STUDIES OF THE MICRONUTRIENTS ZINC, MANGANESE AND SILICON IN

CUCUMBERS (*CUCUMIS SATIVUS*)

ANDREW PETER DOMINY

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ABSTRACT

Zinc and manganese have long been considered as essential micronutrients to plant growth, yet the interactions of the two nutrients on growth and development of plants have not been elucidated in their entirety. Silicon is not classed as an essential element, but has been found to improve growth of a number of crops, particularly of the Poaceae family.

A simple water culture hydroponic system was developed to monitor the growth and development of a fruit crop (Cucumber – *Cucumis sativus*) under deficient, adequate and excessive applications of zinc and manganese. Plant growth parameters were monitored including leaf growth, plant height, plant fresh and dry mass, yield, fruit size and fruit mass. Nutrient uptake was also measured using inductively coupled plasma emission spectroscopy, whilst chlorophyll was determined spectrophotometrically. Plant nutrient analyses were also conducted using inductively coupled plasma emission spectroscopy.

Silicon was found to have a beneficial effect on the growth of cucumbers and was incorporated as a treatment for this crop along with zinc and manganese since foliar silicon sprays were able to correct the occurrence of mineral deficiency symptoms. Along with plant growth measurements, nutrient uptake, plant nutrient analysis and chlorophyll determination, plant tissue was also analysed using transmission electron microscopy to establish the impact of silicon applications on the cell ultra-structure of cucumbers. Electron micrographs showed an increased presence of plasmodesmata in treatments excluding silicon. Such increased plasmodesmata connections under silicon deficient conditions could increase translocation of cell solutes due to reduced cell longevity.

Results also confirmed the essentiality of zinc and manganese on plant growth and development as typical deficiency symptoms were observed. Typical toxicity symptoms were also recorded. Rates of uptake of nutrients corresponded with leaf growth and enlargement as well as yield. The chlorophyll concentration was not a clear indicator of nutrient application level. Typically, manganese and zinc interacted with iron, magnesium, calcium and potassium, affecting their uptake into the plant dependent on the level of manganese and zinc applied.

Although non-essential, silicon improved plant growth, but had neither a relationship with the other nutrients evaluated nor affected the physical growth and development of the plants. Manganese and zinc, as essential to plant growth and development, affect the visual appearance of the plant as well as affect the plant biochemically due to their involvement in many growth and development processes.

PREFACE

The experimental work described in this thesis was carried out in the School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal, Pietermaritzburg, from January 1999 to December 2002, under the supervision of Dr. Isa Bertling.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

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I, Andrew Peter Dominy, declare that

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        Horticultural Science
        School of Agricultural Sciences and Agribusiness
        University of KwaZulu-Natal
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DECLARATION 2 – PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis:

Dominy A, and Bertling I., 2004. Manganese, Zinc and Silicon Studies of Cucumber (*Cucumis sativus*) Using A Miniature Hydroponic System. *Acta Hort.* 644, 393-398.

Dominy A., and Bertling I., unpublished. Impact of Silicon Deficiency on Cell Structure in Cucumber (*Cucumis sativus*). In preparation for South African Journal of Plant and Soil.

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CONTENTS

CHAPTER 1

IMPORTANCE OF THE MICRONUTRIENTS ZINC AND MANGANESE IN PLANT GROWTH AND DEVELOPMENT

Plant growth is dependent on the supply of nutrients to the meristems throughout the plant's lifecycle (Moorby & Besford, 1983). Relationships between nutrients and the environment create a very complicated picture of plant nutrition. Typically for individual nutrients, plants respond to application levels of that particular nutrient in a specific pattern, known as the "response curve". This curve can be divided into three defined zones: "Deficient", "Adequate" and "Toxic". As the application of a nutrient increases from zero through the deficient range to adequate, there is an increase in resultant yield of the crop which plateaus on approach to the adequate range. Yield can usually be expected to remain constant through the adequate range as the application level of the nutrient in question increases without further yield increases, also known as luxury consumption. However, on reaching the excessive or toxic range of application, yield declines. Rates of increase/decrease of yield and response to a certain nutrient are crop and cultivar specific.

The rate of growth of plants is related to the internal concentration of nutrients within the plant tissue. This in turn is determined by the availability and uptake of the nutrient by the plant (Moorby & Besford, 1983). The response of crops to excessive or toxic levels of application of a nutrient differs between crops, and crops are often rated according to their sensitivity to that particular nutrient at excessive levels. Crops that are not sensitive to excessive levels of a nutrient are termed tolerant and these plants tend to withstand very high levels of application before being negatively affected by the excessive levels of a certain nutrient (Kabata-Pendias & Pendias, 2000).

Nutrient interactions can occur which affect the availability of these nutrients to the plant. These interactions can occur in a number of forms: the ionic form of the nutrient bonds with another ion of a different nutrient, competition for sites of adsorption on soil particles, sites of absorption into the plant (dependent on the concentration/availability of the ion in the solution), or for transport mechanisms within the plant, or through the function of nutrients within the plant (Robson & Ptiman, 1983).

Soil conditions are extremely complex with many variable factors and influences that affect the nutrition of a crop. In understanding crop nutrition, and, most importantly, micronutrient nutrition of crops, the complexity of soil and the related interactions will influence and affect the results of such a nutrient study (Asher & Edwards, 1983). A nutrient solution provides a homogenous root

environment in which all influencing factors can be controlled so as to provide accurate results and understanding of crop nutrition. A nutrient solution can also ensure constant supply of nutrients at specified levels, thereby eliminating issues such as withdrawal or depletion of nutrients from the solution. Simple water culture systems can suffer large variations or depletion of nutrients depending on, for example, rate of uptake, volume of solution, solution pH, and temperature of the environment. Intermittently renewed solutions do exhibit large variations in nutrient composition with time due to depletion of nutrients by plant uptake between solution changes. This can restrict the usefulness of simple solution culture techniques in studying nutrient uptake by plants (Stiles, 1916). The impact of the depletion on the relevance of the experiment is dependent on the desired accuracy to achieve the experimental objectives, the range of concentrations required in the experiment, the volume of solution in relation to the size of the root system and the rate of nutrient uptake by the plant. Flowing solution culture systems may provide better results under these situations.

Carroll & Loneragan (1969) showed that for zinc (Zn) nutrition, studies evaluating the excessive application of the nutrient could use a simple nutrient solution system; however, studies on adequate or deficient applications of Zn require a more complex system to correct the readily changing nutrient content to ensure experimental accuracy. This may result in erroneous displays of nutritional deficiency symptoms as a result of the rapidly depleting solution for the particular nutrient under study. This is compounded by increased growth rates and nutrient uptake with the ongoing development of plants. Flowing solution culture systems may provide better results under these situations with the flow rate being adjusted according to the rate of nutrient depletion altered according to crop age and growth (Asher & Edwards, 1983).

A negative aspect factor of using nutrient solutions is the substantial loss of natural microbial interaction in nutrient uptake due to the reduced amount of organic substrates exuded by the plant roots (Bowen & Rovira, 1976). Aeration of the nutrient solution and/or the medium is of increasing importance under hydroponic production due to the reliance on water for nutrient uptake. Aeration of the root zone is essential so as to prevent a reduction in nutrient uptake, reduced growth and yield due to limited oxygen availability in the root zone.

Adams (1999) described a variety of factors to consider in formulating a plant nutrient experiment. The nutrient solution should be formulated for the specific crop and the frequency of application adjusted according to the rate of growth and the rooting volume. Regular monitoring of the solution is required to limit the impact of nutrient depletion and EC or pH changes that could

affect nutrient availability or uptake. The use of substrates or a water culture system and the interaction of the system on plant nutrient availability must also be considered. Solution pH and salinity can significantly impact on nutrient availability or nutrient uptake by the plant. The environment in which the plant is grown also impacts on nutrient demand. Factors such as air temperature can increase water uptake and consequently increase the salinity of the solution. Root zone temperature also has an impact on the growth of plants; further, relative humidity of the environment should be controlled.

Zinc and manganese (Mn) are defined as essential elements for plants. This classification is based on three criteria: Firstly, without this element or nutrient, the plant will be unable to complete its lifecycle; secondly, the functions of this nutrient cannot be replaced by another element; thirdly, the element or nutrient must be directly involved in plant metabolism or required for a specific part of a metabolic process (Marschner, 1993; Mengel & Kirkby, 2001). From a producer's point of view, the economic importance of a nutrient has greater relevance than the essentiality of the nutrient to the crop (Bennett, 1983).

The deficiency of an essential nutrient element causes a reduction in normal plant growth and development and will affect the yield of the crop (Bennett, 1983). Deficiency and toxicity symptoms can be classed as chlorosis (yellowing of the leaf, either uniform or interveinal), necrosis (death of plant tissue), lack of new or terminal growth, accumulation of anthocyanins or stunting and reduced growth. Deficiency symptoms can be specific for any nutrient, but similar symptoms could be created or induced by other conditions relating to temperature, soil and water conditions as well as insect damage. Verification of the deficiency is needed by evaluation of the nutrient status of the plant, usually by leaf analysis.

Not only are Zn and Mn essential elements, but they are also classed as micronutrients. Plant nutrients are divided into two categories, namely macronutrients and micronutrients, where the difference between the two categories is determined by the elemental concentration of these nutrients in plant tissue. Macronutrients tend to be found in plant tissues in relatively higher concentrations than micronutrients, however, their essentiality is just as important and independent of the concentration of nutrient found within the plant tissue.

Both elements, Mn and Zn, being micronutrients and essential, are not only required in small amounts but have very unique roles to play in the metabolism of the plant. So as to understand their

roles in plant metabolism, it is important to monitor the impact of application of these nutrients on plant growth and development.

Manganese uptake has been found to differ for different plant species, but it has been generally shown that uptake occurs as Mn^{2+} ion. Being a divalent cation, Mn exhibits similar characteristics to the other divalent cations and competition for uptake between Mg^{2+} and Ca^{2+} can be commonly found (Le Bot *et al.*, 1990, Mengel & Kirkby, 2001), whilst availability can be limited at higher soil solution pH levels (pH levels > 6.5) (Maas *et al.,* 1969, Huang *et al.,* 1993). Manganese on the other hand can have a negative effect on iron (Fe) uptake through non-enzymatic oxidation of the Fe^{2+} ion. Direct competition between Fe and Mn occurs, antagonising Fe absorption and distribution in plants below toxic levels of Mn. Copper (Cu) does also compete for uptake with Mn (Tisdale *et al.*, 1998). However, under high Mn conditions (toxic Mn), both Mn and Fe accumulate in plant roots, as shown in tomatoes (Alvarez-Tinaut *et al*., 1980). The high levels of Fe absorbed remain in the root and are not actively transported to the shoot level, resulting in Fe deficiency symptoms at toxic levels of Mn application.

Helal *et al.* (1990) showed that uptake of micronutrients by plants differed not only by crop, but also varietal differences in uptake were recorded during studies of spinach and bean. In bean cultivars, micronutrient uptake, specifically Mn, Zn and Fe uptake, is affected by root characteristics such as root length, and number of roots per plant. Spinach, on the other hand, did not appear to exhibit such effects. Caution, therefore, with regards to comparing different crops must be exhibited if differing patterns of uptake between them might exist.

Manganese is relatively immobile in plants and tends to be translocated through the phloem to meristematic tissues, and thus tends to be found more abundantly in younger tissues (Kabata-Pendias & Pendias, 2000). Le Bot *et al.* (1990) contradict the common assumption of Mn transport through the phloem tissue by reporting it as transported via xylem vessels. Structurally, Mn resembles magnesium (Mg) as a divalent cation. The highest concentrations of Mn in plant cells can be found in the cytoplasm, while chloroplasts contain the highest Mn concentration of all plant cell organelles (Shkolnik, 1984). Within chloroplasts, Mn plays an important part in the liberation of the oxygen from the water molecule due to the high oxidation-reduction potential of Mn. Since chloroplasts are sensitive to Mn deficiencies, yellowing of leaves is an expected deficiency symptom. Deficiency symptoms of Mn are very similar to that of Mg, except that being immobile in the plant, the interveinal chlorosis of deficient plants is observed on the younger leaves. Kabata-Pendias & Pendias (2000) reported that under deficient conditions, low concentrations of Mn were translocated

from older to younger leaves. The Mn deficiency range is determined as 10 to 20 µg Mn/g dry matter. Below this level, dry matter production, net photosynthesis and chlorophyll are significantly affected (Ohki, 1981). Plant characteristics and availability of Mn to the plant in the growing medium determine the Mn content of plants. Under Mn deficient conditions, reduced uptake of Ca may also be observed (Shkolnik, 1984), possibly indicating a positive interaction between these elements.

Many enzymes are Mn activated; therefore many biochemical processes are affected by the Mn nutritional health of the plant. Some of these processes include the Krebs Cycle, biosynthesis of ascorbic acid, respiration, nitrogen metabolism, activation of peptidases for protein synthesis, carotenoid synthesis, biosynthesis of RNA and DNA, photophosphorylation, and, most importantly, photosynthesis where Mn plays various roles but primarily acts as an electron donor (Shkolnik, 1984). It is also only in the leaves of plants that a complex compound of Mn with a high oxidation-reduction potential is found. Of interest, too, is the relationship between auxin and Mn, where under the presence of indoleacetic acid (IAA), it is believed that Mn stimulates protein synthesis for continuous growth.

Manganese toxicity can occur at a range from 200 µg/g (in maize) to 5300 µg Mn/g (in sunflower), where it is associated with a 10% reduction in dry matter yield (Edwards & Asher, 1982). Ruffy *et al.* (1979) found that these values are temperature-dependent with higher Mn levels required to induce toxicity symptoms at higher temperatures. Manganese toxicity symptoms are characterised by brown spots on older leaves surrounded by chlorotic areas (Horst & Marschner, 1978a), additionally an excess of Mn can induce Fe, Mg and Ca deficiency in plant tissue. Manganese toxicity is also related to auxin deficiency, as is IAA oxidase activity (Kabata-Pendias & Pendias, 2000). The most common symptom of Mn toxicity is Fe chlorosis, but other symptoms include leaf puckering (wrinkles, folds or bulges), necrotic brown spots and uneven distribution of chlorophyll in older leaves. Toxicity under field conditions is usually a result of excessive availability of Mn to the plant under acid conditions, or due to poorly aerated soils.

Le Bot *et al*. (1990) were able to induce Mn toxicity symptoms in young tomato plants within seven days of application of a 50 µM Mn solution. Typical Mn toxicity symptoms were found, but it was also noted that brown/black spots occurred along the veins of the older leaves, then progressing from older to younger leaves. Roots also exhibited toxicity symptoms by appearing brown in colour. Increasing application of Mn from 10 to 50 µM did not significantly affect plant dry matter, but root mass was significantly heavier in the 50 µM treatment. Le Bot *et al.* (1990) also reported that the highest Mn concentrations were observed in the roots and that high levels of Mn application

affected Mg and Ca uptake negatively, while no effect on potassium (K) nutrition was observed. One hindrance in obtaining consistent results was the temporal depletion of nutrients in the solution by the rapidly growing plants (Le Bot *et al.*, 1990).

Zinc is taken up by the plant as the Zn^{2+} ion, and uptake can be reduced at lower temperatures (Schmid *et al.*, 1965). Competition for Zn^{2+} uptake was observed with Cu^{2+}, Fe^{2+} and Mn^{2+}, as these ions are of similar ionic size (Bowen, 1969; Giordano *et al.*, 1974), and, therefore, compete for the same carrier site (Tisdale *et al.*, 1998). The same authors state that the pH of the soil solution or the nutrient solution does affect Zn availability to the plant, whereas at higher pH values (greater than pH 6.5), Zn availability becomes significantly reduced (Tisdale *et al.*, 1998). High levels of phosphate can induce Zn deficiency in plants, whilst flood conditions may also create Zn deficiencies due to increased pH or antagonism between Fe plus Mn vs. Zn (Swietlik, 1999). Sensitivity to Zn shortage has also been found to be crop-specific. The rate of absorption of Zn differs greatly among plant species and growing media (Kabata-Pendias & Pendias, 2000). The concentration within a plant also differs, roots have also been found to contain more Zn than shoots in spinach and barley.

Zinc deficiency is characterised by reduced growth, interveinal chlorosis of younger leaves, small leaves and reduced internode length, whilst some plants exhibit rosetting of the youngest leaves. As the deficiency worsens, plants become stunted and exhibit shoot dieback, defoliation and severe leaf chlorosis (Swietlik, 1990). Root growth may also be negatively affected in some species. Zn toxicity on the other hand exhibits inhibition of root and shoot growth, reduced leaf size, general chlorosis of the plant - the latter as a result of induced Fe deficiency (Shkolnik, 1984; Marschner, 1993; Kaya *et al.*, 2000). Kaya *et al.* (2000) also found that the leaf chlorophyll *a* and *b* levels under Zn-deficient conditions were higher than under toxic Zn conditions. A negative impact on crop productivity can also be expected under either deficient or toxic conditions. Like Mn, Zn is relatively immobile in the plant and under deficient conditions Zn is not redistributed within the plant (Shkolnik, 1984). Kabata-Pendias & Pendias (2000) reported that redistribution of Zn may occur under sufficient conditions in some species, but under deficient conditions, these same species exhibit immobility of Zn.

Zinc participates in many processes in the plant, including IAA synthesis, activating of numerous enzymes, and involvement in stomatal opening (Shkolnik, 1984; Swietlik, 1990; Marschner, 1993). Zinc has been reported to play an important role in the formation of generative organs and fruit bearing of crops (Shkolnik, 1984). It is an important component of many metalloenzymes and enzyme systems as well as an activator of metalloenzyme complexes. It has also

been found to participate in the biosynthesis of porphyrins and haemoproteins, including cytochrome (Shkolnik, 1984).

Zinc deficiency and the intensity of Zn deficiency symptoms have been found to be influenced by the intensity of the exposure of the plant to light intensity. Under low light intensity, Zn deficiencies may not be observed, whilst under high light conditions, the intensity of the symptoms increases (Zhang & Wu, 1989).

Zinc is also aligned with auxin production, regulating plant growth and development. It has, however, been reported that Zn can be replaced in certain enzymes by other metal elements, such as Co, Mn and lead (Pb) (Bennett, 1983).

The response of horticultural species to high root temperature varies, not only in growth and development, but also in nutrient absorption (Klock et $al.$, 1996). The Mn concentration of tomato dry mass was highest at 27°C, whilst the Zn concentration was unaffected by temperature; continuous exposure to temperatures above 30°C caused a reduction in growth as well as Zn and Mn absorption. Muskmelons, however, showed the highest levels of Zn and Mn at a root zone temperature over 36°C and continuous exposure to temperatures exceeding 30°C resulted in increased growth and absorption of Mn and Zn by the plant (Klock et $al.$, 1996).

Numerous studies have shown possible correlation between Mn and Zn nutrition, with an involvement of Fe. These studies are, therefore, set to elucidate and understand these relationships further by assessing the interaction of these nutrients over differing application levels on the growth and development of a vegetable fruit crop, $Cucumis$ $sativus$.

CHAPTER 2

INFLUENCE OF THE MICRONUTRIENTS MANGANESE, ZINC AND SILICON ON THE GROWTH AND DEVELOPMENT OF CUCUMBER PLANTS (*CUCUMIS SATIVUS* VAR. TYRIA)

2.1 INTRODUCTION

The scope of this study was to identify the importance of Zn and Mn in the growth and development of a fruiting crop. The cucumber plant (*Cucumis sativus*) is a typical fruit vegetable with a short production cycle from germination to fruit harvest. This allows for relatively quick turnaround of experiments, easy repetition of results where necessary. The plants do not need complex pruning and training techniques. Typically, cucumbers produce fruit that are ready for harvest 30 to 40 days after germination (Anon., 1997).

The large cucumber seed its very quick germination and the fast subsequent plant growth allow for *in situ* germination of the seed without the need for first producing seedlings of the plant. This eliminates the need for a growing medium and the entire growth of the plants can be subjected to the desired nutritional control from germination of the plants using water cultivation (Anon., 1997). Typically, cucumbers are produced under hot-house conditions with hydroponic production methods, utilizing a variety of growing media including rockwool, sawdust and coir. Being inert media, the cucumber plants are dependent on the adequate supply of nutrients through the nutrient solution for each growth stage of the crop. A wide range of cultivars is available to producers, varying in adaptability, fruit size, colour, uniformity and disease resistance. The cultivar Tyria used in these experiments is a cucumber commonly planted in South Africa (Derek Askew, *pers*. *com*.)

