Kelpak®, as well as researchers in the case of the triglycerides. Although Maxifo and Trykoside are micro-organism based products and, as such, differ from the other two mentioned bio-stimulants, it seems imperative that a large scale study be undertaken to compare the potential of these products, and others available on the market, to improve the productivity of a variety of crops over more than one season. It also seems important to include C3 and C4 crops in the event that crops might react differently to the active substances contained in different commercial bio-stimulatory products. In this study attention should also be given to possible synergystic effects by applying the products in combination. In doing this it could once and for all be established which products have the most potential to consistently improve crop production and this will assist extension officers to supply scietifically based information.

CHAPTER 5

EFFECT OF LIQUID MAXIFLO AND TRYKOSIDE ON THE VEGETATIVE GROWTH AND YIELD OF A POTATO AND TOMATO

5.1 INTRODUCTION

Trichoderma seems to control soil borne pathogens on turfgrass by a combination of antibiotic production and hyperparasitism (Lo *et al.*, 1998). Possible active substances involved in the initial control mechanism include enzymes, peptaibols (antibiotic peptides) and volatile antibiotics after which pathogens are invaded by hyperparasitism (Lo *et al.* 1998). *Trichoderma* can also protect turfgrass directly against pathogens. The effectiveness of the T-22 strain for pathogen suppression on golf courses was confirmed in a four year study by Lo *et al.* (1996). The strain was tested in growth chambers as well as under field conditions. Other studies also showed that *Azospirillum* stimulated root growth, causing increased absorption and more efficient utilization of the nitrate fertilizers that were applied (Hartmann and Zimmer, 1994; Fallik *et al.*, 1994). According to Kenny (2001), *Azospirillum* significantly increased both plant size and yield of tomatoes and green peppers under field conditions, and reduced the occurrence of diseases in tomatoes.

In the case of potatoes, Saikia & Azad (1999) reported that *Trichoderma* spp. are found on the root surface once applied and supplies protection against pathogens. Its fungicidal effect was confirmed by Etebarian *et al.* (2000) who reported that cell-free metabolites of *T. virens* (strain DAR 74290) completely inhibited the growth of *Phytophthora erythroseptica in vitro*. *T. virens* and Trichodex, a commercial formulation of *T. harzianum* T39, were tested for their ability to protect potato and tomato plants from disease caused by *P. erythroseptica* in glasshouse experiments. Trichodex and *T. virens* (DAR 74290), alone and combined, reduced disease severity in shoots and roots of potatoes

ten weeks after inoculation with the pathogens. The yield of potatoes from plants treated with *P. erythroseptica* and *T. virens* (DAR 74290) was significantly greater than in controls inoculated with the pathogens alone. Also Saikia and Azad (1999) reported that both *Trichoderma spp.* and Dithane M-45 (mancozeb) showed significant disease control of *Phytophthora infestants* on potato in experimental plots while *Trichoderma viride* was most effective.

The objectives of this study was to investigate the effect of Maxiflo and Trykoside on the growth and yield of tomatoes and potatoes, two high value crops, under South African conditions.

5.2 MATERIALS AND METHODS

5.2.1 Trial site

Potato and tomato trails were conducted at the experimental farm of the Department of Soil, Crop and Climate Sciences of the University of the Free State near Kenilworth in the Bloemfontein district (29°01'00"S, 26°08'50"E) during the 2003 growing season.

5.2.2 Experimental design

A randomized complete block design (RCBD) was used for both the potato and tomato trials. For tomato, six different treatments were randomly applied and in the case of potato, three different treatments were randomly applied. Each treatment was replicated five times for both crops.

The soil on which the experiments were conducted was a sandy loam soil. In the case of potato, N, P and K were applied as 2:3:2 (22) at a rate of 700 kg ha^{-1} and urea at 400 kg ha^{-1}. Fertilizer application for tomato was as follows: 300 kg N ha^{-1}, 75 kg P ha^{-1} and 460 kg K ha^{-1}. Irrigation commenced immediately after applying the fertilizer to avoid volatilization.

Tomato seedlings cultivar (Nemoneta) were transplanted in moist soil with an in row spacing of 35 cm and between row spacing 150 cm which represents a plant population of approximately 12 500 plants per hectare. The planting density for potato was 55 000 plants per hectare. The potato trial was planted mechanically. The plot size of potato was 96 m^2 while that of tomato was 16.8 m^2 .

The soil on the experimental farm of the University of the Free State was analyzed beforehand and showed the following composition:

Table 5.1: Some physical and chemical properties of the topsoil from the field trial / site

Phosphorus (P)	35 ppm
Potassium (K)	175 ppm
Calcium (Ca)	716 ppm
Magnesium (Mg)	114 ppm
Sodium (Na)	42 ppm
Zinc (Zn)	2.7 ppm
$pH_{(H_2O)}$	6.1
$pH_{(KCl)}$	4.9

Table 5 .2: Summary of the treatments applied on the potato trial

Treatment	Concentration	Spray information
A. Control		
B. Maxiflo	1L ha^{-1}	Spray at planting and every week thereafter for four weeks
C. Trykoside	1L ha^{-1}	Spray at planting and every week thereafter for four weeks

Table 5.3: Summary of the treatments applied on the tomato trial

Treatment	Concentration	Spray information
A. Control		
B. Trykoside Low dosage (T)	1 L ha^{-1}	Spray at planting (seed treatment) and every two weeks thereafter (13 sprays in total)
C.Trykoside High dosage (T)	1 L ha^{-1}	Spray at planting (seed treatment) and every two weeks thereafter (13 sprays in total)
D. Maxiflo + Trykoside	1 L + 1 L ha^{-1}	Spray at planting (seed treatment) and every two weeks thereafter (13 sprays in total)
E. *ComCat*® + Maxiflo + Trykoside	100g + 1 L + 1 L ha^{-1}	Spray at planting (seed treatment) and every four weeks thereafter (3 sprays in total)
F. *Kelpak*®	2 L ha^{-1}	Spray at planting (seed treatment) and three additional sprays every four weeks

Suspensions of both Maxiflo and Trykoside as well as the positive controls *ComCat*® and *Kelpak*® were applied at a rate of 1000 L water ha^{-1} for tomato using a knapsack spray. Foliar sprays commenced on the 11th of December, 2003. For potato the Maxiflo and Trykoside suspensions were applied at a rate of 890 L water ha^{-1} and foliar sprays commenced on the 10th of October, 2003. All treated plants received standard fertilizer at planting. The seeds were inoculated or treated with Maxiflo and Trykoside before planting at the rate of 1L ha^{-1} separately.