More recent cultivars of "English" cucumbers are parthenocarpic or gynoecious, therefore neither requiring insects nor hand-pollination to develop fruit (Anon., 2002). This is an ideal trait for the production of a fruiting crop in a controlled environment where no artificial pollination or manipulation of the flowering and fruiting process is required.

During the summer season, cucumbers require a nutrient solution of pH 6.0 (Harris, 1966). As the plant is not very specific in its nutrient requirements, a general modified Shive's solution can be used as a nutrient solution for cucumber production. The pH can be monitored and adjusted to a close to 6.0 to optimise nutrient availability for the plants. Environmental conditions affect the growth and development of cucumber plants. Daylength can affect the development of male flowers with longer days favouring such male flower development (Robinson & Decker-Walters, 1997). High

light intensity is needed for optimum yields. Furthermore, Krug & Liebig (1980) found that temperatures above 28°C result in lower growth rates of cucumbers, whilst maximum growth rates are achieved with temperatures of 25°C. Slack& Hand (1983) correlated leaf area and stem length to average growing temperature, and found that temperature increases result in corresponding increases in leaf area and stem length, ultimately increasing yield.

Cucumber has been found to be particularly sensitive to Mn deficiencies and tends to exhibit acute curling and twisting of chlorotic leaves with plant death occurring within three weeks (Hewitt & Watson, 1980). Zinc deficiency symptoms of cucumbers have been described by Locascio (1993) as prominent on new growth, where leaves are small and show interveinal chlorosis which gradually turns to necrosis. Shortened internodes may also develop. Manganese deficiencies are visible in the younger tissues where leaves exhibit, as in Zn deficiency, interveinal chlorosis, which gradually extends to older leaves. Zinc toxicity on the other hand can be observed as reduced top and root growth, while Mn toxicity is seen in reduced growth rates and necrotic leaf spots. Under field conditions, Zn toxicity in cucumbers can be reduced by increasing the organic matter in the soil, allowing the organic matter to buffer the Zn levels of the medium (Swietlik, 1990).

Whilst not being classified as essential for growth, cucumbers benefit greatly from the addition of silicon (Si) to the nutrient solution. Production of cucumbers under controlled conditions eliminates the source of Si to the plants. Evaluation of the Si requirements and the impact of Si application on cucumber plants are therefore required. Silicon is the second most abundant element in the earth's crust and is available to the plant as silicic acid (H_4SiO_4), (Tisdale *et al.*, 1998). Silicon has been found to be important as a nutrient for monocotyledons, such as sugar cane and rice. Availability of Si is determined by the presence of aluminium (Al) and Fe oxides, liming and soil moisture content. Under high nitrogen (N) application regimes, lower levels of Si in the plant tissue are recorded necessitating the need for additional Si to be applied to the crop. Silicon is present in the soil solution as monosilicic acid ($Si(OH)_4$), and it is in this form that Si is taken up into the plant through the roots (Jones & Handreck, 1967). Uptake of Si is not just a function of availability in the soil or nutrient solution, but appears to be influenced strongly by the uptake and movement of water through the plant by transpiration.

Perhaps the most commonly reported aspect of Si nutrition is its ability to reduce the susceptibility of plants, particularly cucumber, to fungal diseases such as powdery mildew (*Podospaera xanthii*), the most common leaf disease in cucumber production (Miyake & Takahashi, 1983). Under deficient conditions, cucumber plants are easily infected with powdery mildew, and

plant growth was also reduced. Cherif & Belanger (1992) found suppression of infection by *Pythium ultimum* disease (damping-off) of cucumber when Si was applied. Cucumbers are classed similarly to rice and sugar cane as Si absorbers, since the growth responses to Si application are similar to rice. Results of the studies by Miyake & Takahashi (1983) suggest that Si exerts a beneficial effect on cucumber plants. Silicon growth responses include increased yield. Plants grown under Si-deficient conditions exhibit symptoms of wilting - similar to *Fusarium* wilt infection. Belanger *et al.* (1995) reported that the reduction of disease incidence by application of Si lead to the growth response due to healthier plants. Epstein (1999) concluded that growth stimulation of Si was due more to protection from detrimental effects of abiotic and biotic stresses. For disease management, Menzies *et al.* (1992) found that foliar applications of potassium silicate were more effective than application through nutrient solutions.

Samuels *et al.* (1993) found Si did not affect the rate of fruit growth or size in cucumber, but that plants without Si application produced smoother fruit than those supplied with Si. The dull appearance of Si supplied fruit is a result of the silification of fruit trichomes. Once deposited though, Si cannot be remobilized within the plant (Samuels *et al.*, 1991). The concentration of Si in different parts of the plants varies greatly, not just within a species, but also between plant species (Jones & Handreck, 1967). Deposition in different tissues and positions within tissues is related to rates of transpiration, with highest concentrations found in tissue with higher rates of transpiration.

It has been reported that under toxic Mn conditions, the development of necrotic spots on the leaves of plants is reduced when Si is applied to the crop. Si uniformly distributes the excessive Mn across the leaf preventing the development of such necrotic spots (Horst & Marschner*, 1978b). Marschner *et al.* (1990) report the enhancement of plant growth by Si due to increased tolerance to high Mn concentrations, increased resistance to fungal attack or high mechanical stability of stems and leaf blades and, thus, better light interception. Miyake & Takahashi (1983) found reduction in growth and fruit yield as well as chlorosis in cucumber grown without Si supply. The growth stimulating effect of Si is inversely related to the P content of the leaves of the plants when grown under deficient conditions. Excessive phosphorus (P) levels are also common with Zn deficient conditions; therefore, an interaction between the uptake and use of these two nutrients is evident. Leaf senescence is also delayed by Si applications, even under high light intensity. Malformation of young leaves is described as a symptom of Si deficiency in cucumbers, but it is also a symptom of Zn deficiency under high light conditions. Si is found to be deposited in the cell walls as amorphous silica which, in turn, can increase cell elasticity (Marschner *et al.*, 1990). Yoshida (1965) found that

with increasing application of Si, there was a corresponding reduction in transpiration rate. This was suspected to be the result of enhanced cuticular efficiency due to the silica being deposited on the epidermal cells.

Miyake & Takahashi (1978) identified Si deficiency in tomato and found that symptoms of deficiency could only be established under certain conditions, including optimum day and night temperatures, high light intensity, aerated conditions and low planting density. They described tomato deficiency symptoms to include development of symptoms after the first flower bud break, leaflets exhibited outward curving, warping, hardening and thickening, retarded growing point development, yellowing of leaves with necrotic spots developing on the older leaves spreading to younger leaves, and, in severe conditions, plants began to dry up starting at the base of the plant. Miyake & Takahashi (1983) found similar symptoms in cucumber plants as well as reduced pollen fertility and increased incidence of powdery mildew under Si deficient conditions. Also, cucumber plants are not rejective, like tomato plants, and will absorb considerable amounts of Si based on its availability in the nutrient solution. Si-rejective plants limit the uptake of Si into the plants at the surface of the root.

Adatia & Besford (1986) described similar symptoms of Si deficiency in cucumber plants. Their research also established differences in leaf structure, shape and disposition, leaves tended to experience delayed senescence and were darker green in colour. At higher light intensities, applications of Si to cucumber plants resulted in shorter petioles, increased fresh and dry weight per unit area and chlorophyll concentration. There was no effect on leaf area, but increased fresh and dry root mass was also established. Light intensity also plays an important role in Si nutrition.

This study, therefore, was therefore designed to identify and report on the relationship between uptake of Mn, Zn and Si on plant growth and development of *Cucumis sativus,* including the establishment of nutrient deficiency and toxicity symptoms.

2.2   MATERIALS AND METHODS

A miniature hydroponic system consisting of 5.5 L plastic containers with sealable lids was used for the experiments. All chemicals were supplied by Sigma® (St Louis, Missouri, U.S.A.). A hole was made in the lid through which the cucumber plants could grow. Four litre of the nutrient solution were placed in each container which was continually aerated by a small fish tank air pump. A

modified Shive's solution (Table 1) was used as the basis for the nutrient solutions supplying each crop (Franco & Loomis, 1947). The basic Shive's solution as outlined was considered the normal nutrient requirement for *Cucumis sativus.* Manganese and Zn levels were adjusted according to the aims of each experiment whilst Si was applied as a foliar spray to the designated treatments. Manganese and Zn applications (Table 2) for the different experiments were applied as $MnCl_2$ and $ZnSO_4$. Silicon was applied at a rate of 498.75 mg/l as $Na_2O_3Si.9H_2O$, with 1 mL/L spray of Tween 20 surfactant to the designated treatments. In each experiment, a standard application of either Mn or Zn was used as a control; this application was 6.12 mg/L $MnCl_2$ and 1.16 mg/L $ZnSO_4$. Initially, distilled water was supplied as nutrient solution until the seedling roots had reached the solution. The solution was then replaced with the standard treatment required for the container concerned. A single cucumber seed (cv Tyria; Hygrotech Seed (Pty) Ltd, Pretoria, South Africa) was placed on a piece of moist filter paper above the hole in the lid. A glass container was placed over the filter paper, to maintain humidity around the germinating seed. The glass container was removed once the roots from the germinated seed had reached the distilled water in the plastic container (3 to 4 days). Once the primary leaves began to emerge, the distilled water was replaced with nutrient solution. The nutrient solutions were replenished on a weekly basis for the first four to six weeks and thereafter all solutions were changed when the nutrient solution levels dropped below 500 mL remaining solution. Solutions were changes for all treatments if a solution EC exceeded 3.0 mS/cm.

Table 1. Modified Shive's solution used for the hydroponic nutrient solution (Franco & Loomis, 1947)

Nutrient	Application (mg/L)
KH_2PO_4	195.46
K_2SO_4	54.44
KNO_3	530.73
$MgSO_4$	462.19
$Ca(NO_3)_2$	974.33
$FeSO_4$	11.21
H_3BO_3	3.14
$CuSO_4$	0.174
Na_2MoO_4	0.125
$CoSO_4$	0.193

Experiments were conducted in a controlled environment growth chamber of 24^0C, RH of 30 - 60%, and 12 hour day length. Light (307.02 µmol m^2 s^{-1}) was supplied by 120 "Growlamps®", (Philips, Eindhoven, Netherlands), and 12 100 W incandescent lights. Temperature and humidity was monitored using HOBO® data loggers. Due to the limited size of the growth chambers, the experiments were split up into several experiments to ensure adequate replication and sample size for statistical validation of the experiments. Each experiment consisted of two replications with six treatment combinations, except for the deficiency experiment where there were eight treatment combinations. Each experiment was arranged in a randomised complete block design. There were four experiments conducted in these experiments, one deficiency experiment, two Mn toxicity experiments and one Zn toxicity experiment. A second Mn experiment was conducted as the Mn levels in the first Mn toxicity trial did not exhibit typical Mn toxicity symptoms.

Table 2. MnCl$_2$ and ZnSO$_4$ applications for the differing experiments using Shive's solution

Experiment	Treatment Level	MnCl$_2$ (mg/L)	ZnSO$_4$ (mg/L)
Deficiency	1	0	0
	2	6.12	1.16
Manganese Toxicity	1	6.12	1.16
	2	12.24	1.16
	3	24.48	1.16
	4	48.96	1.16
	5	97.92	1.16
Zinc Toxicity	1	6.12	1.16
	2	6.12	9.28
	3	6.12	18.56

Electrical conductivity and pH were measured on a daily basis using a Hannatech pH/EC probe (Hanna Instruments, Woonsocket, Rhode Island, U.S.A.), and the nutrient solution pH was maintained between 5.3 and 6.5 with 0.5 M KOH. Leaf width and length were recorded of every second leaf on each plant every three to four days using digital callipers. Leaf number was recorded according to leaf emergence with leaf 1 being the first true leaf produced by the plant. Flower and fruit numbers were recorded together with the leaf measurements once the plants had begun flowering and fruiting.

Leaf measurements provide an accurate image as to the impact of any factor on the growth and development of a plant. Since leaf emergence is rapid in cucumbers resulting in subsequent leaves being very close together - particularly in the earlier stages of growth - ease of access to leaves can be reduced, additionally differences between leaves may not be as distinct (Robinson & Decker-Walters, 1997). Therefore, every second leaf was measured from emergence until senescence or the termination of the experiment.

Once fruit were ready for harvest, these were collected from the plant and a variety of measurements undertaken, including fruit length, mass, width, the number of fruit harvested per plant, and these data were compared between the treatments. Fruit number, including the number of aborted fruit by the plants, was also recorded. The plants were also rated on a scale of 1 to 10 every three days to provide a measure of the general health of the plants. The rating took into account the health and appearance of the plant, the percentage leaf curl, leaf die back and necrosis. The experiment was terminated once the plants reached a maximum height of 1.8 m, 38 to 45 days after germination due to the limitation of space within the growth chambers.

Nutrient solution samples were placed into sterilized 50 mL plastic containers of each fresh solution applied and just prior to solution changes and stored in a cold room at 5^oC. These samples were then analysed using inductively coupled plasma emission spectroscopy, (ICP). A Liberty Spectrometer System, Liberty 150 AX Turbo Inductively Coupled Plasma Emission Spectrometer, supplied by Varian (Walnut Creek, California, U.S.A.), was used with an ultrasonic nebuliser, U-5000 AT^+ (Cetac Technologies Incorporated, Omaha, Nebraska, U.S.A.). The elements K, Ca, Fe, Mn, Zn, Mg and Si were analysed on the wavelengths 769.896 nm, 318.128 nm, 259.940 nm, 259.373 nm, 206.200 nm, 293.654 nm and 251.611 nm, respectively (Zarcinas & Cartwright, 1983). Si was measured in the solution to determine whether there was any contamination of the solution.

Tissue samples of plants from each experiment were taken and analysed to determine the chlorophyll concentration of each leaf, using a Beckman DU-65 Spectrophotometer (Beckman, Irvine, California, U.S.A.) that was first standardised according to Lichtenthaler (1987). Fresh green leaf material was homogenised at 6700 rpm with an Ultra-Turrax blender (IKA, Wilmington, U.S.A.) and then sieved through a nylon cloth. The filtrate was first centrifuged at 500 g for 10 minutes and then at 20 000 g for 30 minutes using an IEC Model B-22M laboratory centrifuge. The pellets were re-suspended in 1 mL of water. The carotenoids were extracted from the re-suspended pellets with 4 volumes of chloroform-methanol 3:1 (v/v). The solution was thinly marked across the base of a type 60 (aluminium backed, silica gel TLC plate, Merck & Co, New Jersey, U.S.A.) 20 cm x 20 cm thin layer

chromatography (TLC) plate. The pigments were separated in the dark in a solvent tank using 20% v/v ethyl acetate in diethyl ether. Once separated, the bands were removed from the TLC plate and eluted through Whatman No. 4 filter paper with 80% acetone. An 80% acetone solution was used as a standard. Complete wavelength scans were conducted on each of the chlorophylls and wavelength peaks determined. For the Beckman DU-65 Spectrophotometer the determined wavelengths were 470 nm, 662.5 nm and 665 nm. These wavelengths were then used to determine, spectrophotometrically, the chlorophyll concentration of the leaves. The following calculations were therefore used where C_a, C_b, C_{a+b} and C_{x+c} are the concentrations of chlorophyll a, b, a and b, and total carotenoids according to Lichtenthaler (1987), respectively:

$$C_a = 12.25A_{662.5} - 2.79A_{665}$$

$$C_b = 21.50A_{650} - 5.10A_{662.5}$$

$$C_{a+b} = 7.15A_{662.5} + 18.71A_{665}$$

$$C_{x+c} = (1000A_{470} - 1.82C_a - 85.02C_b)/198$$

Cucumber leaf samples were extracted and quantified as follows: Five 1 cm disks of fresh leaf material were obtained and placed in a glass boiling tube with a pinch of washed silica. In the dark, 8 mL 80% acetone was added and the tissue was ground until the flesh was colourless. The extract was filtered into a centrifuge tube and made up to 10 mL with 80% acetone. The extracts were spun using a multispeed desktop centrifuge, HT-800, at 4000 rpm. Using glass cuvettes and the wavelengths obtained from the standardisation of the spectrophotometer, the chlorophyll and carotenoid concentrations were quantified. A blank standard was obtained using 80% acetone solution.

Plant condition was also evaluated and plant height, leaf measurements, fruit measurements, fresh mass and dry mass were recorded. The experiment was terminated after 8 to 9 weeks, upon which final leaf measurements, plant height, fruit length and diameter, fresh mass and dry mass were taken. Both, fresh and dry mass (FM and DM, respectively), were recorded for the different plant parts. The "above ground" portion of the plants was termed "leaf", "below ground" parts "roots" and the fruit produced by the plants were weighed and measured separately.

Once dry, the nutrient content of the plant tissue was then determined using ICP. An amount of 1 g dry, ground tissue was placed in a glazed, high form porcelain crucible and ashed for 2 h at 500^oC and then allowed to cool. The ash was wet with 10 drops of distilled H_2O and 3 to 4 mL HNO_3:H_2O (1:1) was added. The excess HNO_3 was evaporated at 100 to 120^oC on a hot plate. The

crucible was returned to the furnace and ashed for a further 1 h at 500°C. After cooling, the ash was dissolved in 10 mL HCl:H$_2$O (1:1) and placed into a 50 mL volumetric flask (Helrich, 1990). The solution was made to volume with distilled water and analysed by ICP.

The Si concentration of plant tissue was also determined using ICP. The method was adapted from Van der Worm (1987) and Woolley (1957). Dry ground plant tissue (300 mg) was ashed in porcelain crucibles for 3 h at 550°C. The ash was then washed into 100 mL volumetric flasks with 50 mL of a 0.08 M H$_2$SO$_4$ solution. An amount of 2 mL 40% HF solution was added and the suspension shaken for 1h and left overnight. The volumetric flask was made up to 100 mL with 0.08 M H$_2$SO$_4$. An aliquot was then removed and placed in a 100 mL polyethylene bottle with 50 mL 0.32% H$_3$BO$_3$ solution. This solution was then used to determine the Si concentration of the tissue using ICP. Solutions of HF and H$_2$SO$_4$ diluted with H$_3$BO$_3$ were mixed with differing levels of Na$_2$O$_3$Si solution containing 1000 mg Si and used to calibrate the equipment.

Digital photographs were taken weekly throughout the growth of the plants as a visual record and to highlight and illustrate the symptoms of deficiency or toxicity observed in the experiments. All measurements taken were analysed by ANOVA and/or regression using GenStat – Seventh Edition (Discovery Edition 3). A 5% significance level was used for all statistical analyses.

2.3   RESULTS

2.3.1 Deficiency Trials

The impact of deficiencies of the various nutrients on the growth and development of the cucumber leaves was primarily identified in the measurement of leaf length. During the course of the experiment, the cucumber plants produced between 23 and 33 leaves. Leaves 5, an older leaf, and 19, a younger leaf, illustrate the impact of the treatments on the growth and development of the plants over time. Leaf length showed larger variations due to the treatments which were easier to evaluate and was therefore used in comparing treatments.

In the micronutrient deficient experiment, plants supplied with Si did not show any difference in leaf length. However, exclusion of Mn and Zn from the nutrient solution had a negative impact on the size of the leaves. The shortage of both, Mn (Figure 1) and Zn, significantly affected the growth of the cucumber. An interaction between the shortage of Zn and Mn was also discovered on younger (leaf 19) and older (leaf 5) leaves.
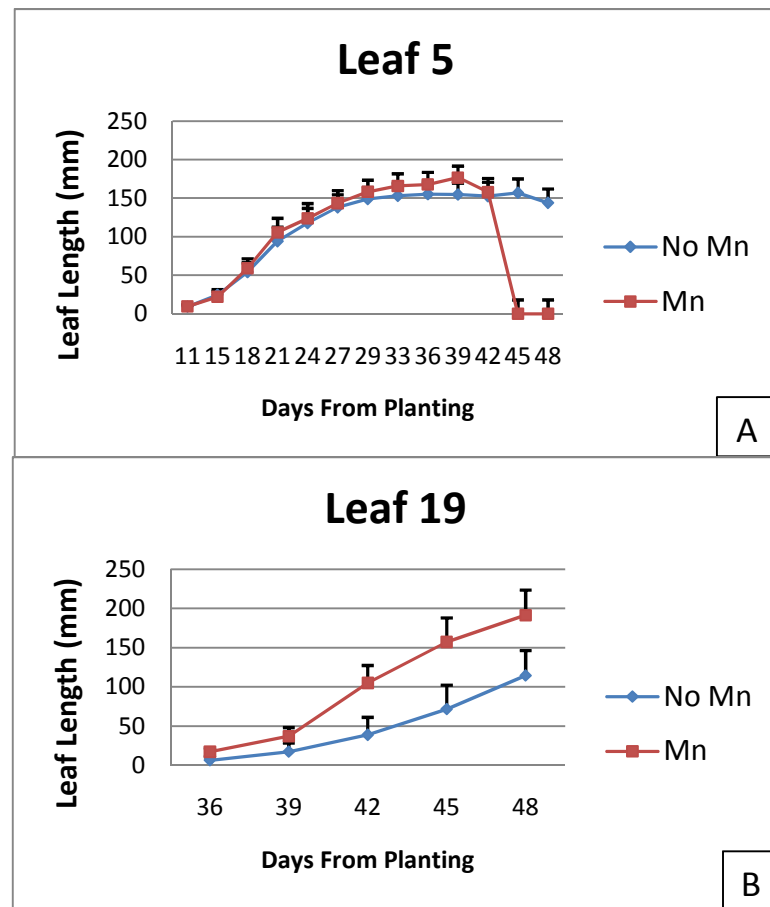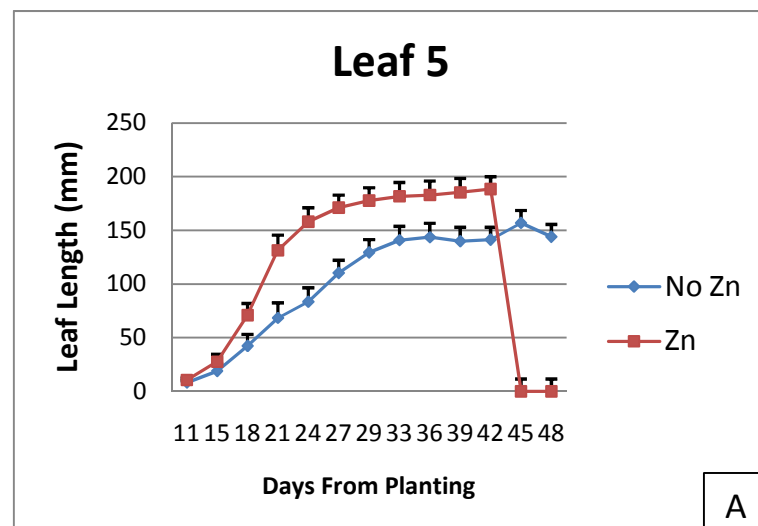
Figure 1. Impact of Mn deficiency on cucumber leaf growth, cv Tyria, of older (Leaf 5, A) and younger (Leaf 19, B) leaves

Zinc, being essential for auxin production and activation in the plant, stimulated leaf extension, resulting in significant differences between the two treatments (Figure 2). The observed reduction in leaf length of older leaves (Leaf 5, Figure 2) in the Zn applied treatment is caused by early senescence of the leaves measured.
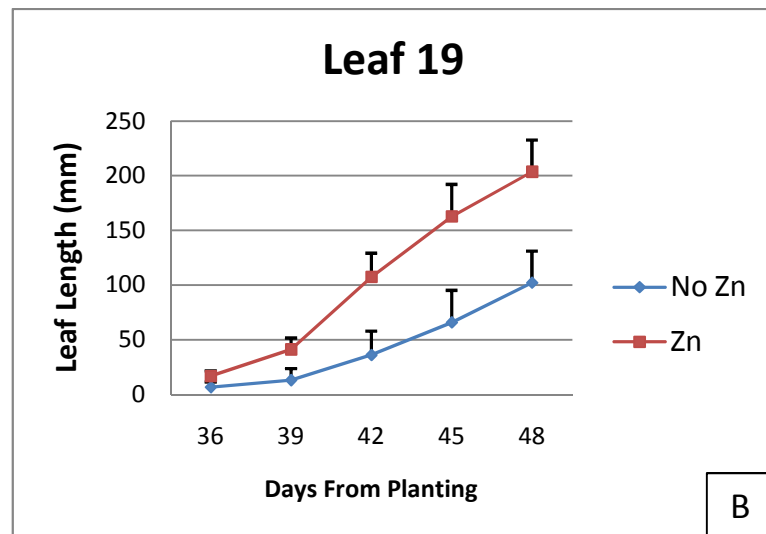
Figure 2. Impact of Zn deficiency on older (Leaf 5) and younger (Leaf 19) leaf growth of cucumber cv Tyria

Silicon treatments did not significantly affect leaf growth and development in any way and no differences were observed between the two treatments on any measured leaves. Interactions between Mn and Zn treatments were observed, but Si did not play a role in this (Figure 3). During the early growth phase, although differences were observed, some of which were significant, these differences were not as clear as observed later in the growth of the plant (Leaf 19).

In addition to the noticeable differences in leaf growth and development, the plants produced distinguishable visual differences as symptoms of either deficiency or toxicity depending on the experiment concerned (Table 3). Digital photographs captured for each experiment helped identify typical deficiency or toxicity symptoms for cucumbers of Zn (Figure 4), Mn (Figure 5) and Si (Figure 6).
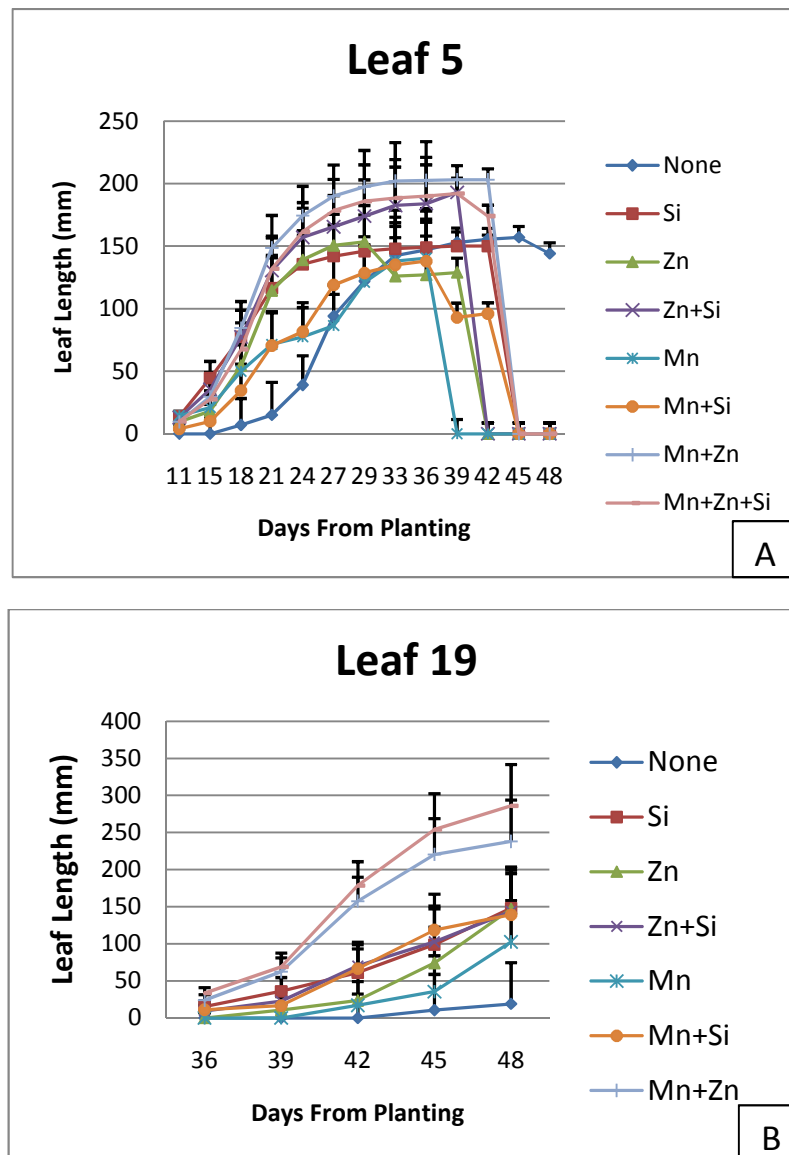
Figure 3. Effect of applied micronutrients on extension of old (A, Leaf 5) and young (B, Leaf 19) leaves in cucumber plants cv Tyria

Table 3: Observed deficiency symptoms for manganese, zinc and silicon in cucumber plants

| Element	Symptom
Manganese	lighter green coloured leaves, predominant interveinal chlorosis; leaves exhibit senescence of the leaf margins, leaf margins turn white and dry out resulting eventually in dead tissue; leaves are smaller in size (Figure 4)
Zinc	leaves are larger than Mn deficient plants; internodes are shortened; younger leaves are lighter green in colour; no interveinal chlorosis (Figure 5)
Silicon	leaves exhibit a downward and inward curling; leaves tend to droop and are not held out horizontally; leaves have marginal interveinal chlorosis, with light green spots on the leaves; symptoms appear more prevalent in the presence of Mn (Figure 6)
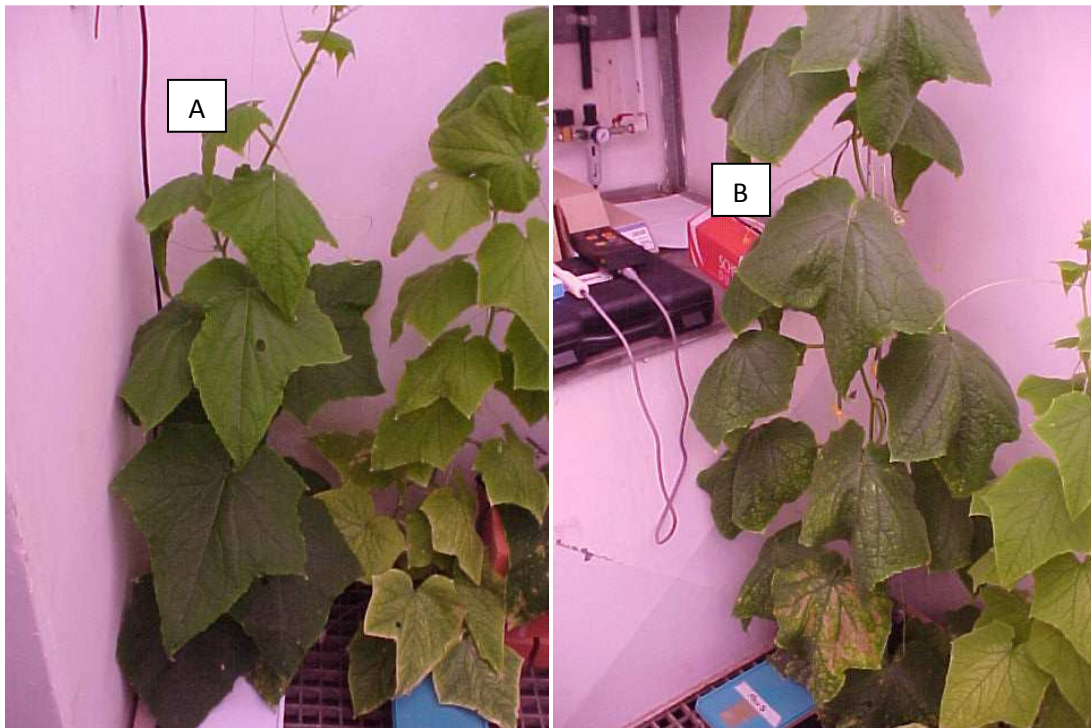
Figure 4. Zinc deficiency symptoms of cucumber plants cv Tyria, Mn+Si (plant A) versus Zn+Mn+Si (plant B) at 18 leaves stage
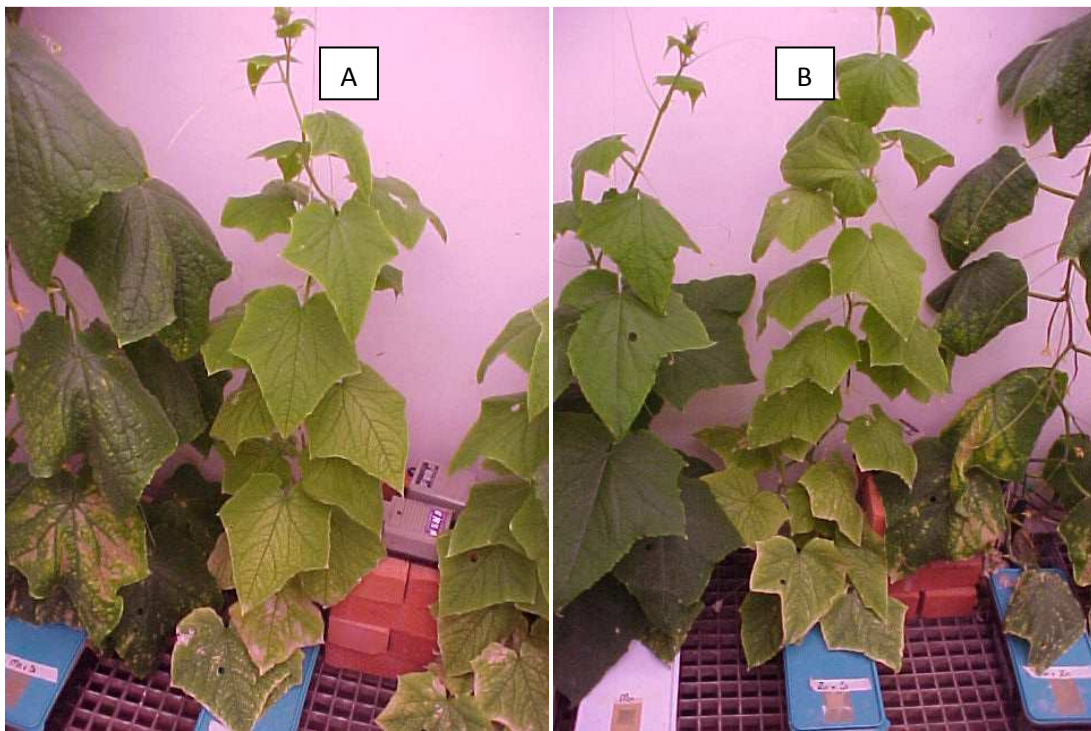
Figure 5. Manganese deficiency symptoms of cucumber plants cv Tyria, Zn only (plant A) and Zn+Si (plant B) at 18-leaves stage
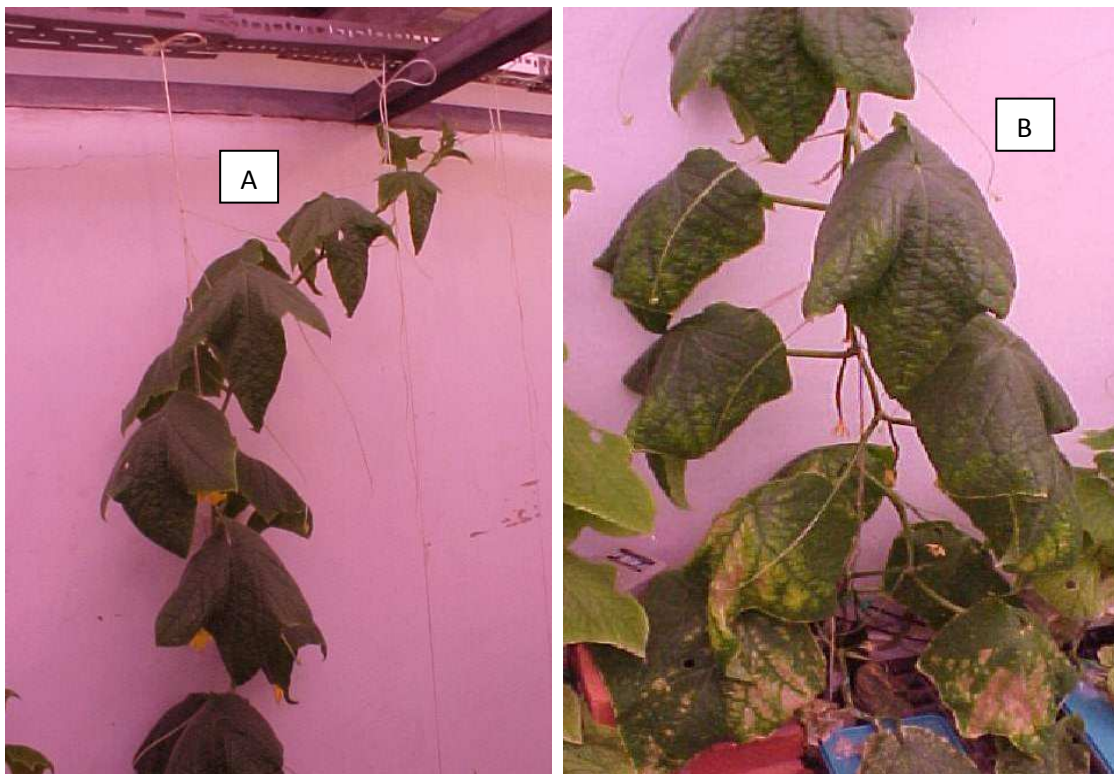

Figure 6. Silicon deficiency symptoms of cucumber plants cultivar Tyria, Mn+Zn (both plants) at 23 leaves stage on younger (A) and older (B) leaves

Leaves exhibiting symptoms of leaf curl from Si deficiency were evaluated on the percentage curvature of the leaf in relation to leaf width. Similar to leaf length measurements, leaf width was affected by treatment (Figure 7). In older leaves width was significantly affected by the Mn treatments. However, with younger leaves, the differences recorded showed significance with Mn and Zn applications (from deficient to adequate) whilst there was also an interactive effect when both Mn and Zn were present in the treatment producing significantly larger leaves. No significance in leaf width was recorded with or without the application of Si to plants.

Although the curling of the leaves has been described as a symptom of Si deficiency (Miyake & Takahashi, 1978) in the growth and development of cucumber plants, the percentage leaf curl showed no significance with the inclusion of Si in the deficiency experiment in general (Figure 8). The only treatment, therefore, that showed a significant difference in the application of Si was Mn+Zn, where addition of Si to the treatment significantly reduced the amount of leaf curl. This significance was more prevalent in older, larger leaves. Leaf 22 did also tend to exhibit greater leaf curl than leaves either smaller than it or slightly older.