5.2.3 Parameters used to quantify vegetative growth and yield

Potatoes

5.2.3.1 Number of stolons, stems and leaf canopy area

The number of stolons per plant was counted in separate plots treated in the same way as plots used for yield assessment. Plants were lifted from the soil using a garden fork and the soil removed. Over a four week period the total number of stolons per plant were counted every week. The same plants were used to count the number of stems formed over a four-week period.

Leaf canopy area was measured using a 1 m^2 grid specially constructed with square tubing and strung with nylon rope in 100 blocks of 100 cm^2 each. The grid was placed over the leaf canopy and the 100 cm^2 portion of the grid that was fully covered by leaves was noted with one credit. Portions that were not fully covered were noted with fractions closest to 0.25, 0.5 and 0.75. The sum of the collected area data was calculated and expressed as leaf canopy area in m^2. The procedure was repeated four times per plot and the average calculated.

5.2.3.2 Total tuber yield and sorting of tuber size

Potato tubers were mechanically harvested and the total yield determined by weighing using a commercial mass meter and expressed as ton ha^{-1} for small, medium and large tubers as well as the total yield. Sorting of tubers into three sizes were done mechanically. All spoilt tubers were removed by hand.

Tomatoes

5.2.4.1 Number and mass of fruits

The number of fruits on seven plants per plot (inside row to eliminate side effects) was counted and the weight determined in kg $plot^{-1}$ after sorting the fruits into categories of small, medium and large.

5.2.4.2 Insect control

In tomatoes and potatoes cutworm, American bollworm and other insects were controlled with cutworm bait and Chlorpyrifos at a rate of 10 g 10 m^{-2} and 20 ml 10 L^{-1}, respectively, according to the instructions on the manufacturers. Three treatments during the trial period were sufficient for total control of insect pests.

5.2.4.3 Disease control

In tomato bacterial and fungal diseases were controlled by means of a standard preventative spray program using Tamaron at the rate of 1 ml per 4 L of water (every three days), Parathion at 75 ml per 100L (every two weeks), Dithane WG at 20-30 g per 10 L (every three days), Bravo at 20-35 ml per 10L (after every three days) and Calmabon at a rate of 250 g per 10L (every two weeks). The spraying program was successful and no disease incidence was observed. No disease control was performed on potatoes since seed inoculated with Maxiflo and Trykoside, due to the parasitic activity of the products, was believed to be sufficient to prevent disease infection.

5.2.5 Harvesting time and methods

Potatoes were harvested by hand 20 weeks after planting. Immediately after harvesting tuber fresh mass was determined for each plot using an electronic kilogram unit scale and subsequently classified according to size (These include large, large / medium, medium, small, rejects and % class) using a mechanical sorter supplied by the farmer. Tomatoes were harvested by hand once a week over an eighteen week period. Only fruit that visibly changed colour were

harvested from five plants per plot in the middle row to exclude possible side effects.

5.3 RESULTS

5.3.1 Potato

5.3.1.1 Vegetative growth

5.3.1.1.1 Canopy area

Four weeks after emergence both the Maxiflo and Trykoside treatments tended to decrease the canopy area (Figure 5.1). During later growth stages the canopy area measured was rather inconsistent, no clear pattern emerged and no statistically significant effect was observed.

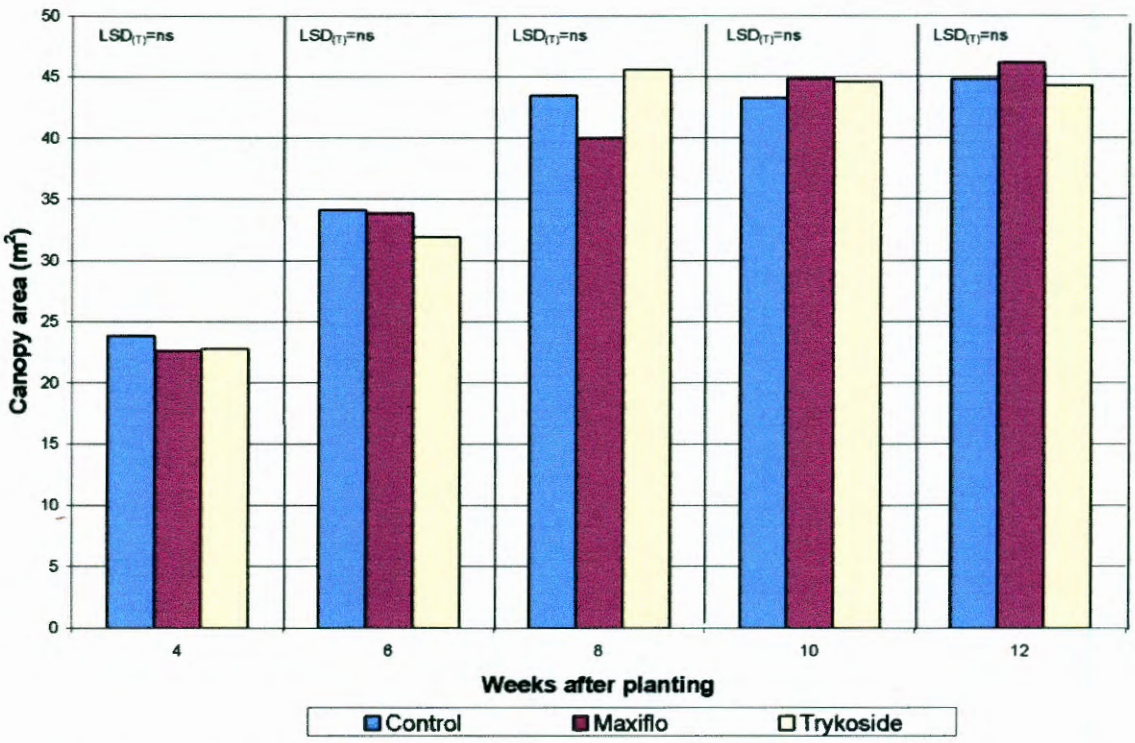

Figure 5.1: The effect of Maxiflo and Trykoside applied separately on the canopy area (m^2) of potato

5.3.1.1.2 Stem counts

No significant differences in stem counts between Maxiflo and Trykoside treated as well as the untreated control were observed (Figure 5.2).