The scores and ratings based on the visual appearance of the leaves were also evaluated at the termination of the experiment. No significant differences were observed between treatments within the deficiency experiment. No other leaf measurements taken at the same time showed any significant difference between treatments.
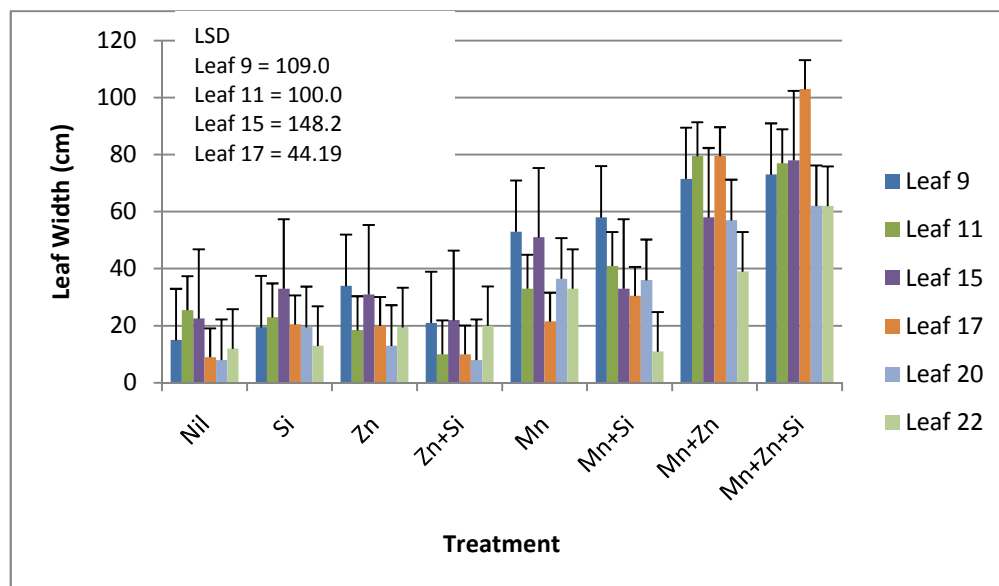

Figure 7. Leaf width of cucumber cv Tyria grown in hydroponic solutions of varying nutrient composition at termination of the experiment
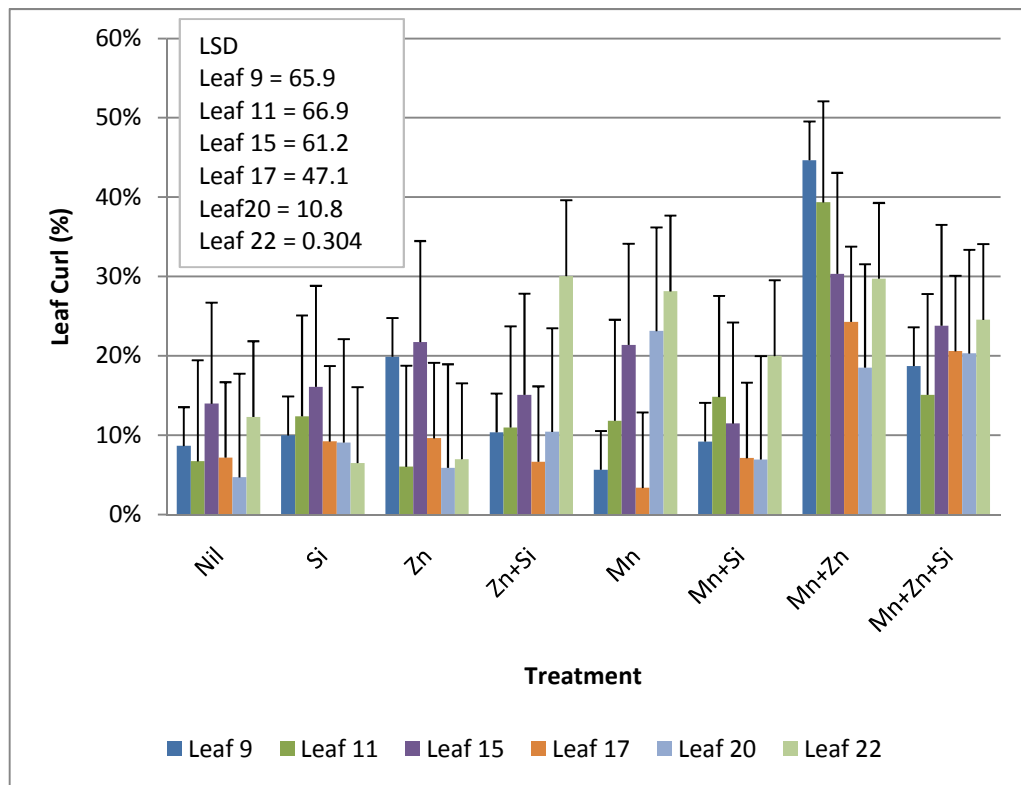
Figure 8. Leaf curl of cucumber leaves, cv Tyria, plants grown in hydroponic solutions of varying nutrient composition at termination of the experiment

At termination of the experiment, plants were cut into sections, these were above and below water level, and measured and weighed to determine differences between the treatments applied to the plants. The addition of either Mn or Zn, or both elements together significantly improved the growth of the plants, increasing height (Figure 9). The addition of Si did not have any significant effect on plant height.
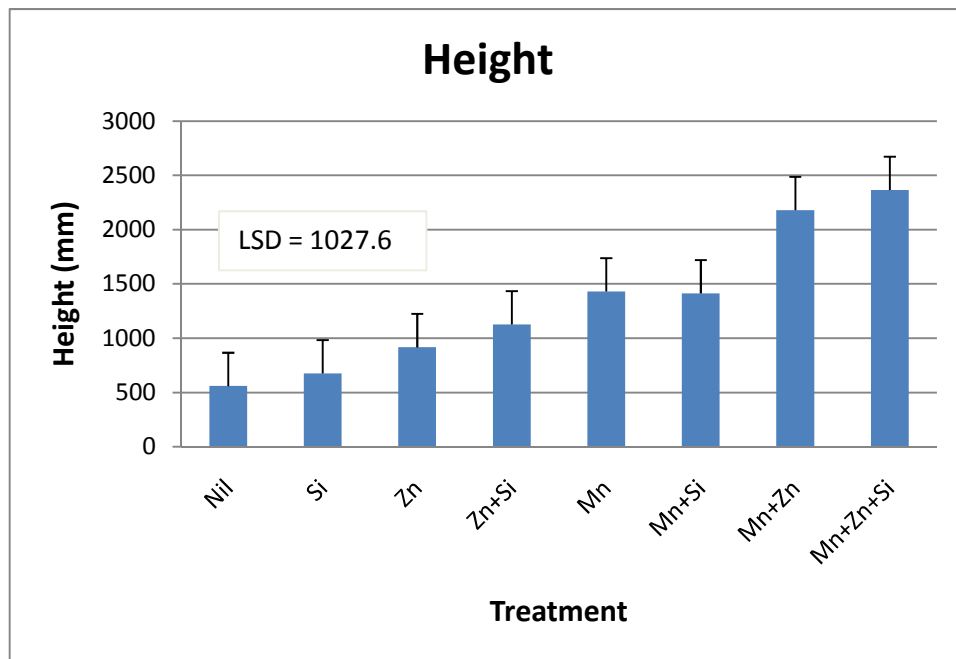
Figure 9. Plant height as affected by nutrient shortages in the deficiency experiment

The deficiency experiment showed increasing plant mass with the addition of Zn, Mn and a combination of the two (Figure 10). The application of Mn appeared to have a stronger effect on the growth and development of the plants than the Zn application alone. Zinc significantly increased leaf FM and DM (Figure 10a, b), but had no effect on fruit or root development. Manganese, on the other hand, significantly increased the FM and DM of the leaf portion of the plants, the roots and the fruit. Silicon applications did not significantly affect FM or DM of any plant part. No treatment significantly affected the moisture content of any plant part measured (Figure 10 a, b).

The application of Mn, Zn and the combination of the two nutrients significantly increased the number of fruit that matured as well as the total number of fruit produced by the plant, whether they matured or not (Figure 11). Silicon applications, however, had no significant effect on the development of fruit in this experiment.
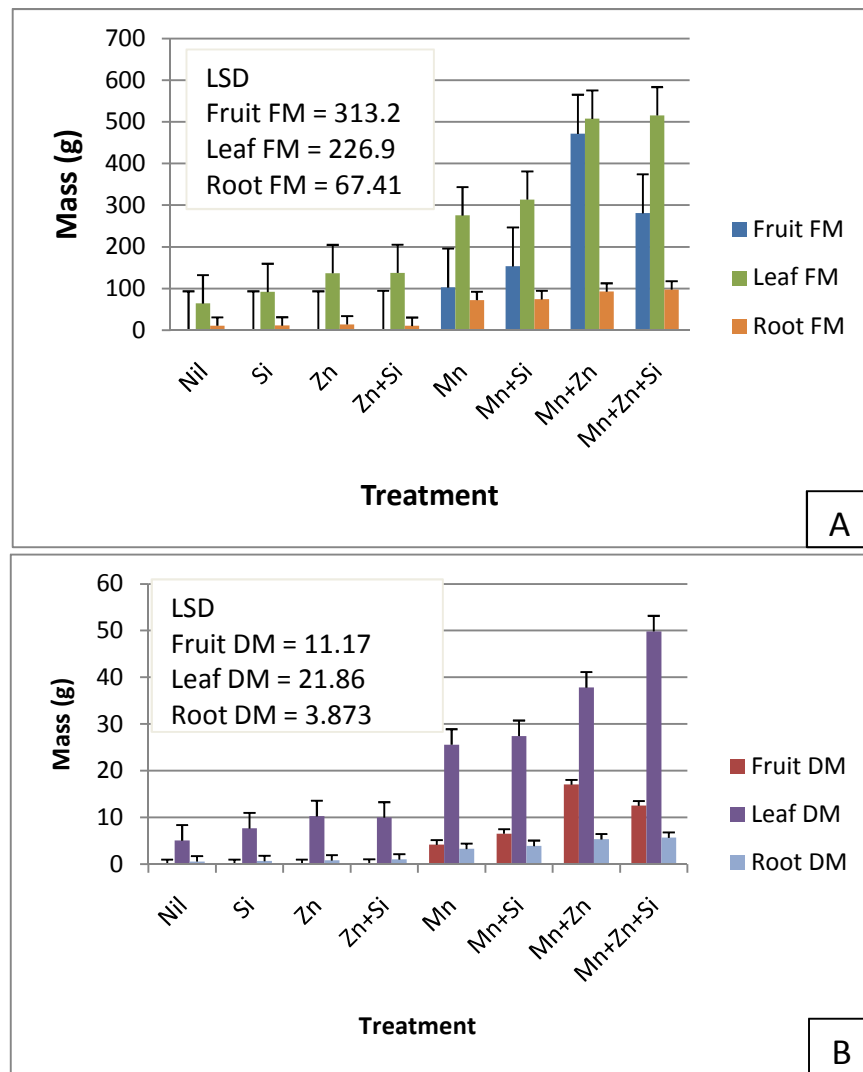
Figure 10. Fresh (FM, A) and dry mass (DM, B) measurements for various plant parts at the termination of the deficiency experiment
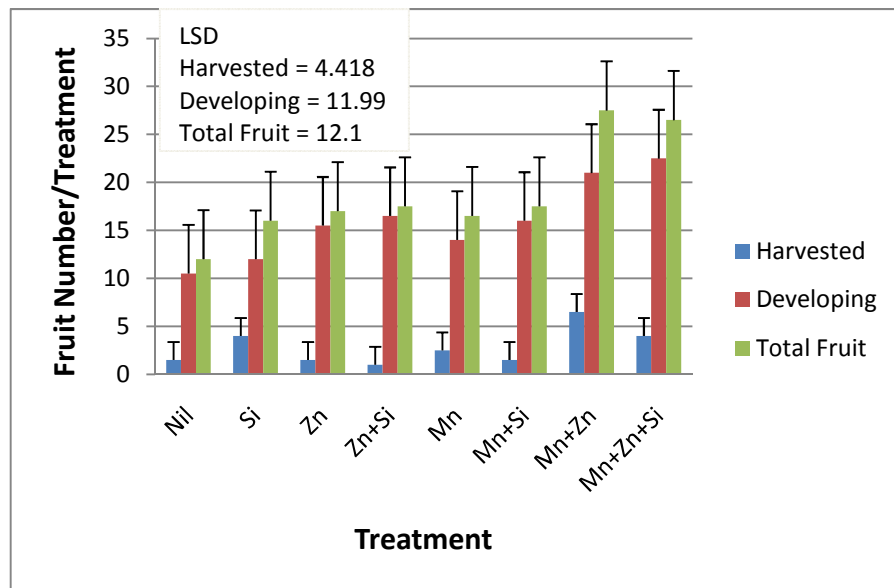
Figure 11. Fruit number of cucumber plants cv Tyria grown under Si, Zn and Mn deficient conditions

Plant condition was visually rated on the plants during the course of the experiments, assessing the condition of the plants over time (Figure 12). Significant deterioration of the conditions of the plants occurred with time. Some significant differences between the treatments were observed, but these were not consistent over time. Treatments incorporating Mn appeared to receive better scores than those without, whilst the plants of the poorest condition were rated highest and lacked both Mn and Zn.
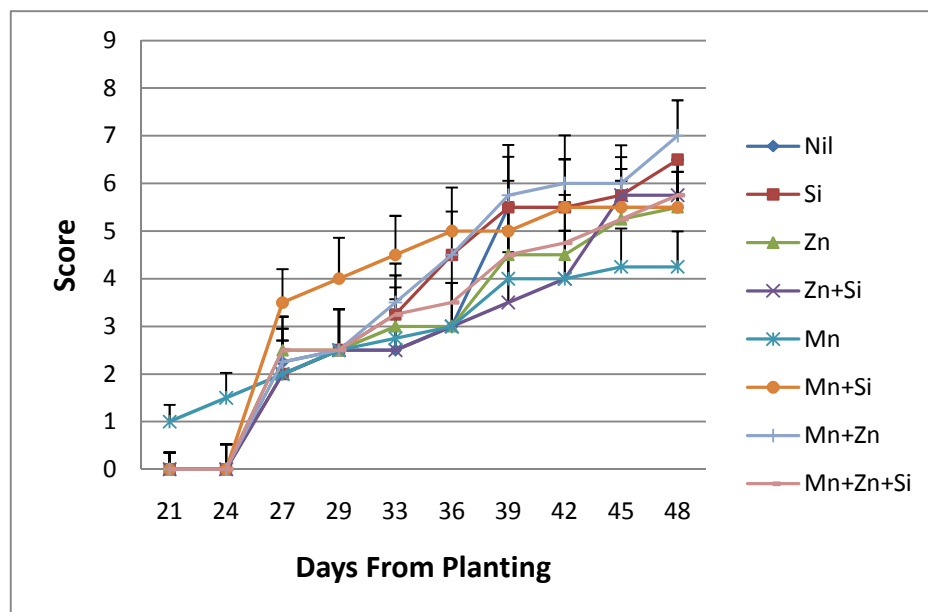

Figure 12. Rating of the deficiency development in cucumber cv Tyria over the experimental period (Score 0 = healthy and 9 = dead) for each treatment

Daily monitoring of nutrient solution pH did not show any significant differences between treatments in the deficiency experiment except for the duration of the last solution change (Figure 13). At each solution change the pH was set to the same range (6.0 to 6.1). Different nutrient applications, whether sufficient or deficient, did not impact the change in pH levels of the solution during the course of that week or until the solution was changed. However, during the last week before termination of the experiment, Mn and Zn as well as the combination of the two, significantly affected the change in solution pH. This change, however, was at no time dependent on the amount of water or nutrient solution taken up by the plant (data not presented).
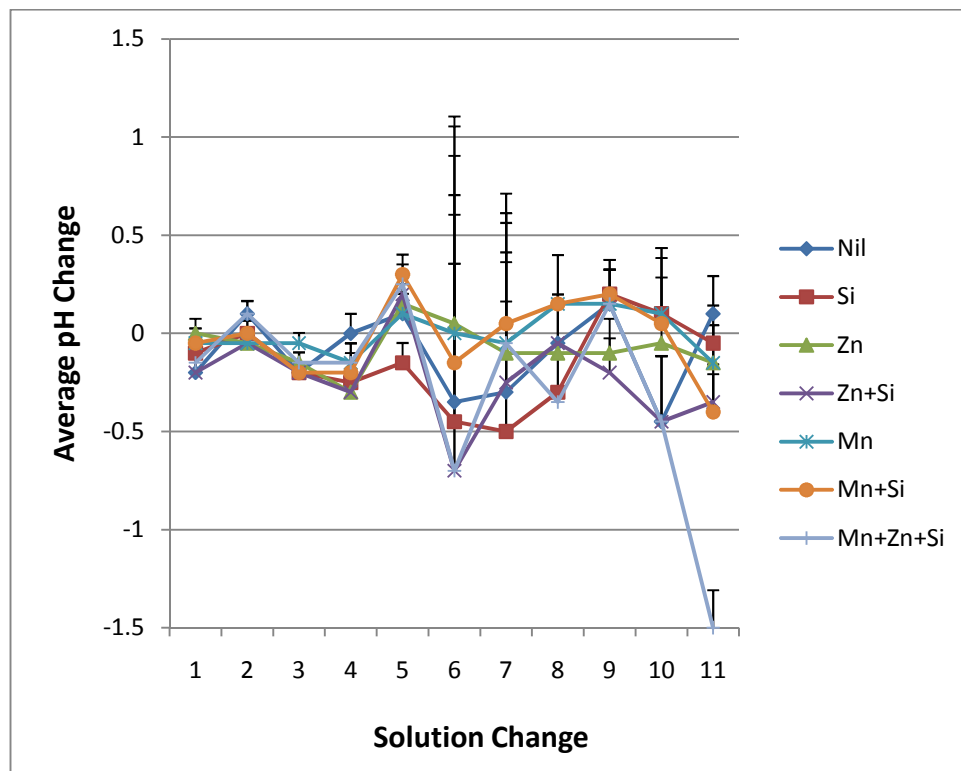

Figure 13. Change in pH between each solution change (deficiency experiment) for each treatment

Along with pH, the daily measurement of the electrical conductivity (EC) of the solutions was also recorded to establish whether there was any impact of the treatments on the solution pH or EC. It was found that the significant differences between the treatments were not related to the amount of water or nutrient solutions used by the plants, but were directly impacted by the treatments applied to the plants (Figure 14). Water use by the plants did not have an effect on the change of EC of the solution, but did, however, impact on the actual EC of the solution. As the plants grew larger, the impact of plant size and the plant's requirement for water and nutrients significantly affected the EC of the solutions. Significant differences were therefore only observed for solution changes 10 and

11 and could be seen in the application of Mn and Zn to the nutrient solutions. Statistical analysis showed that Si was not involved in the observed differences.
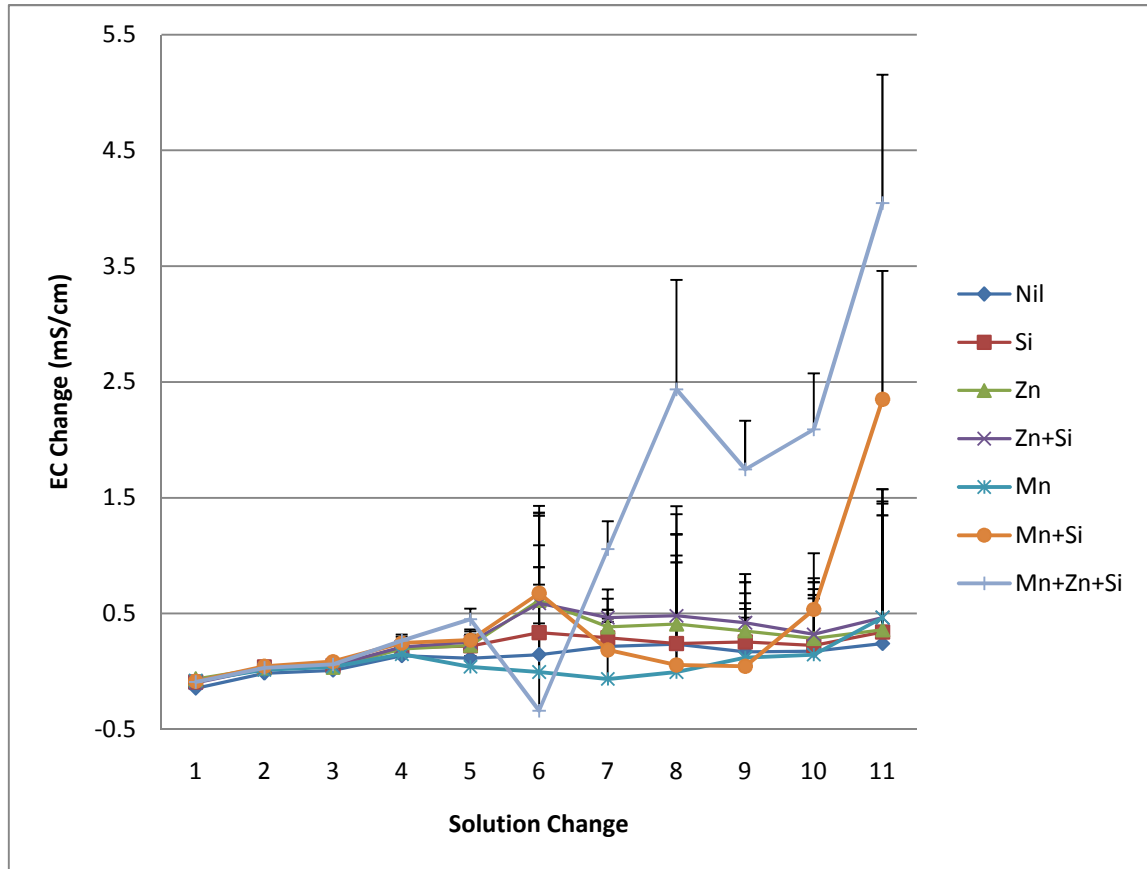

Figure 14. EC changes between solution changes for the cucumber deficiency experiment for each treatment

Due to the relationship between EC and water uptake by the plant, nutrient composition comparisons were carried out using the water use figures by the plants, where significant. Not all nutrients measured showed significant differences in uptake with water use. It was established that the uptake of Mg, Ca and K from the solution by the plants was not significantly affected by the presence or absence of Zn, Mn or Si from the solutions or application to the plants. For all nutrients measured, their uptake by the plant was a function of the uptake of water by the plant and, hence, was used within the deficiency experiment as a covariate to the analyses.