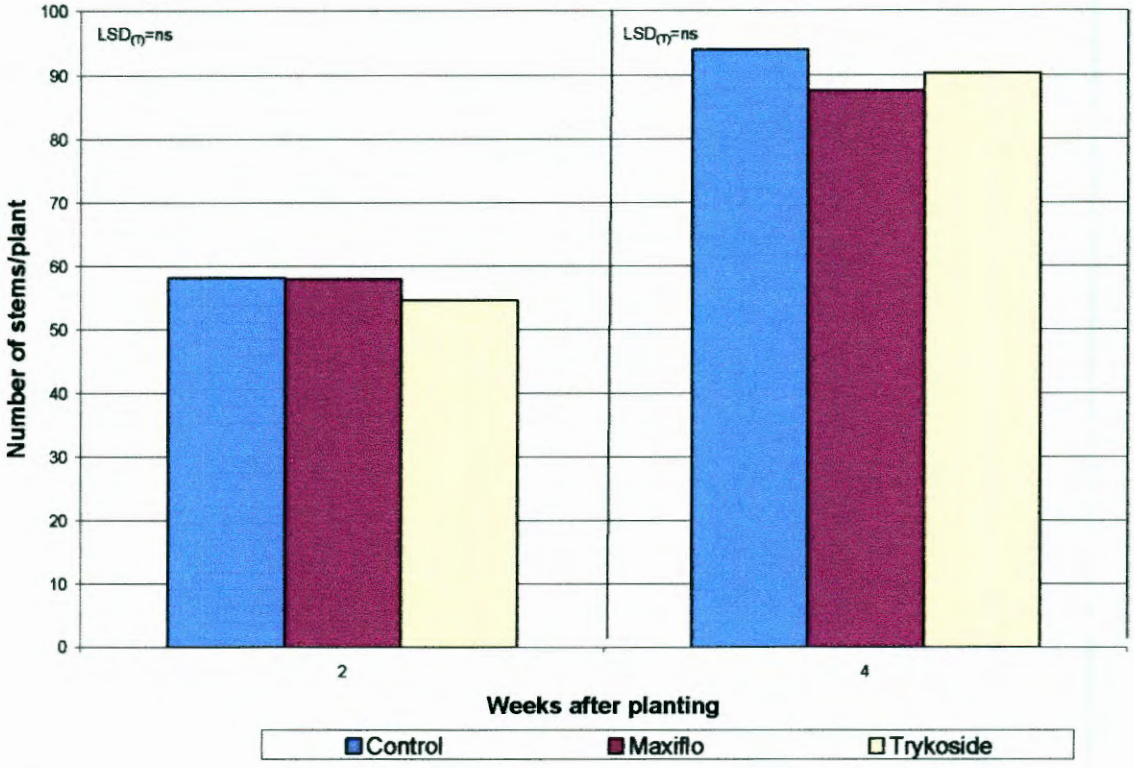

Figure 5.2: The effect of Maxiflo and Trykoside applied separately on the number of potato stems per plant.

5.3.1.1.3 Stolon counts

The results showed non-significant differences between the number of stolons per plant (Figure 5.3) probably due to huge standard deviations calculated. At the first count, four weeks after emergence, as well as at the third count, eight weeks after emergence, both Trykoside and Maxiflo seemed to have a slight enhancing effect on the number of stolons per plant. However, this was not the case at the second count. Further, the lower control counts at four and eight weeks and the extremely low control counts after ten weeks, possibly indicate that more tubers

have been formed from untreated seeds implicating the Maxiflo and Trykoside treatments to have had an inhibitory effect on tuber formation (see .5.3.1.2.2).

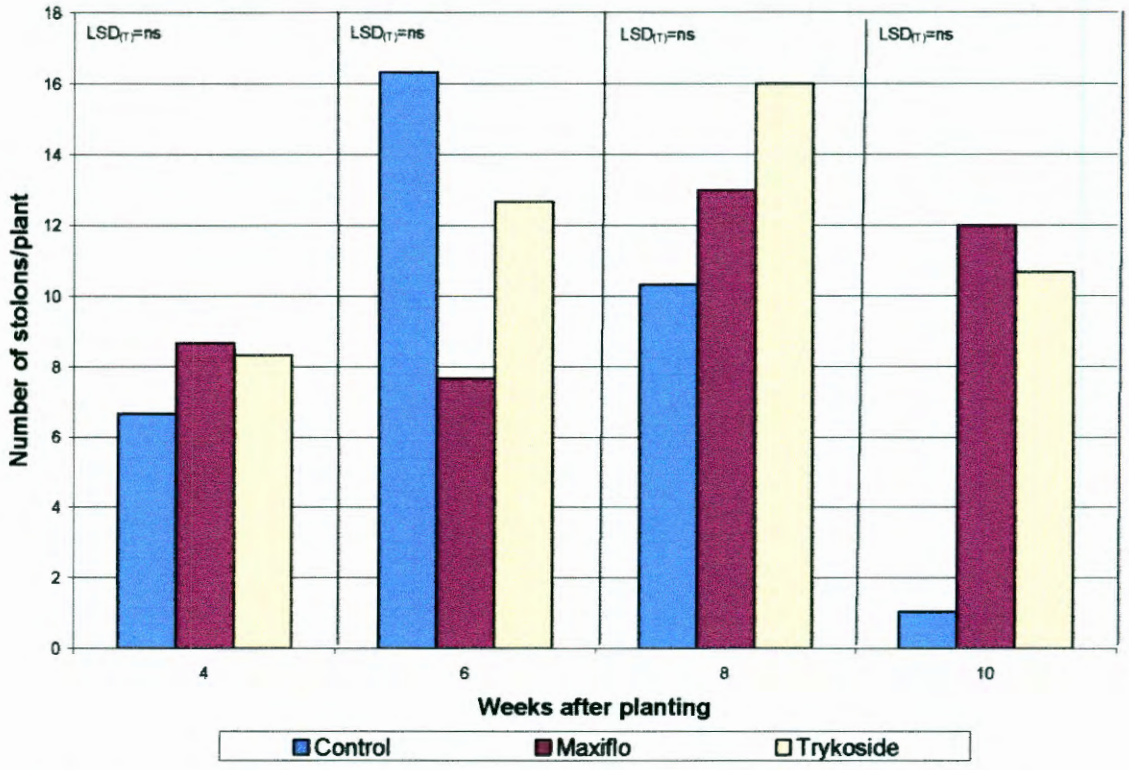

Figure 5.3: The effect of Maxiflo and Trykoside applied separately on the number of potato stolons per plant.

5.3.1.2 Yield

5.3.1.2.1 Classification of potato tubers according to size

Compared to the untreated control, especially Trykoside had an increasing effect on the medium, medium/large and large size potato tubers (Figure 5.4). Maxiflo had no increasing effect on tuber size. Statistical analysis was not performed on this specific data.

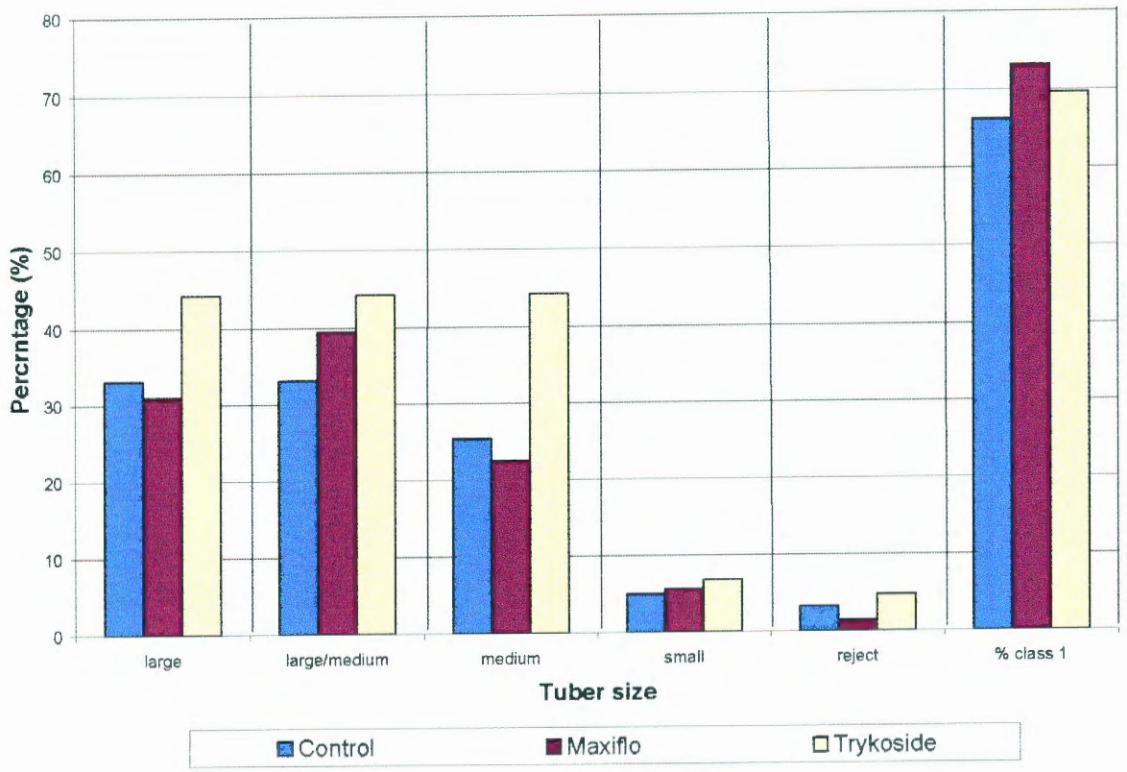

Figure 5.4: The effect of Maxiflo and Trykoside applied separately on the potato tuber size at final harvest.

5.3.1.2.2 Total yield

Differences between the two treatments (Maxiflo and Trykoside) as well as the untreated control were not statistically significant with regard to total tuber yield (Figure 5.5). However, both the Maxiflo and Trykoside treatments tended to have a decreasing effect on the total yield. Extrapolation to yield per hectare from this small statistical trial was extremely low and it is difficult to draw any conclusions.