Some of the nutrients measured displayed significant differences in daily uptake by the plants in the deficiency experiment (Table 4). The presence or absence of Zn in the nutrient solution significantly affected the daily uptake of Zn by the plants. Of interest though, was that the presence of Mn in the solution had an interactive effect on the daily uptake of Zn, significantly increasing Zn

uptake in its presence. The presence of Mn, therefore, had a synergistic effect on the uptake of Zn under deficient conditions.

As can be expected, the presence of Mn in the nutrient solution had a significant effect on the daily uptake of Mn by the plant. Iron was the only other applied nutrient analysed to show significant differences in daily uptake by the plant. In the presence of Zn, there was a significant increase in the uptake of iron by the plants. Certain nutrients analysed showed significant differences between treatments (Mn, Fe, Mg and Zn; Figure 15). Although Si is presented here too, the differences between treatments were not significant, despite Si being applied as a treatment. The application of Mn did significantly increase the amount of Mn in the plants ($p \leq 0.01$); there was also an interactive effect with Zn, where the presence of both Zn and Mn resulted in much higher levels within the plant tissue. Magnesium was also found to benefit from the presence of Mn, where increased presence of Mn significantly ($p \leq 0.05$) increased the uptake of Mg by the plant. The presence of Mn, Zn or a combination of the two significantly increased the amount of Zn found in the plant ($p \leq 0.01$). Calcium and K did not show any response to the treatments.

Table 4: Daily uptake of nutrients which displayed significant differences in the deficiency experiment (*= $p \leq 0.05$, **= $p \leq 0.01$)

	Daily Nutrient Uptake (ppm)			
Treatment	Zn	Si	Mn	Fe
Nil	2.13	0.00555	0.8	2.553
Si	3.30	0.00655	0.59	2.445
Zn	4.90 *	0.00644	0.9	1.54 *
Zn+Si	8.96 *	0.01168	7.69	2.309 *
Mn	15.99 **	0.01689 *	62.46 *	3.189
Mn+Si	17.91 **	0.01519 *	49.32 *	4.197
Mn+Zn	33.81 **	0.02681 *	57.04 *	4.287 *
Mn+Zn+Si	27.90 **	0.02558 *	57.6 *	3.491 *
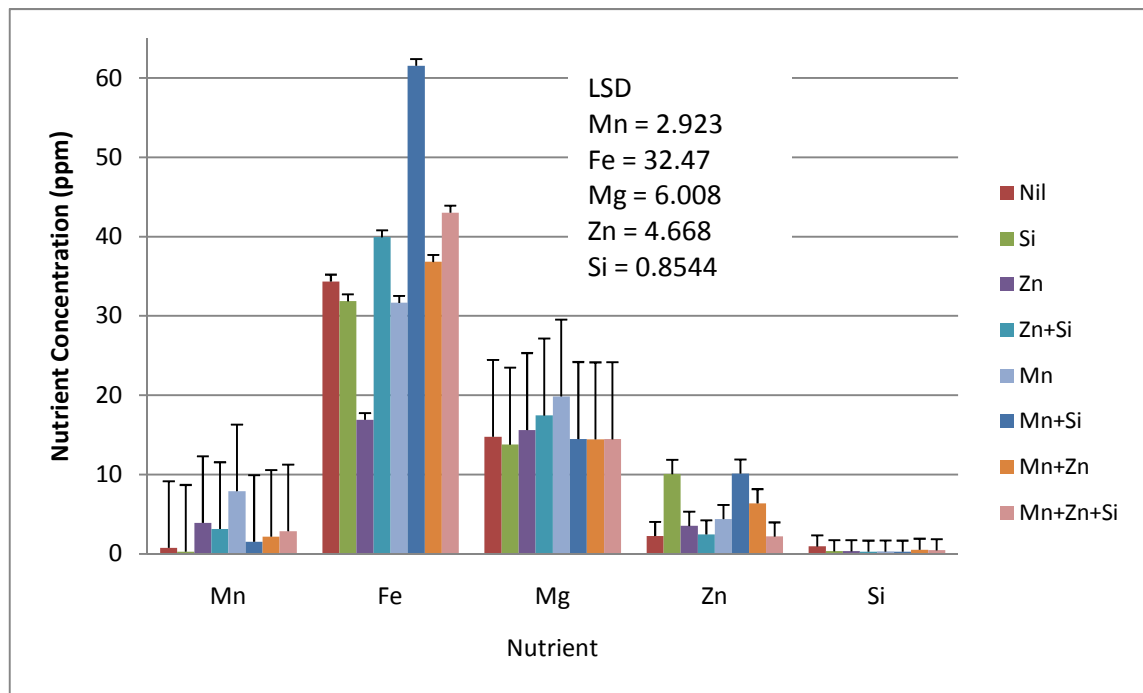
Figure 15. Nutrient concentration in cucumber plants grown in different nutrient solutions for each treatment

Mn alone influenced the concentration of chlorophylls and total carotenoids in the leaves when compared with Zn and Si (Figure 16). The application of Mn to the nutrient solutions significantly ($p \leq 0.01$) increased chlorophyll and total carotenoid (C_{x+c}) concentrations of the leaves.

Figure 16. Chlorophyll and carotenoid concentration of cucumber cv Tyria leaves as impacted by Zn, Mn and Si applications

.3.2 Toxicity Trials

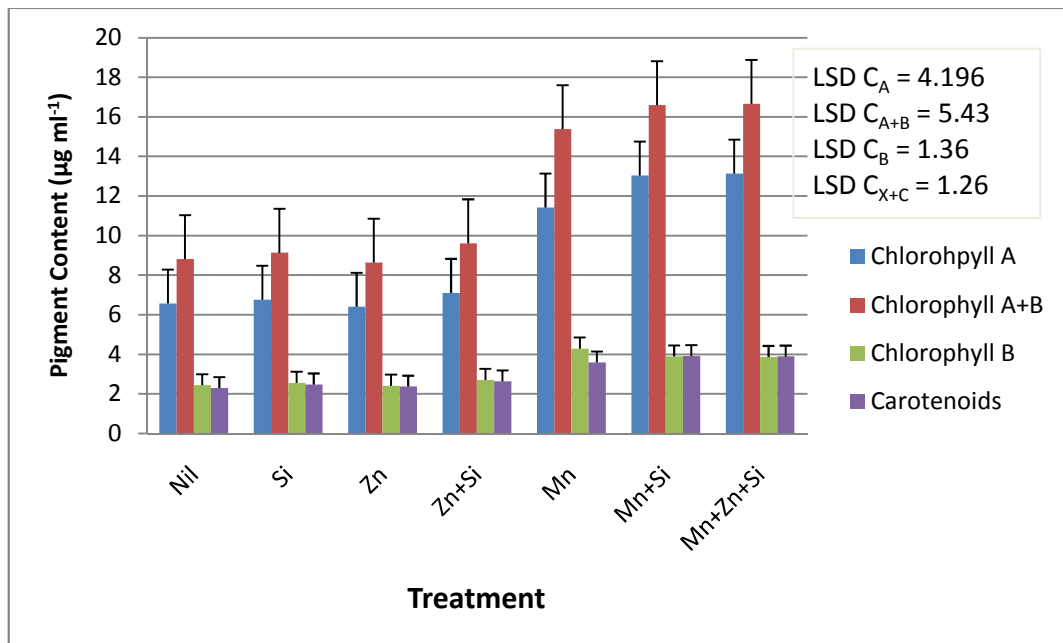The first Mn toxicity experiment (Mn Tox 1) did not exhibit any of the expected toxicity symptoms, such as leaf necrosis or stunting of growth. The plants did appear to be almost as healthy as the control plants at both levels of toxicity (double and four times the application rate, Mn2 and Mn4, respectively). For this reason a further Mn toxicity experiment (Mn Tox 2) was conducted with 8 and 16 times (Mn8 and Mn16, respectively) the levels of applied Mn as the control. As with the deficient experiment, the application of Si showed no significant impact on the leaf growth. The Mn toxicity applications of the first experiment failed to consistently show significant differences between and within measurements. Significant differences were, however, found in the size (length) of the youngest and oldest leaves of the plants only (Figure 17).
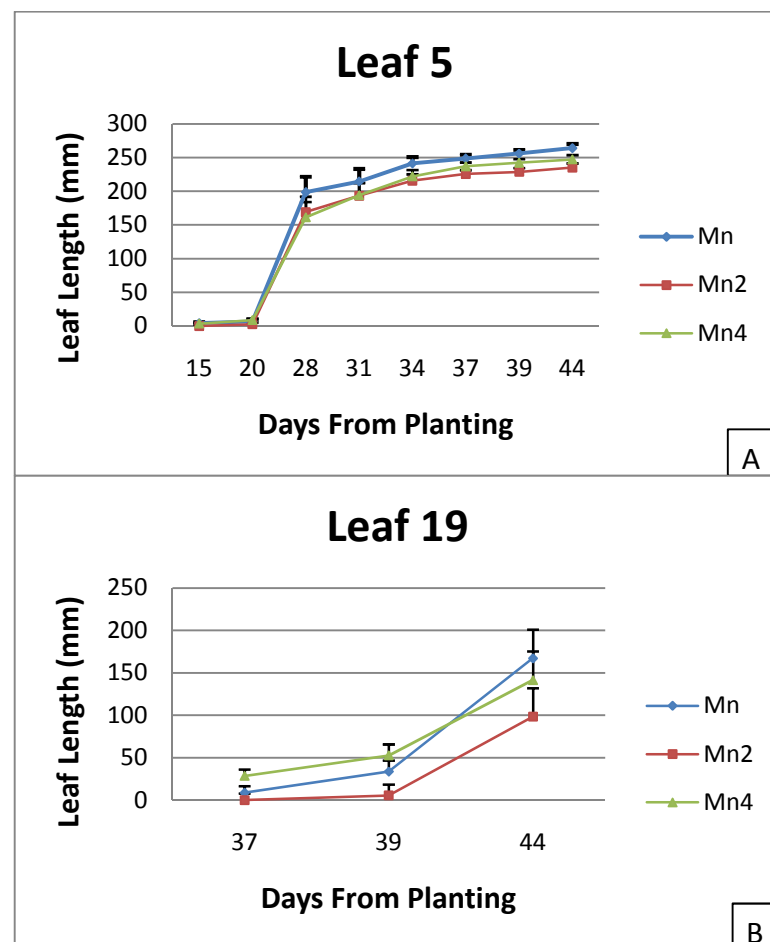

Figure 17. Effect of increasing Mn applications to super-optimal levels (Mn2 and Mn4) on leaf growth of leaves 5 and 19 (Mn Tox experiment 1) of cucumber cv. Tyria plants

Significant interactive effects between the Mn and Si applications were observed but only in the younger leaves of the plants. There were interactive effects of Mn and Si on leaf growth of leaf 5 in Mn Tox 1 (Figure 18).
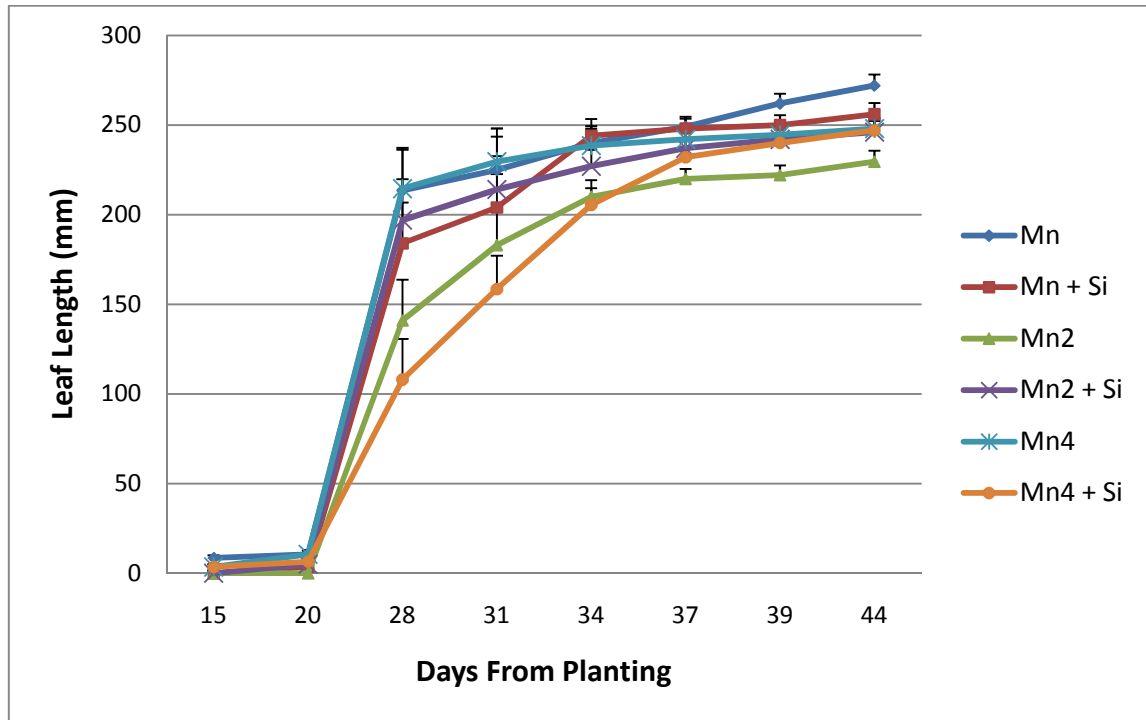

Figure 18: Interactive effects of Mn and Si on leaf length growth for leaf 5 (Mn Tox experiment 1) of cucumber cv. Tyria plants

The differences between treatments are best seen as reduced expansion rates of the leaves with the higher Mn applications. When Mn levels were raised even further (Mn Tox 2), the same impact of Si on leaf growth and extension was observed. As with Mn Tox 1, the excessive application of Mn had an impact on the expansion of the leaves by reducing their size. This impact was observed earlier in the life of the leaves and resulted in earlier leaf senescence. As the applied Mn levels increased (Figure 19) leaf length was reduced and leaf senescence occurred earlier.

In younger leaves of the plant, the high levels of Mn slowed the rate of leaf extension substantially as well as the rate of leaf emergence, particularly in the high level of application (Mn16). Early leaf senescence was also clearly exhibited in the interactive effects of the application of Mn and Si. All excessive applications of Mn and Si had early leaf senescence and showed slower growth than the control. The application of Si to these excessive treatments did, in the Mn8 treatment for the younger leaves, reduce the impact of the Mn treatment (Figure 20).
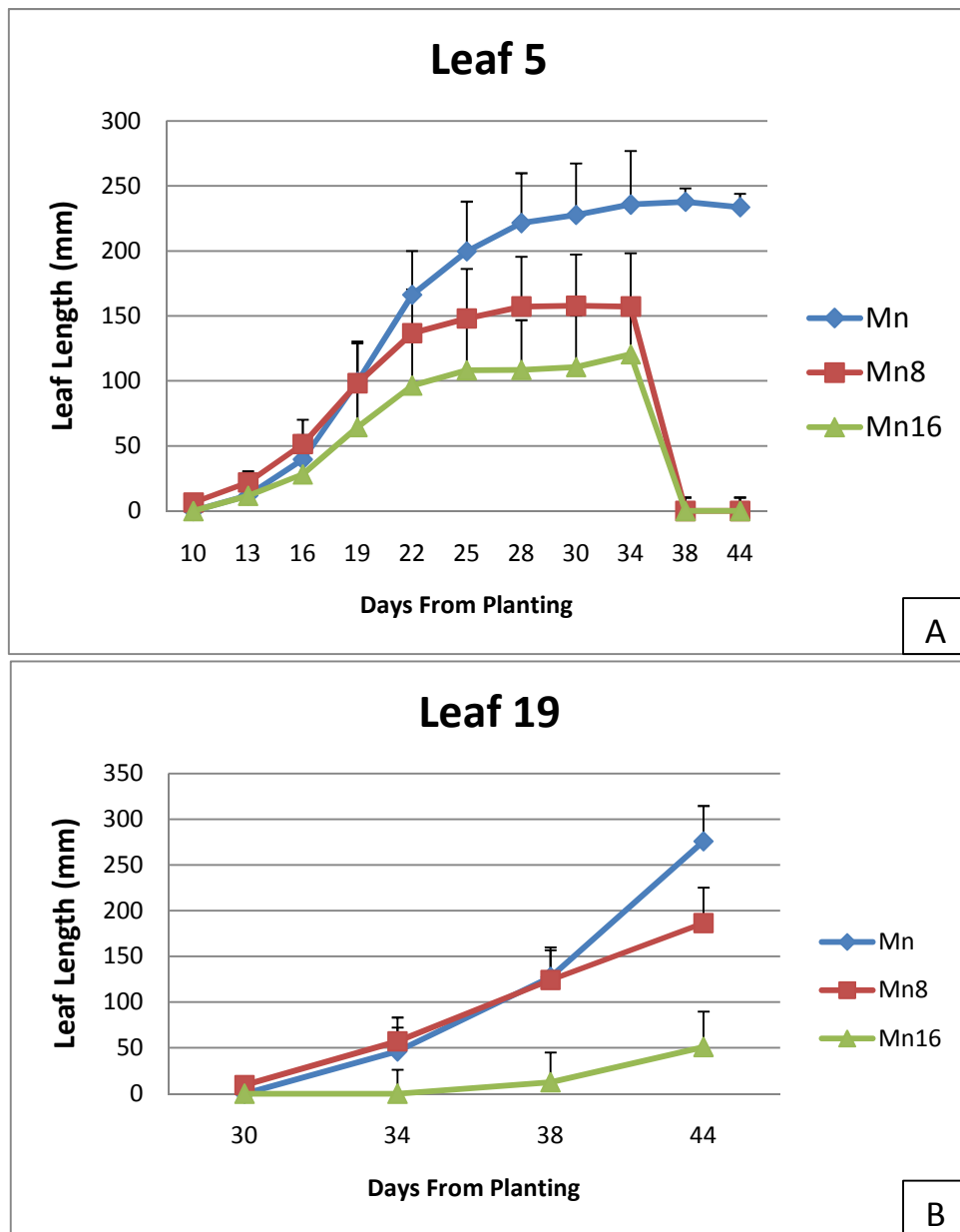
Figure 19. Impact of Mn application (recommended and 8x and 16x recommended rate) on leaf length (Mn Tox experiment 2) of cucumber cv Tyria plants
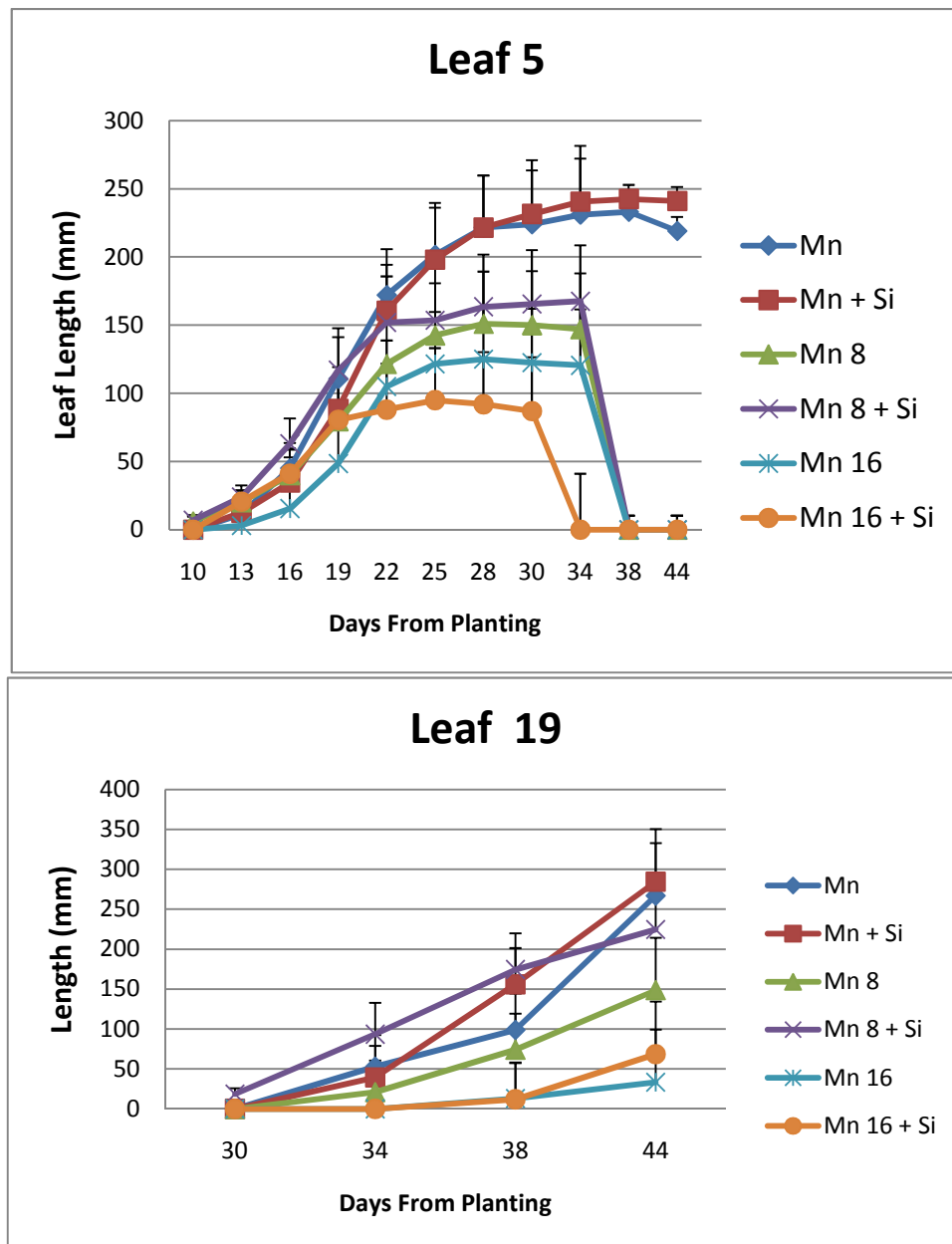
Figure 20. The impact of Mn (recommended and 8 or 16 times the recommended rate) and Si applications on the development of leaf length (Mn Tox experiment 2) of cucumber cv Tyria plants