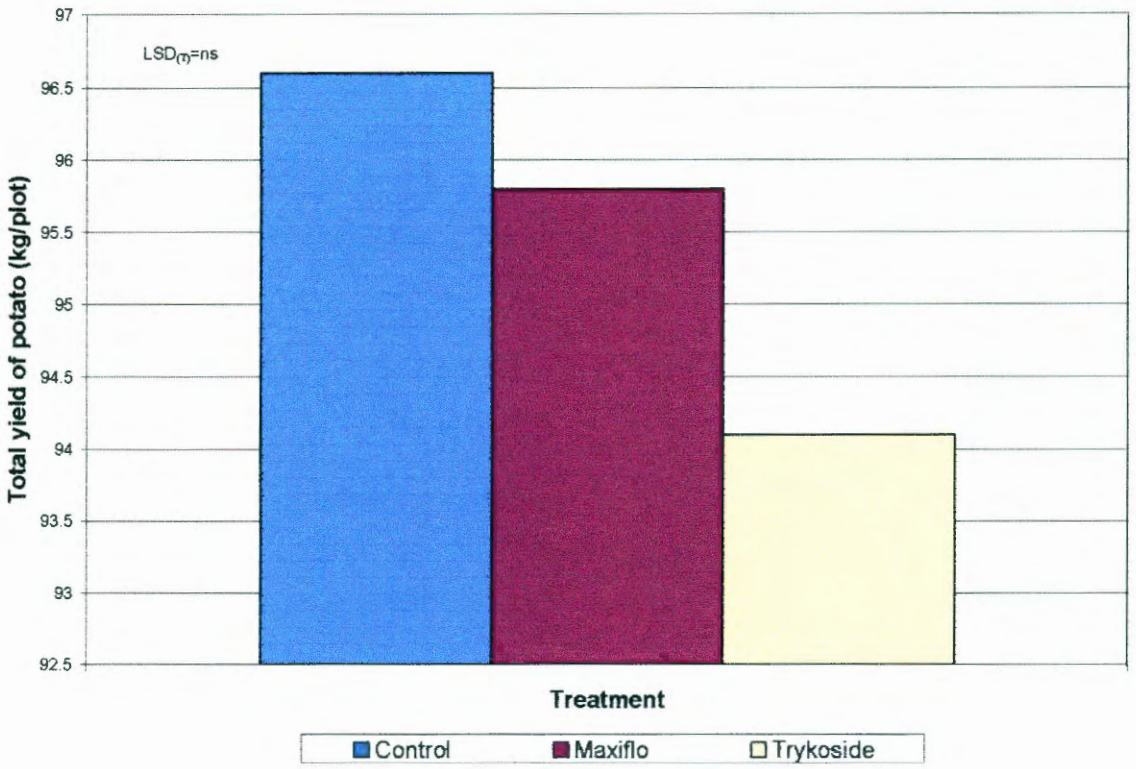

Figure 5.5: The effect of Maxiflo and Trykoside applied separately on the total potato tuber yield

5.3.2 Tomato yield

5.3.2.1 Small, medium, large size and total yield of tomato

As shown in Figure 5.6, no significant differences between treatments in terms of either fruit size or total yield were observed. Except for the *Kelpak*® treatment that had no effect, all other treatments tended to reduce the total yield compared to the untreated control. This was especially visible for the large size fruits.

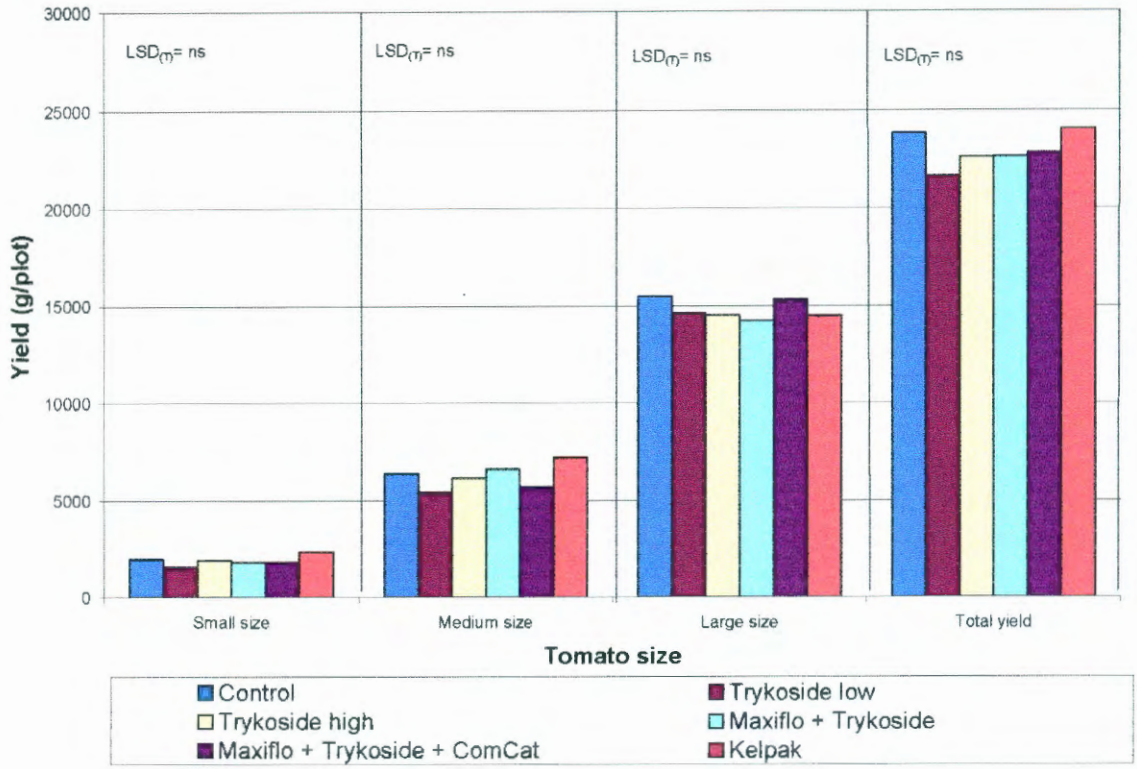

Figure 5.6: The effect of Maxiflo and Trykoside both separately and in combination on the yield of small, medium and large size tomato fruits as well as the total yield.

5.4 DISCUSSION

In potatoes, compared to the untreated control, separate foliar spray treatments with neither Maxiflo nor Trykoside showed a significant effect on vegetative growth in terms of canopy development, stem counts and stolon counts per plant. This is consistent with other crops tested, e.g. the leaf vegetables cabbage and lettuce, and indicates that the effect of the two products manufactured from the bacterium *Azospirillum* and the fungus *Trichoderma* respectively, exerts its influence, if any, via other means. Of these the role of both organisms as parasitic controllers of pathogens is probably best known and most researched.

All applications commenced with a seed treatment of potato tubers at planting and was followed up with foliar spray treatments after emergence. Compared to the untreated control, most treatments tended to reduce the total potato yield. The possibility exists that the spraying programme that was followed, as recommended by the manufacturers, was too intense in terms of number of sprays. This possibility necessitates a follow-up study where the effect of either a single seed treatment or less foliar applications on growth and yield can be investigated.