As not to repeat the poor responses shown in Mn2 and Mn4 (Mn Tox 1), the Zn toxicity experiment used applications of 8 and 16 times (Znx8 and Znx16, respectively) and the control level of Zn. Excessive applications of Zn negatively impacted on the extension of the leaves. The differences, although significant on older leaves, were marked on younger leaves, where even leaf emergence was delayed under very high applications of Zn (Zn16) (Figure 21).
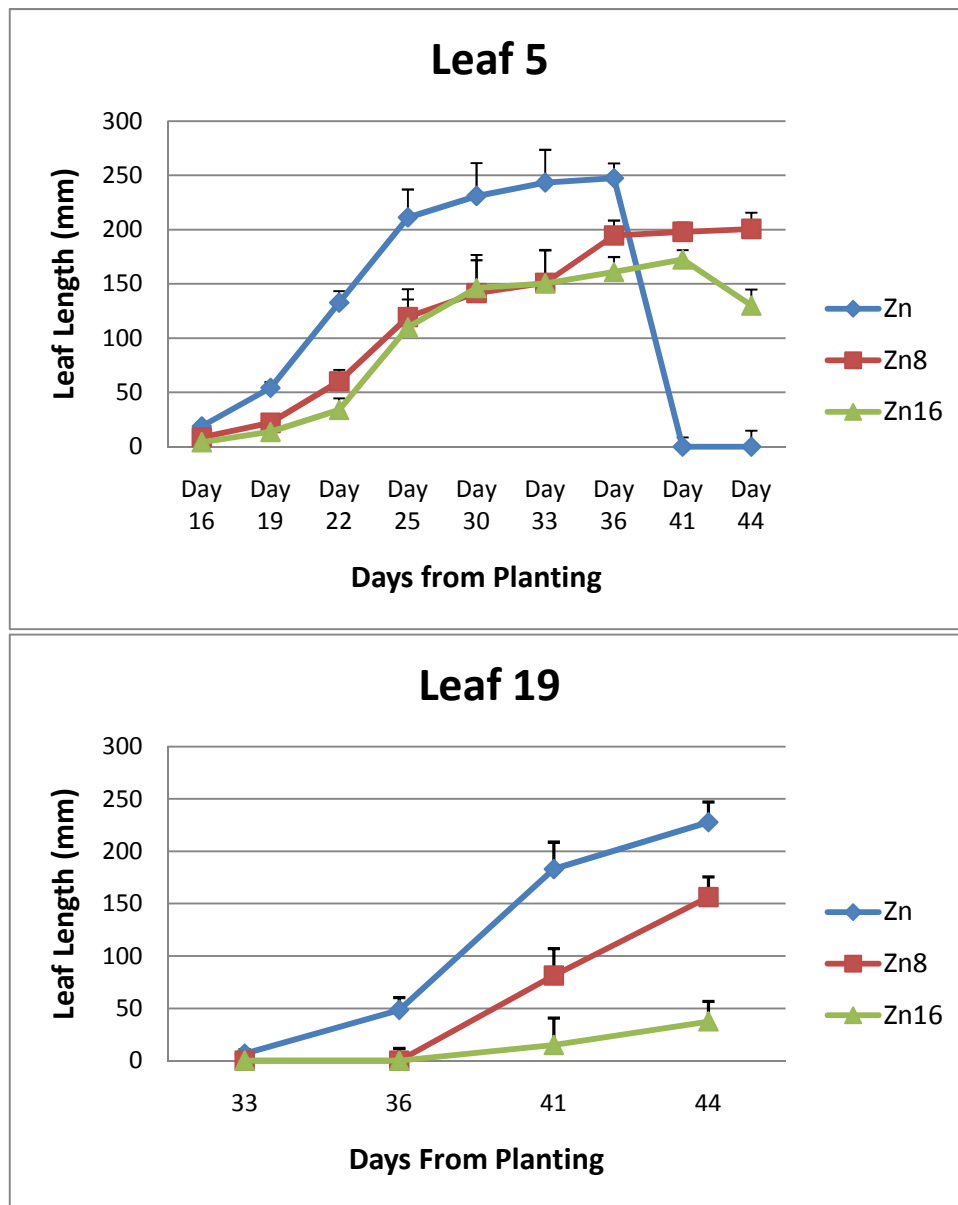
Figure 21. Impact of Zn applications (recommended rate and 8 (Zn8) and 16 x (Zn 16) of recommended rate) on the growth of cucumber cv Tyria leaves

Interactive effects of Si under excessive Zn applications were also observed (Figure 22). As with the Mn toxicity experiments, older leaves only began to show significant differences later in their life cycles as the growth in the treatments with toxic Zn levels began to slow. Younger leaves showed marked differences in growth and development as the applied Zn levels were increased. At higher concentrations, leaf emergence was delayed whilst leaf growth and extension were considerably slowed. On the interactive side, Si applications to the Zn8 treatment created significant differences to the untreated control. The Si application appeared to stimulate the growth of the leaf, negating or lessening the impact of the excessive application of Zn. This enhanced leaf development was

consistent across all leaves measured in this treatment. No such significant additive effects were observed with the application of Si within the control or the Zn16 treatments.
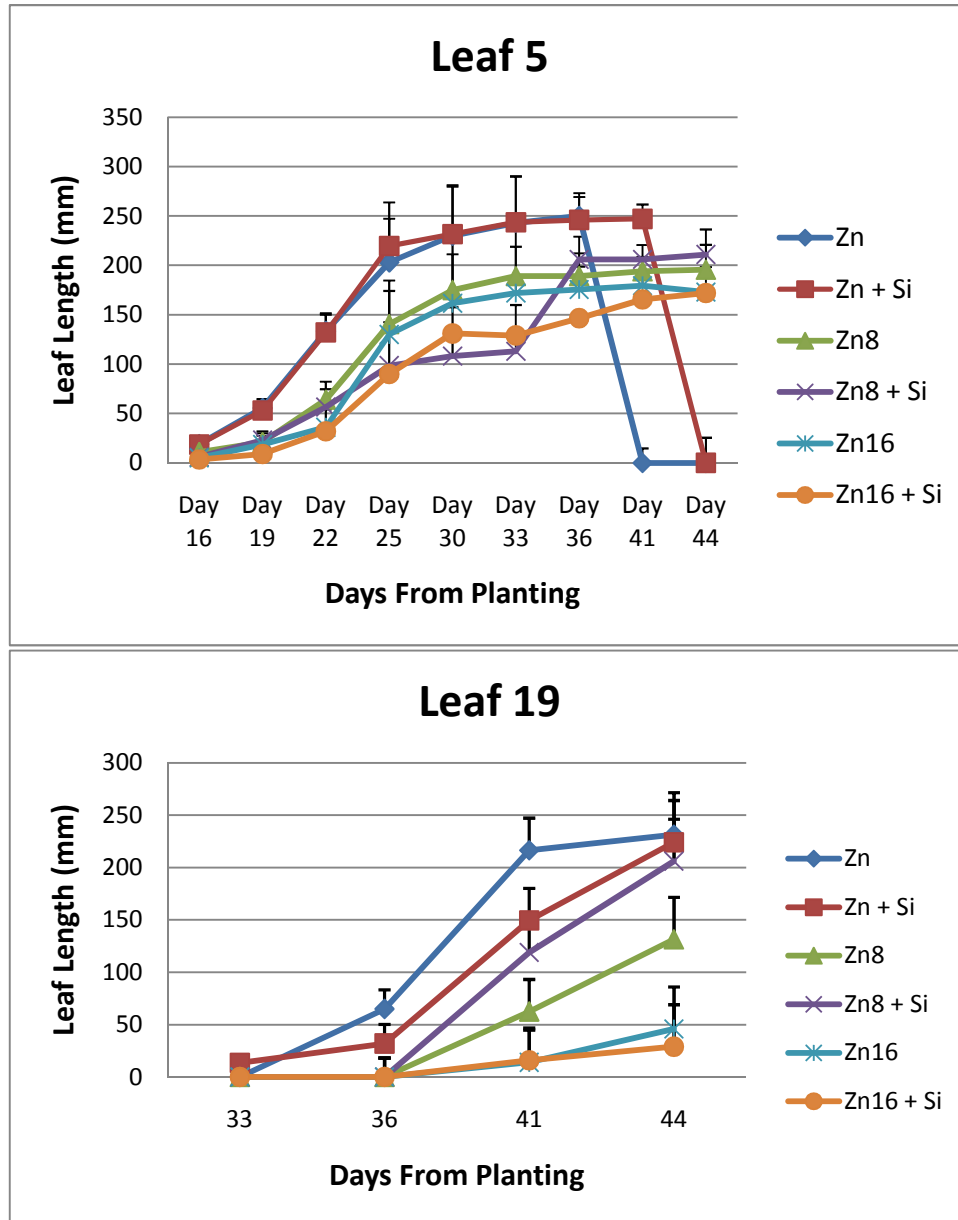
Figure 22. Effects of the addition of Si and Zn (recommended rate and 8 x (Zn8) and 16 x (Zn 16) of recommended rate) to cucumber cv. Tyria plants

Due to the nature of the Mn Tox 1 experiment, toxicity symptoms were not as prevalent and distinguishable. To determine whether there was Mn toxicity in the solutions, a cabbage plant was placed into each solution next to the cucumber plant to express nutrient toxicities sooner due to a greater sensitivity of cabbage to the toxicity (Mengel & Kirkby, 2001). Toxicity symptoms were observed in the treatments, but the expression was very weak.

However, more defined symptoms were observed in Mn Tox 2. Plants tended to be light green and chlorotic on all leaves. Chlorosis was confined to interveinal regions of the leaves. Plants were stunted in growth and leaves were significantly smaller in size when compared with sufficiently supplied plants. At very high Mn concentrations, the leaf margins turned white in colour and necrosis began leading to the onset of leaf senescence. The young emerging leaves were pale yellow to white in colour with little green colour even in the veins. Interveinal necrotic spots developed on older leaves which coalesced as the leaf senesced (Figure 23). At higher levels of Mn (Mn16) leaves tended to curve inwards, a symptom not present in the lower toxic applications.

Figure 23. Mn8 (plant A) and Mn16 (plant B) (8 and 16x recommended application rates of Mn, respectively) depicting manganese toxicity symptoms for cucumber plants cv. Tyria

Symptoms of Mn toxicity (Figure 24) were less defined than observed without the application of Si (Figure 23). Addition of Si resulted in leaves not as yellow, fewer necrotic spots on the leaves were observed, but the plants remained stunted.

Excessive applications of Zn (Zn8 and Zn16 treatments) resulted in symptoms typical for zinc toxicity, i.e., interveinal chlorosis of all leaves and necrotic leaf margins with necrosis spreading backwards towards the leaf petiole. The intensity of the chlorosis increased with the amount of Zn

applied to the plants as well as with leaf age. Plants appeared stunted with shortened internodes and smaller leaves (Figure 25).
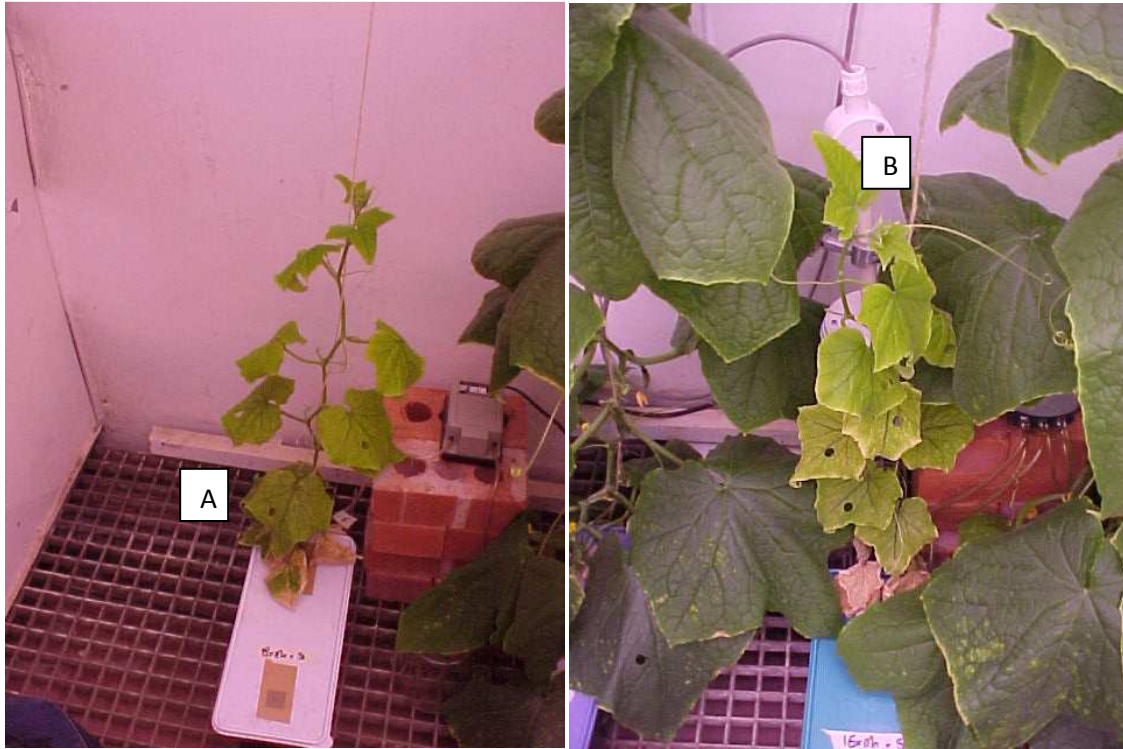
Figure 24. Toxicity symptoms of Mn (Mn8 (plant A) and Mn16 (plant B)) treatments on cucumber cv Tyria plants under addition Si addition on
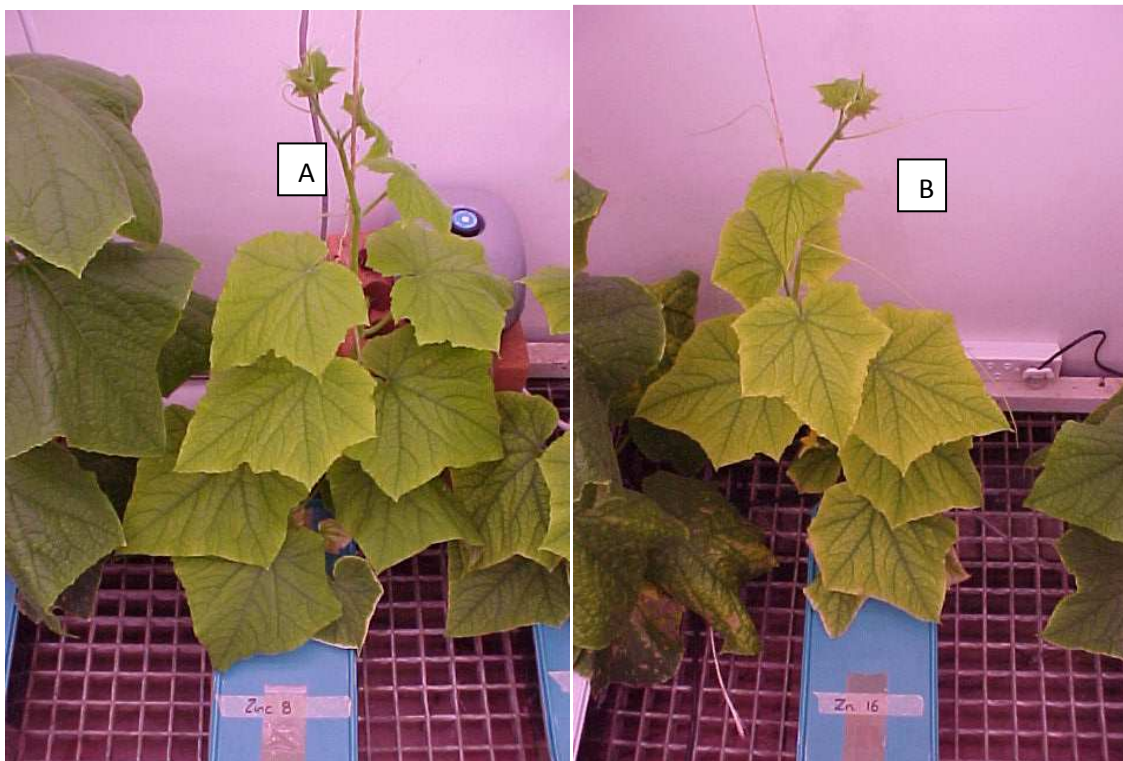
Figure 25. Toxicity symptoms of Zn applications to cucumber plants (Zn8 (plant A) and Zn16 (plant B))