However, despite the possible over application, especially Trykoside had a positive effect on the number of medium/large and large potatoes harvested improving the marketability of the crop. Another positive aspect that needs to be mentioned is that inoculation of the potato seed before planting with both Maxiflo and Trykoside kept both the tubers and vegetative growth of plants free from disease infection throughout the life cycle (results not shown). This is especially significant in the light of the fact that no disease control was administered. According to Dix (1964), *Trichoderma spp* are considered to be inhabitants of root surfaces and it may be applied for protection against diseases such as *Botrytis* grey mould (Elad, 1994) when applied to the tuber surface before planting. Elad (1994) demonstrated the control of *Botrytis* grey mould by *Trichoderma harzianum*, isolate T39 (which is the active ingredient of Trichodex) in a range of crops. However, there is a need for further research to confirm these results.

Although no relationship between the seed inoculation and foliar application of potatoes with either Maxiflo or Trykoside was observed in terms of disease control, it seemed from the results that seed inoculation before planting can be recommended from a practical farming perspective. Seed inoculation will probably be more acceptable by the farming community from an economical perspective as less product will be needed while seed treatment is also less complicated than foliar spray treatments.

From this study, especially on potatoes, it seems that the advantage of applying biological agents such as *Azospirillum* and *Trichoderma* lies in their ability to control pathogens. From an organic farming perspective, this might be the way to go. According to Elad & Chet (1995), the application of *Trichoderma harzianum* has been used extensively as a bio-control agent and it is capable of controlling a large variety of phytopathogenic fungi that are responsible for major crop disease. This is in support of the recommendation suggested higher up.

In tomatoes, although both Maxiflo and Trykoside did not have a significant effect on the total fruit yield, both tended to improve the number of medium and large size fruits during some of the harvest periods. This is not consistent with the findings of (Kenny (2001) who reported that treatment of tomatoes with *Azospirillum* (the active ingredient of Maxiflo) significantly increased both the size and yield of tomato as well as green pepper fruits under field conditions. The author further observed a reduction in the occurrence of diseases in tomatoes. Bashan *et al.* (1989) also reported that *Azospirillum brasilense* increased the growth and yield of tomato plants. Again a possible over application of the products might have been a reason for the yield inhibition in tomatoes.

In light of the results from this study and other reports, the possibility exists that the Maxiflo and Trykoside concentrations used in this study might not have been optimal and needs to be verified in a follow-up study. What is important to note is that the objectives for applying one or both of these products should be formulated beforehand. If the objective is to control infection by bacterial or fungal infection or both, the trial layout and parameters measured should be adapted in future research. In the latter instance glasshouse trials are suggested as it will be easier to control. However, if the objective is to improve yields in crops, a series of concentrations should be tested, despite the current recommendations of the manufacturers, in order to ascertain the optimum.

CHAPTER 6

GENERAL DISCUSSION

Legislation restricting the use of many synthetic crop protectants in recent years as well as the banning of copper containing synthetic pesticides in Europe, has lead to increased organic farming practices (Rizvi & Rizvi, 1992). This accelerated the search for natural chemicals from plants, also known as green chemicals (Gorris & Smid, 1994). The application of micro-organisms such as the fungus *Trichoderma* and the bacterium *Azospirillum spp* to obtain this goal is a well established technique in organic farming systems. However, the natural products manufactured from these organisms in the past were mostly wettable powders. A company Axiom Bio-Products Pty Ltd, recently established in South Africa, manufactures two products namely Maxiflo (*Azospirillum* based) and Trykoside (*Trichoderma* based) in liquid form. The effect of these two products on the growth and yield of six economically important crops was investigated in this study by strictly following the instructions of the manufacturers. Two commercially available natural products, *ComCat*® and *Kelpak*®, were used as positive controls where possible in order to ascertain the impact of Maxiflo and Trykoside, both separately and in combination, by comparison.

Both *Azospirillum* and *Trichoderma* have been known for their parasitic effect against soil borne pathogenic organisms for at least two decades (Papavizas & Lumsden, 1980; Papavizas, 1981; Schroth & Hancock, 1981; Cook & Baker, 1983). However, Okon & Labandera-Gonzalez (1994) reported that *Azospirillum* is a N_2-fixing rhizobacterium which live in close association with plants and are capable of increasing the yield of important crops grown in various soils and climatic regions. Significant yield increases in the order of 5-30% have been reported. This was attributed to improved root development and enhanced water and mineral uptake. Based on preliminary field results, the manufacturers of the liquid formulations of Maxiflo and Trykoside recently postulated that the

liquidizing process might have played a role in releasing an "X-factor" with bio-stimulatory properties from these organisms. For this reason the emphasis was placed on foliar applications in this study in an attempt to test this postulate. This approach differed from previous approaches where either seed or soil was treated with the products.

The leafy vegetables cabbage and lettuce responded differently to foliar treatments with Maxiflo and Trykoside when applied either separately or in combination. In lettuce, root fresh mass was reduced considerably when Maxiflo and Trykoside were applied separately while this was not as marked in the case of cabbage. Further, when Maxiflo and Trykoside were applied in combination the inhibiting effect on root growth was not observed. Despite the effect on root growth, both products tended to increase the final head yield of cabbage but not in lettuce, compared to the untreated controls. From these findings it seemed that different crops, even though the growth pattern might show similarities, reacted differently to treatment with the two natural products which makes it difficult to make recommendations. To test the consistency of treatments there is a need for further research.

However, according to Agwah & Shahaby (1993), inoculation of lettuce by *Azospirillum* species significantly increased the leaf nitrogen content and the dry weight, but had no effect on fresh weight, leaf length and yield. Agwah & Shahaby (1993) found that *Azospirillum brasilense* (strain sp7) also contributed to an increase in the vitamin C content. With regard to the response of lettuce to treatment with Trykoside, the finding of this study were not consistent with the findings of GuoJing *et al.* (2001) who recently reported that *Trichoderma* application resulted in promoting plant growth, root-colonizing ability, yield and quality. The authors ascribed the response to alleviated pathogen infection by *Trichoderma* which indirectly had an affect on the measured growth and yield parameters. From this no deduction can be made on the possible direct involvement of an "X-factor" contained in the liquid formulation of Trykoside.

Results obtained from the experiment with peas, a legume crop, differed entirely from those observed with cabbage and lettuce. Maxiflo applied separately and together with Trykoside had a significant effect on seed yield while Trykoside on its own had no effect. Further, where *ComCat®*, a commercial bio-stimulant used as a positive control was added to the Maxiflo/Trykoside mixture, significant enhancement in pod fresh mass, pod number and seed mass was observed. As *ComCat®* is known to enhance flower formation in crops (Schnabl et al., 2001) this would supply a simple explanation for the results obtained with the three way application treatment. As the main enhancing effect of Maxiflo was on seed yield only, it might be indicative of a different mechanism of action than that of *ComCat®*. Further, combination treatments might indicate a synergistic effect between products in the case of peas.

Only a few reports on the effect of *Azospirillum* on the yield of legumes are available in the literature and none on the effect of *Trichoderma.* Field inoculation of garden peas and chickpeas with *A. brasilense* produced a significant increase in seed yield, but did not affect the dry matter yield (Sarig *et al.,* 1986). The author suggested that the increase in yield was the result of increased nitrogen fixation after inoculation with *Azospirillum.* However, according to Steenhoudt & Vanderleyden (2000) *Azospirillum spp.* secretes phytohormones that might explain the possible involvement on an "X-factor" in stimulating vegetative growth (Burdman *et al.,* 1998). Bashan & Holguin (1997a, b) also reported on the growth regulating properties of *Azospirillum* and the possible involvement of phytohormones secreted by the bacterium. However, no mention in literature has been made of a possible secreted chemical or hormone that might meet the description of an "X-factor" for *Trichoderma.* Traditionally, *Trichoderma* inoculants are applied to the soil. Zheng & Shetty (1999) reported that soil sanitation with *Trichoderma viride*, *T. harzianum* and *T. pseudokoningii* increased the germination rate of pea seeds by 20, 40 and 15%, respectively, compared

with untreated potting soil. However, no mechanism of action was mentioned by the authors.

In wheat, the same tendency of Maxiflo, Trykoside and *ComCat*® to increase the dry kernel yield at final harvest as was seen in peas was not observed again indicating that different crops react differently to treatment with these bio-products. What is especially confusing is the fact that Trykoside, while *Trichoderma* is not known for secreting growth promoting substances, significantly enhanced the root volume of wheat. Further, the results obtained with wheat in this study contradicted previous findings reported in the literature (Sarig *et al.,* 1984; Okon, 1985; Baldani *et al.*, 1986; Boddey *et al.,* 1986; Kapulnik *et al.,* 1987b; Wani, *et al.*, 1988; Saubidet & Barneix, 1998). In the entire list of latter references substantial kernel yield increases in wheat after treatment with *Trichoderma* have been reported. In the present study high standard deviations between replicates were encountered that could explain the statistical non-significance obtained.

Neither Maxiflo nor Trykoside applied separately to potatoes (family *Solanaceae*) showed any effect on vegetative growth. This is consistent with other crops tested, e.g. the leaf vegetables cabbage and lettuce, and indicates that the effect of the two products manufactured from the bacterium *Azospirillum* and the fungus *Trichoderma* respectively, exerts its influence, if any, via other means. Of these the role of both organisms as parasitic controllers of pathogens is probably best known and most researched. With regard to potatoes, even though no significant differences between treatments were observed in terms of growth and final yield, no disease incidence was either observed in this study. From this perspective it is advisable to inoculate potato seed with a Maxiflo-Trykoside combination as it can have a significant influence on the reduction of production costs in terms of disease control. For example, *Phytophthora erythroseptica* that is causative of pink rot in potato, also cause root and stem rot in tomato (Grisham *et al.,* 1983; Gillings & Letham, 1989). *Trichoderma virens* (DAR 74290) was

reported to provide some protection in terms of potato seedling survival, whereas disease severity was reduced by both *T. virens* and Trichodex compared to controls inoculated with the pathogen alone (Gillings & Letham, 1989).

Further, compared to the untreated control, treatment with both products tended to reduce the total tuber yield. According to Harmley and Clinch (1966) and Leclerc *et al.* (1994), growth regulators had failed to induce tuberization when the sucrose supply was inadequate. It has been stated that the use of growth retardants rather than bio-stimulants have improved the micro-tuber formation of potato (Forti *et al.,* 1991; Leclerc *et al.,* 1994; Harvey *et al.,* 1991). On a positive note, especially Trykoside enhanced the number of medium/large and large potato tubers improving the marketability of the crop. In tomatoes, also belonging to the family *Solanaceae,* the same tendency of Maxiflo and Trykoside to improve the number of medium and large size fruits was observed although it had no effect on the total yield.

The yield results obtained on tomatoes in this study were statistically non-significant and this was also contradictory to the report by Bashan *et al.* (1989) in terms of significant growth and yield increases after treatment with *Azospirillum brasilense* in a wettable powder format. Recently Kenny (2001) confirmed that *Azospirillum spp.* significantly reduced the occurrence of diseases in tomatoes and green peppers and simultaneously increased both plant size and yield under field conditions. According to Elad & Chet (1995), the application of *Trichoderma harzianum* has been used extensive as a bio-control agent and is capable of controlling a large variety of phytopathogenic fungi that are responsible for major crop disease in tomato. Terry *et al.* (2000) reported that tomato plants inoculated with *Trichoderma spp.* differed significantly from those just receiving mineral fertilizer only in terms of the general appearance of the plants. Inoculation of *Trichoderma* increased plant height, shoot diameter, root length and fresh and dry weight of plants. Sharon *et al.* (2001) showed that tomato plants treated with the bio-control agent (T-203) and grown in nematode infested soil exhibited a

drastic reduction in root galling compared to the control. These reports strongly suggest that the advantage of applying parasitic micro-organisms to agricultural crops lies in its capability of controlling pathogens. However, the reports on growth and yield enhancement under the influence of these micro-organisms also point towards a possible "X-factor" involved.