Visually, application of Si to Zn toxicity treatments did not significantly alter the growth and development of plants. Where nutrient solution was in contact with the growing point of the cucumber plant, toxic levels of Zn significantly influenced the natural growth and development of the plants (Figure 26), resulting in stunted and necrotic plants. The Zn8 plant illustrated was more severely damaged by contact with the solution as it was continually wet whilst the Zn16 plant was only periodically wettened by the solution.
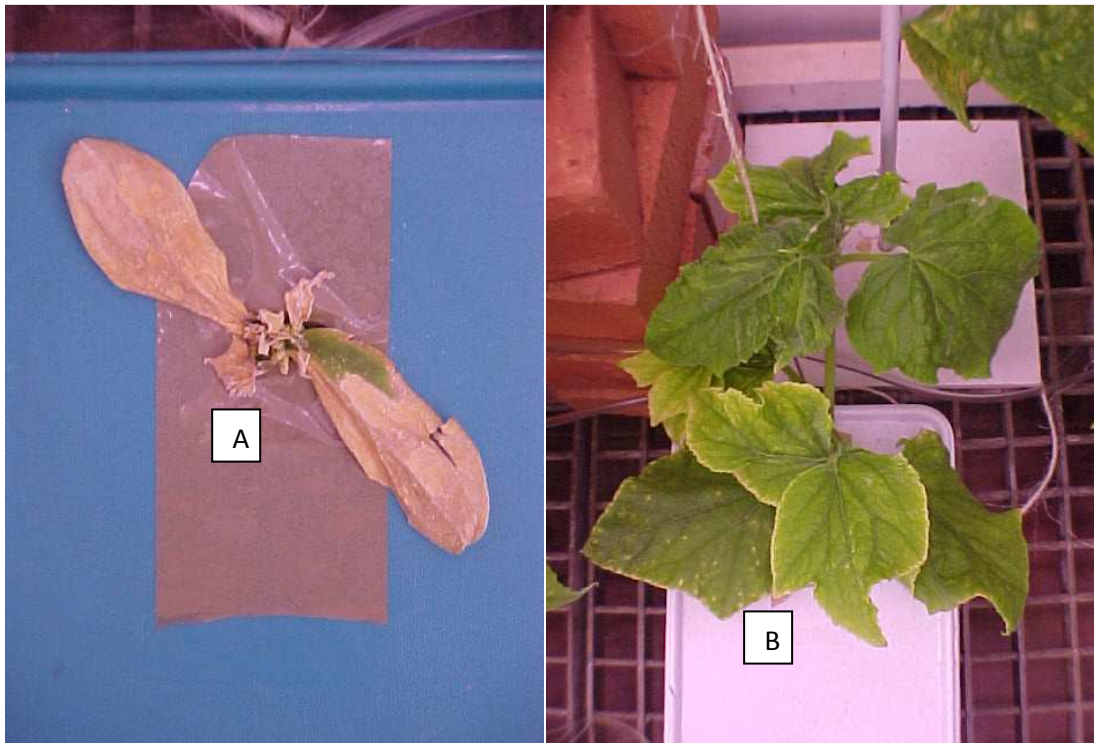
Figure 26. Impact of toxic Zn nutrient solution on the growing points of the plants for extended periods (Zn8 (plant A) and Zn16 (plant B))

The Mn toxicity (Mn Tx 1 and Mn Tx 2) experiments showed significant differences, firstly between treatments, but more noticeably between experiments (Figure 27). Unlike in the deficiency experiment, Si applications tended to significantly improve leaf growth as recorded by leaf width. Application of Si resulted in a tendency to increased leaf width in older and younger leaves across treatments. No other common significances were found in Mn Tx 1 with these measurements. Unlike Mn Tx 1, Mn Tx 2 did not reveal any significant differences between Si applications on leaf width. Significant differences between leaf width measurements were observed though where increasing levels of Mn significantly reduced the growth of the leaves.
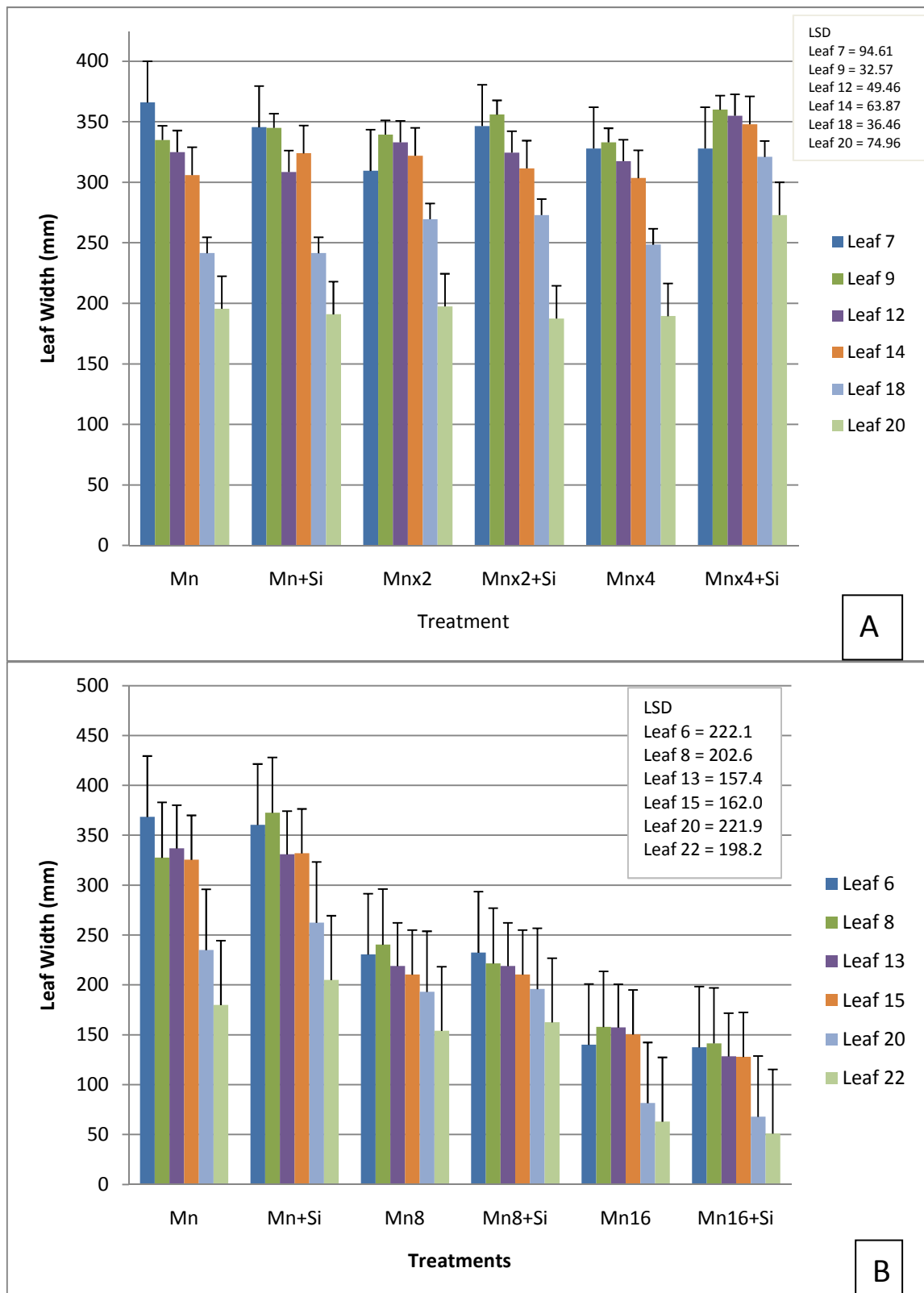
Figure 27. Differences in leaf width at experiment termination of Mn Tx 1 (A) and Mn Tx 2 (B) experiments (Mn applications increasing from recommended to 2, 4, 8 and 16x recommended rates) of cucumber cv Tyria plants

Leaf curl was also prevalent in the Mn Tx 1 and Mn Tx 2 experiments. The greatest prevalence of leaf curl was found in the largest leaves, leaf 7 (Figure 28). In general though, Si applications did not appear to affect the amount of leaf curl, except at very high dosages of Mn, Si addition substantially reduced curling.
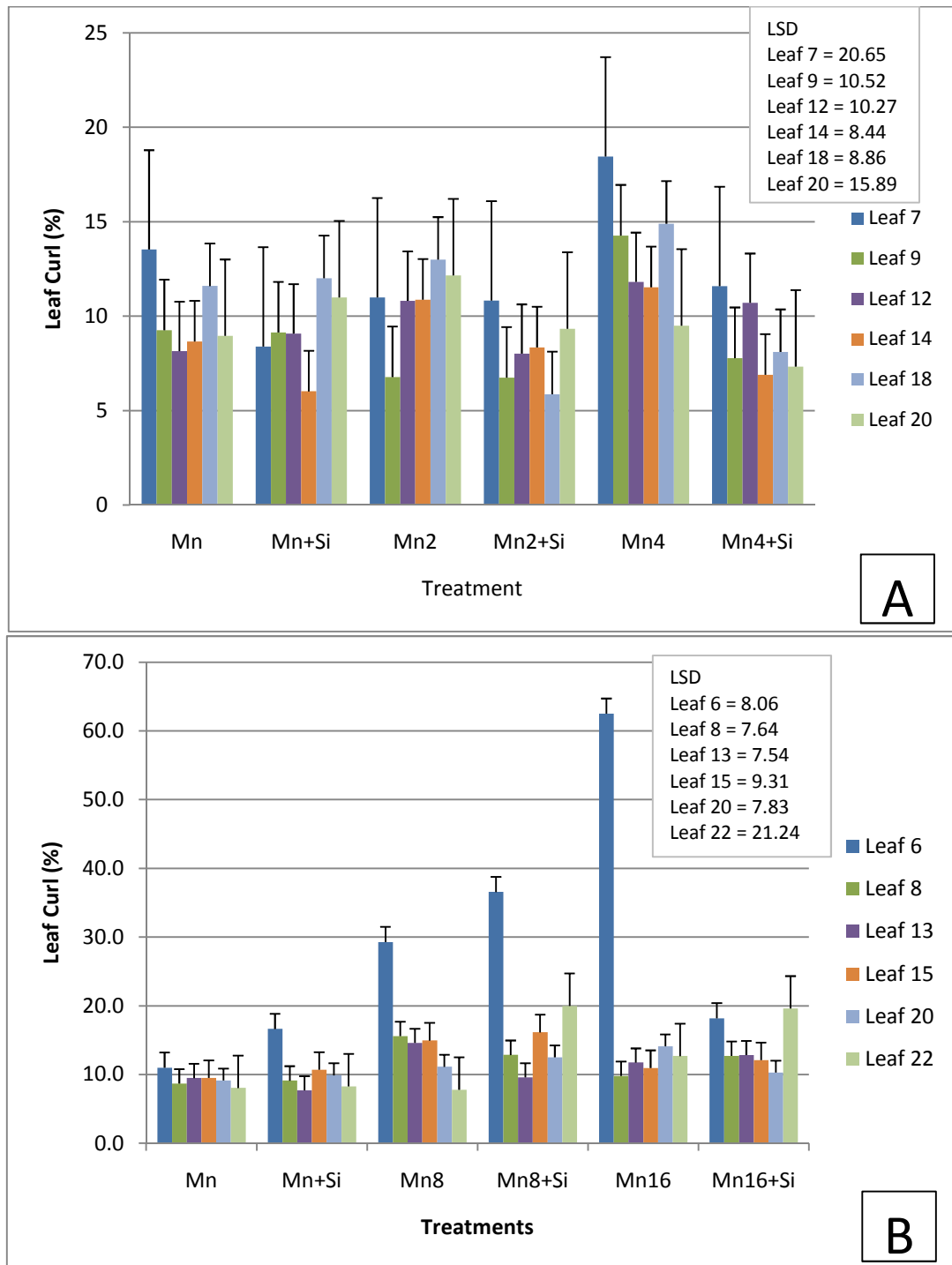
Figure 28. Percentage leaf curl in Mn Tx 1 (A) and Mn Tx 2 (B) (Mn applications increasing from recommended to 2, 4, 8 and 16x recommended rates) of cucumber cv Tyria plants

Scored ratings were based on qualitative features of the leaves and their general appearance and health. Scored ratings provide a measure of the condition of the plants and the impact of the application of the treatments. The scores also show the limited impact of the toxic applications in Mn Tx 1 (Figure 29). Higher scores were awarded to older leaves of the plant and no significant differences were found between Si or Mn applications. The damage and health of the leaves was far worse in Mn Tx 2 showing the severity of the impact of the very high levels of Mn to the plants (up to 16x of recommended Mn rates). Only on leaf 6 Si appeared to improve the condition of the leaf at the highest level of Mn application. This trend did not continue for any other treatment or leaf. Manganese treatments significantly affected ratings, where higher scores were awarded with increasing levels of Mn. Younger leaves (from leaf 20 onwards), did not show the same impact of Mn on leaf condition (not significant).
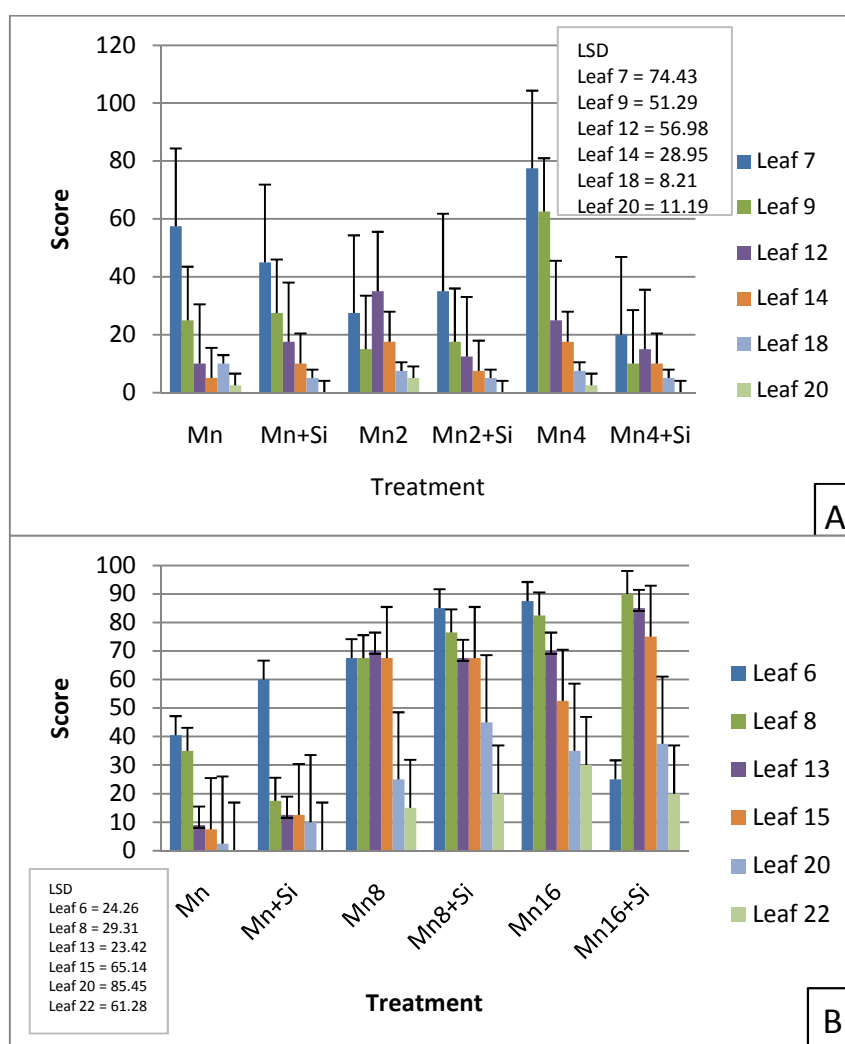
Figure 29. Scored ratings of Mn Tx 1 (A) and Mn Tx 2 (B) of cucumber cv Tyria leaves (Mn= recommended Mn, Mn + Si= recommended Mn plus Si, 2, 4, 8 and 16x recommended Mn rates), where 0 is healthy and 100 is dead

Zinc toxicity experiments clearly showed the impact of the higher levels of Zn on the growth of the leaves. Increasing Zn applications to toxic levels (16 times of standard application) further impacted leaf growth and development (Figure 30). On some leaves, the application of Si significantly increased leaf width, too; this, however, was not consistent in all leaves examined, occurring in leaves 11 and 15 only, and not at the highest levels of Zn application.
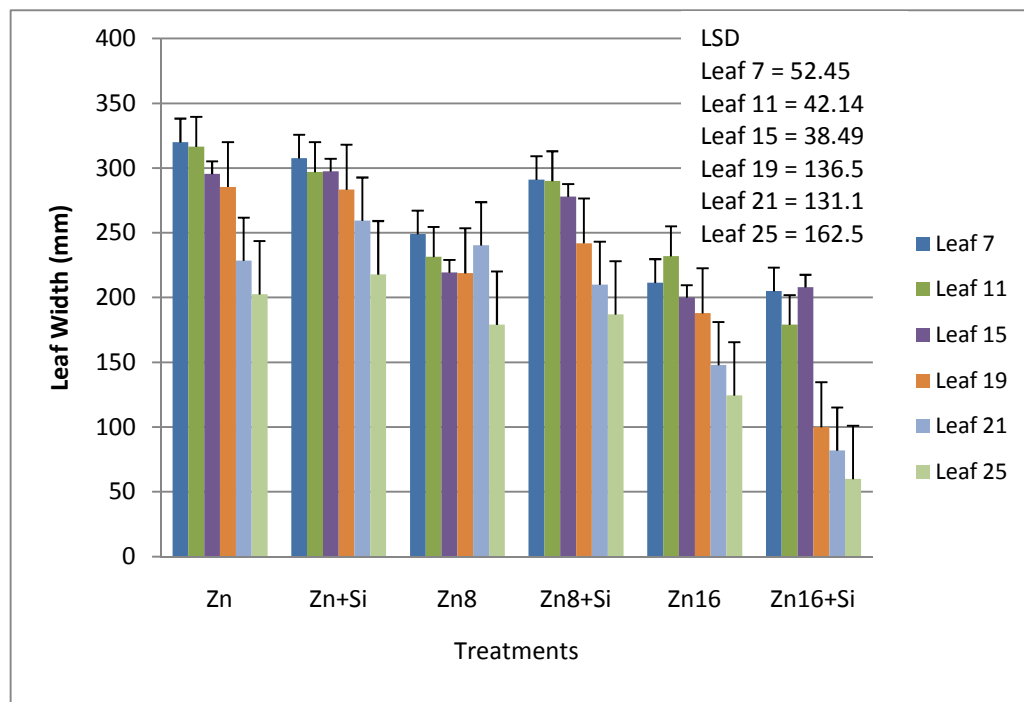
Figure 30. Leaf width at experiment termination of Zn toxicity experiment (recommended, 8 and 16x of recommended Zn rate) of cucumber cv Tyria plants

There were no significant differences between Zn applications in the Zn toxicity experiment for the amount of leaf curl recorded. Plants supplied with excessive Zn concentrations displayed similar leaf curl, regardless of application of Si. Leaf scores recorded at the same time also did not reveal any significant differences between treatments and on leaves of different ages. The scored results took into account leaf defects and necrotic spots or leaf senescence, and not purely leaf chlorosis. Whilst this reflected the health of the plant, Zn leaves tended to remain healthy in appearance despite the general leaf chlorosis.