In summary, and in the light of somewhat contradictory reports found in the literature, the possibility exists that the concentrations of the Maxiflo and Trykoside liquid formulations used in this study might not have been optimal. The one liter per hectare rate used was based on the recommendations of the manufacturers. A follow-up study seems to be necessary where different concentrations can be tested and compared. In the event that an "X-factor" is discovered in future, possibly a hormone or a hormone-like compound released from the organisms during the manufacturing process of the Maxiflo and Trykoside liquid formulations, a series of concentrations will have to be tested. The latter statement is based on experience with hormones and especially the fact that treatment of plants with hormones, to evoke a specific reaction e.g. vegetative growth, is concentration dependant. The latter implies that a concentration below or above an optimal, can lead to an effect opposite than what was envisaged.

Finally, in light of the observation that treating some crops with a combination of the two products Maxiflo and Trykoside led to enhanced growth or yield or both in some, while evoking an opposite response in others, indicate that a follow-up study with different ratios of the products also might to be worthwhile to pursue. What is also important to note is that the objectives for applying one or both of these products should be formulated beforehand. If the objective is to control infection by bacterial or fungal infection or both, the trial layout and parameters measured should be adapted in future research. In the latter instance glasshouse trials are suggested as it will be easier to control.

However, if the objective is to improve yields in crops, a series of concentrations and combination ratios should be tested in order to ascertain the optimum. From this study, especially with respect to potatoes, it seems that the advantage of applying biological agents such as *Azospirillum* and *Trichoderma* lies in its ability to control pathogens. From an organic farming perspective, this might be the way to go.

SUMMARY

The challenge for science is to address the need for adequate food provision and a sustainable future for agriculture. The solution for increasing food production can probably only be obtained through expansion of arable land, by increasing irrigation practices or by increasing harvestable yields on available land through the improvement of agricultural technology. With regard to the latter approach, field experiments were conducted at the experimental farm of the University of the Free State, Bloemfontein, during the 2003 and 2004 growing seasons, to determine the effect of Maxiflo and Trykoside (liquid formulations of *Azospirillium* and *Trichoderma* based products, respectively) on vegetative growth and yield of two leafy vegetable crops (cabbage and lettuce), a cereal crop (wheat), a fruit crop (tomato), a tuber crop (potato) and a legume crop (peas). A randomized complete block design with six treatments (Control, Maxiflo, Trykoside, Maxiflo + Trykoside, *ComCat*® and *Kelpak®)* was applied in all cases. Maxiflo and Trykoside were applied either separately or together. Two commercially available natural bio stimulants, *ComCat*® and *Kelpak*®, served as positive controls. Different growth and yield parameters were used to quantify the effect of the test products in the above six economically important crops. In cabbage and lettuce vegetative growth (plant height, plant diameter and stem thickness) were not affected but a significant increase in head mass was observed. Peas were most responsive to treatment with the bio-products in terms of the increase in yield obtained. Treatment with Maxiflo and Trykoside in combination increased the medium/large and large size fruit yield in tomatoes while exactly the same was observed for tuber size in potatoes. However, in both crops the total yield was not significantly affected. In wheat root growth was stimulated significantly by treatment with Trykoside but no significant yield increase was observed at the 5% probability level ($P<0.05$).

Keywords: Cabbage, lettuce, peas, wheat, tomato, potato, effect, Maxiflo, Trykoside, *ComCat®, Kelpak®,* vegetative growth, yield components.

OPSOMMING

Die uitdaging vir die wetenskap is om die behoefte aan verhoogde voedselproduksie aan te spreek en 'n volhoubare toekoms vir landbou te verseker. Die oplossing kan langs die weg van vermeerdering in landbougrond, verhoogde besproeiing of verhoogde produksie op beskikbare grond gevind word. Ten opsigte van laasgenoemde benadering is veldproewe uitgevoer op die proefplaas van die Universiteit van die Vrystaat, Bloemfontein, gedurende die 2003 en 2004 groeiseisoene om die effek van Maxiflo en Trykoside (vloeibare formulasies van respektiewelik *Azospirillium* en *Trichoderma* gebaseerde produkte) op die vegetatiewe groei en oesopbrengs van twee blaargroentes (kool en slaai), 'n graangewas (koring) 'n vruggewas (tamatie), 'n knolgewas (aartappel) en 'n peulgewas (erte) te ondersoek. Volledige blok ontwerpe is in alle gevalle gebruik en ses behandelings (Kontrole, Maxiflo, Trykoside, Maxiflo + Trykoside, *ComCat*® en *Kelpak*®) toegepas. Maxiflo en Trykoside is apart of saam aangewend. Twee kommersiële bio-stimulante, *ComCat*® en *Kelpak*®, het as positiewe kontroles gedien. Groei en oesopbrengs parameters is gebruik om die effek van die toetsprodukte te kwantifiseer in bogenoemde ses ekonomies belangrike gewasse. In kool en slaai is vegetatiewe groei (planthoogte, plant deursnee en stamdikte) nie beïnvloed nie maar kopmassa betekenisvol verhoog. Erte het die beste op behandeling met die bio-produkte gereageer in terme van die verkreë oesopbrengsverhoging. Behandeling met Maxiflo en Trykoside in kombinasie het tot vermeerdering in die medium/groot en groot tamatievrugte aanleiding gegee terwyl dieselfde tendens met aartappelknolle waargeneem is. Maar, in nie een van hierdie twee gewasse is die totale oesopbrengs betekenisvol verhoog nie. In koring is wortelontwikkeling betekenisvol verbeter deur veral behandeling met Trykoside maar weereens is geen betekenisvolle oesopbrengsverhoging waargeneem by die 5% waarskynlikheidsvlak ($P<0.05$) nie.

Sleutelwoorde: Kool, slaai, erte, koring, tamaties, aartappels, effek, Maxiflo, Trykoside, *ComCat*®, *Kelpak*®, vegetatiewe groei, oes komponente

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