Unlike in the deficiency experiment, Mn Tx 1 did not show significant differences between treatments; however, further increasing Mn levels (Mn Tx 2) resulted in a significant reduction in plant height with the greatest reduction observed in the highest application of Mn (Mn16) (Figure

31). Similarly, excessive levels of Zn also negatively impacted the growth of cucumber plants (Figure 32).
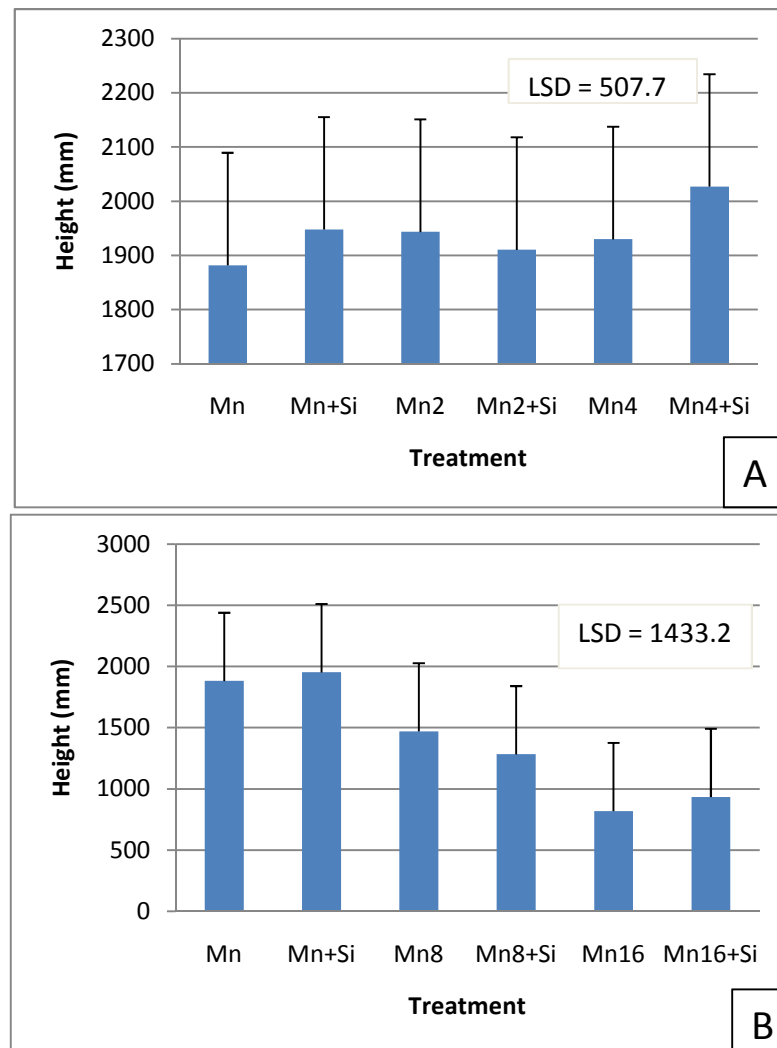
Figure 31. Impact of increasing levels of Mn from recommended to 2, 4, 8 and 16x of recommended rates on plant height in the Mn Tox 1 (A) and Mn Tox 2 (B) of cucumber cv Tyria plants
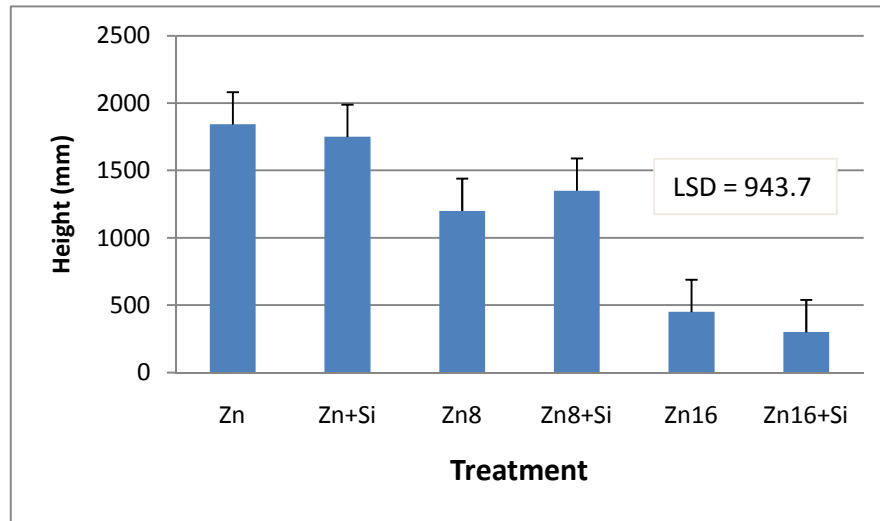
Figure 32. Effect of increasing levels of Zn application from recommended to 8 and 16x of recommended rate of cucumber cv Tyria plants on plant height

Measurement of fresh and dry mass (FM and DM) at the termination of the toxicity experiments showed that the lower Mn amendment, Mn Tx 1, did not result in any significant differences between treatments (Figure 33), unlike Mn Tx 2, which exhibited significant differences between the Mn treatments (Figure 34). Increasing Mn levels to 16 x standard application significantly reduced both, top growth (Leaf FM and DM) and root growth (Root FM and DM), but had no significant effect on the development of the fruit on the plants. Silicon applications did not significantly impact the mass of the plant, or affect the moisture content of the tissue.
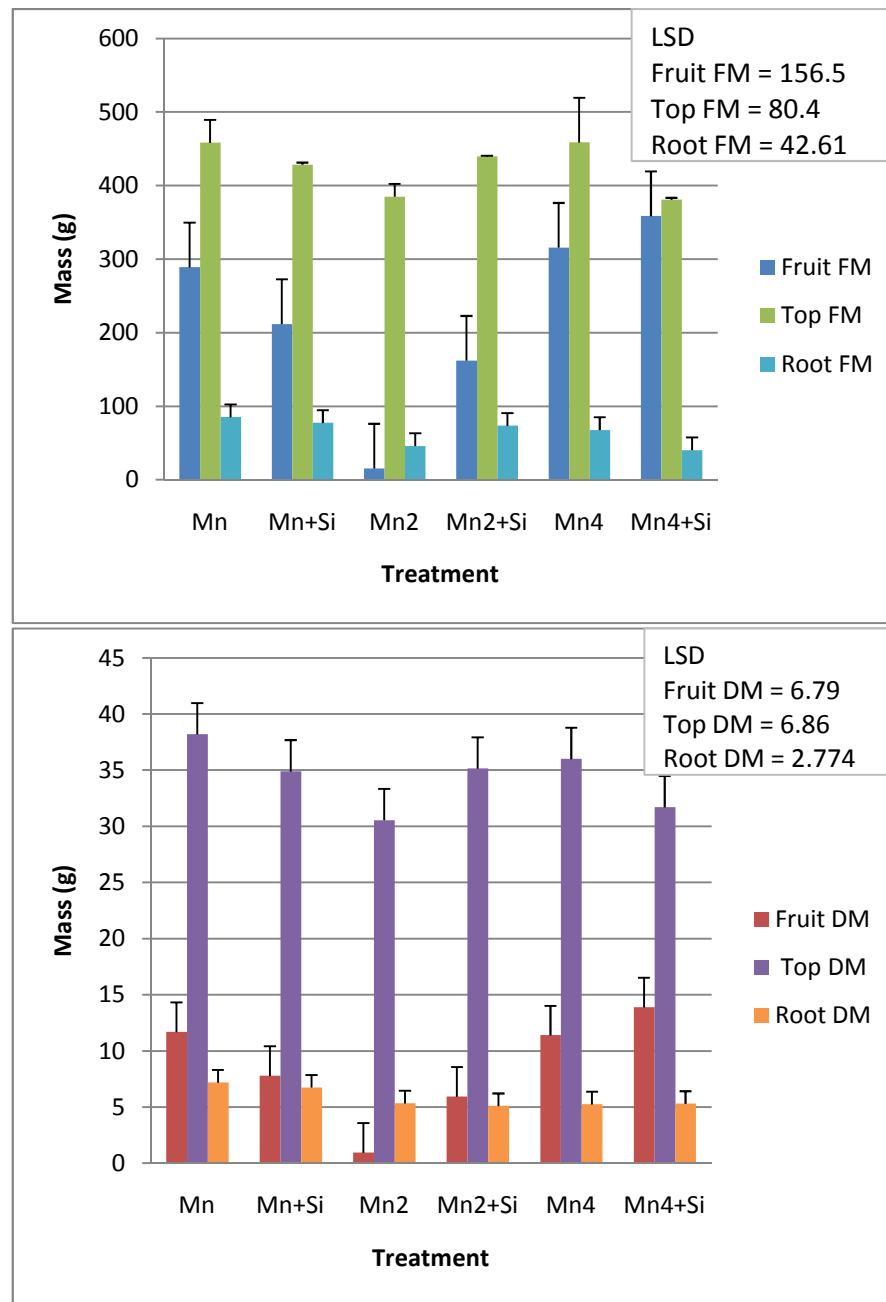
Figure 33. Fresh mass (FM) and dry mass (DM) for various plant parts at the termination of Mn Tx 1 experiment of cucumber cv Tyria plants (Top = above solution portion, Fruit = harvested fruit, Root = below solution level portion)
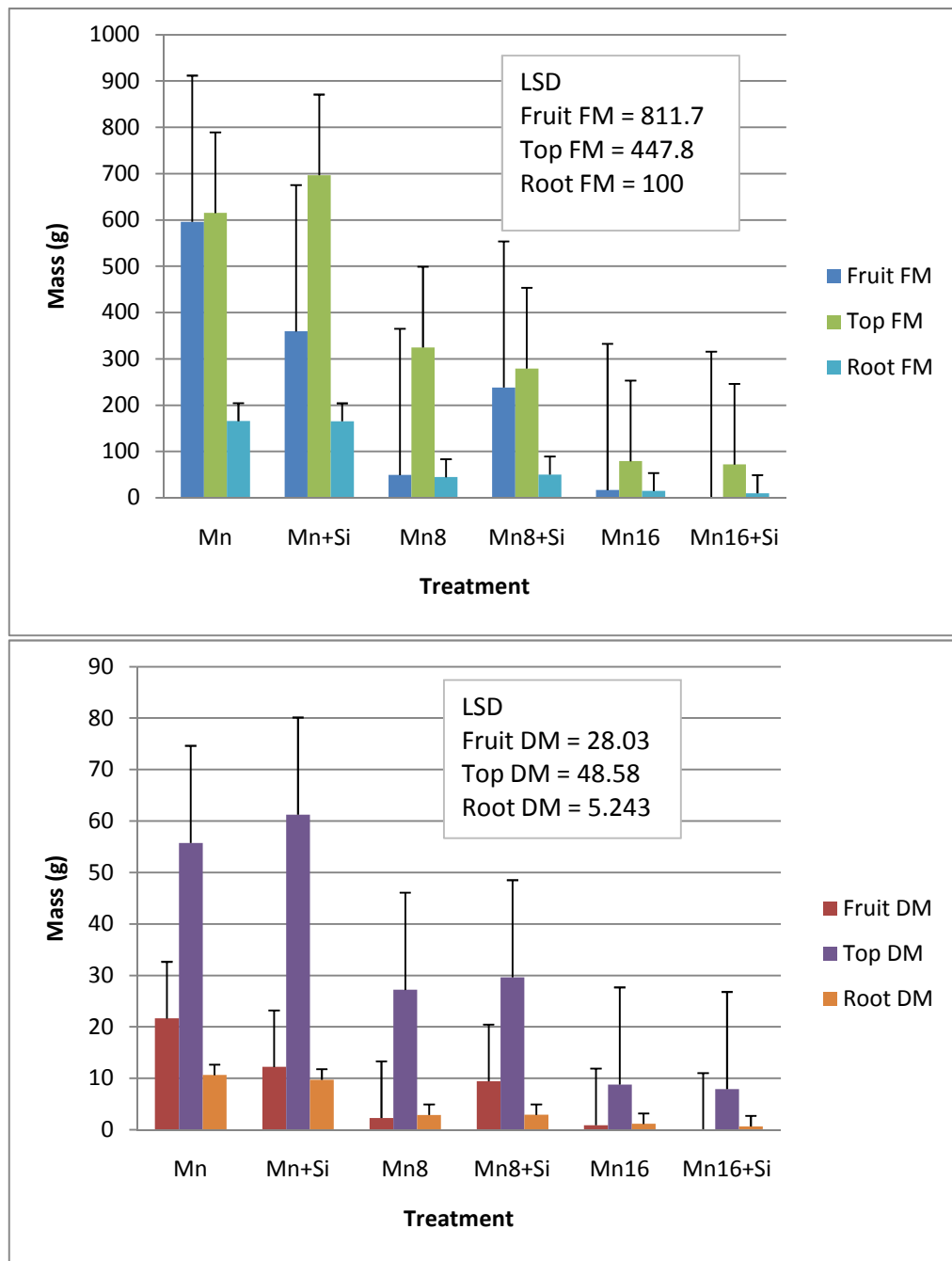
Figure 34. Fresh mass (FM) and dry mass (DM) for various plant parts at the termination of Mn Tx 2 experiment of cucumber cv Tyria plants (Top = above solution portion, Fruit = harvested fruit, Root = below solution level portion)

Toxic Zn applications significantly reduced top and root FM and DM but had no significant effect on the FM and DM of the fruit. Si applications had no significant effect on FM or DM of any plant part and there was no interactive effect between Zn and Si (Figure 35).

As with the other measurements conducted on Mn Tx 1, no significant differences were found between the treatments on fruit count, whether the fruit was developing or matured (Figure 36). Unlike the first experiment and the deficiency experiment, in Mn Tx 2 fruit aborted. Fruit failed to develop once the flower had senesced and entered into senescence. The number of aborted fruit was also recorded, however, no significant differences between different toxicity levels were observed. There were also no significant differences between the treatments, application of increasingly toxic levels of Mn and / or the addition of Si to the treatment, on the number of developing fruit, or the number of fruit that were harvested.
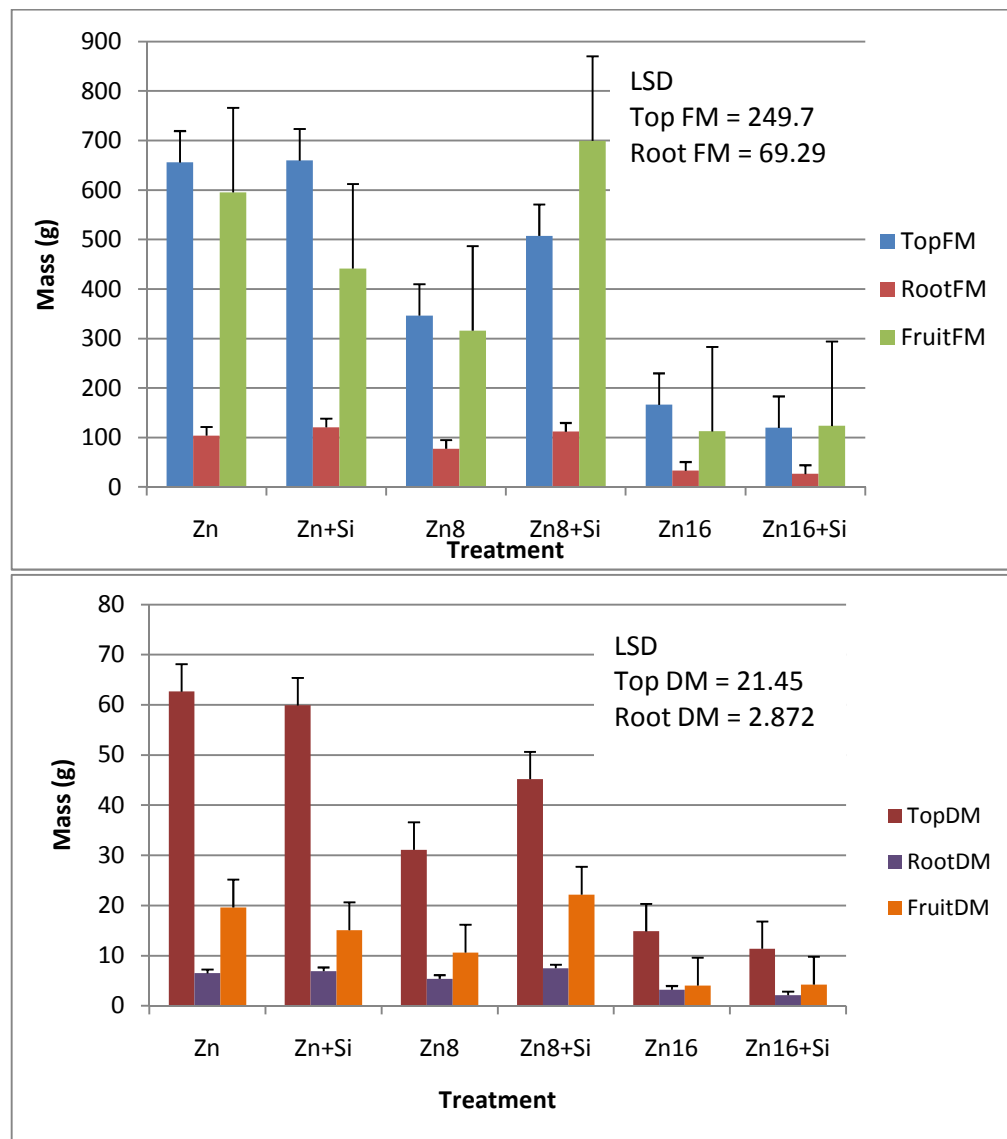
Figure 35. Fresh mass (FM) and dry mass (DM) of various plant parts at the termination of Zn toxicity experiment of cucumber cv Tyria plants (Top = above solution portion, Fruit = harvested fruit, Root = below solution level portion)
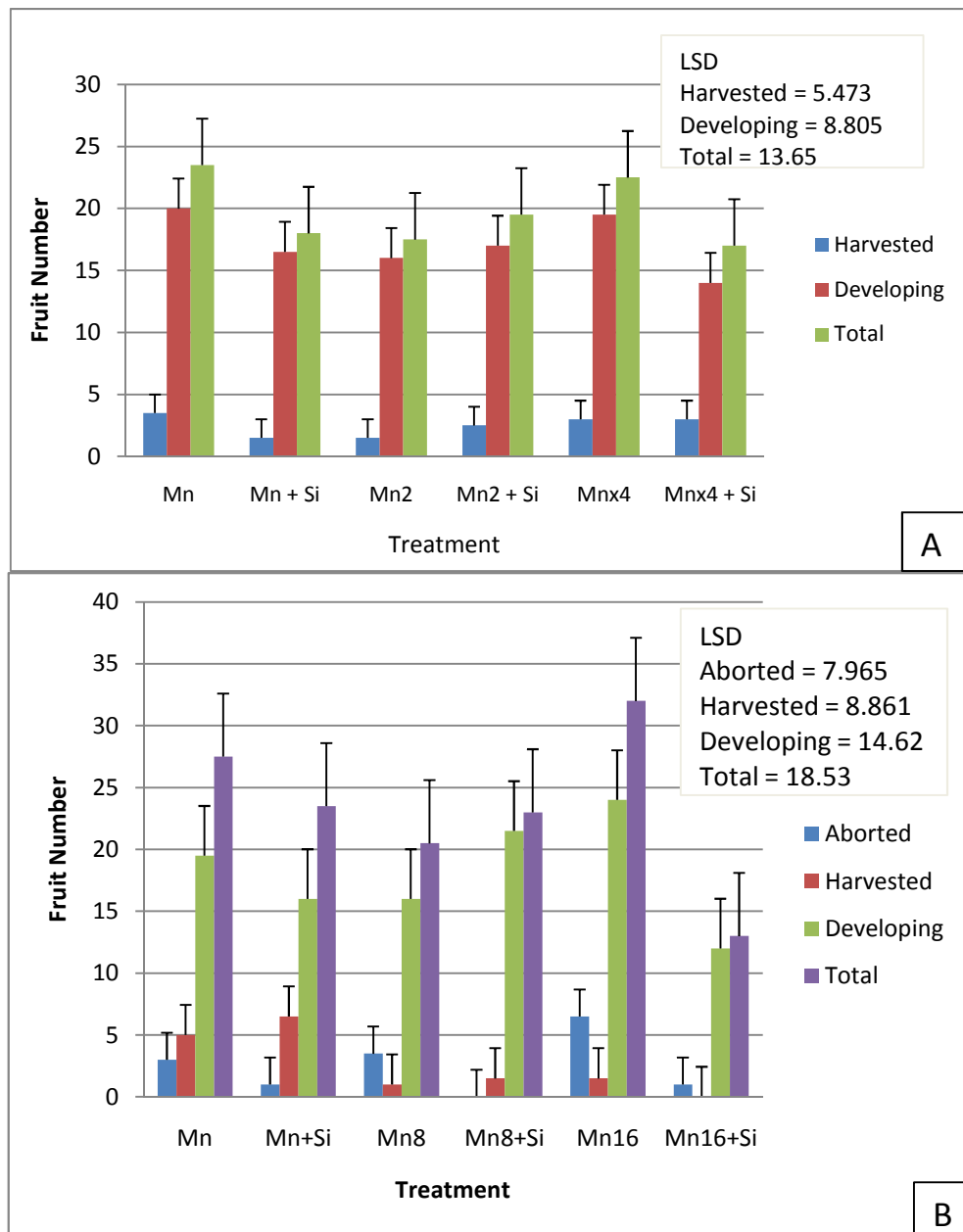
Figure 36. Number of developing, mature and aborted fruit in Mn Tx 1 (A) and Mn Tx 2 (B) experiments of cucumber cv Tyria plants

As in Mn Tx 2, fruit abortion also occurred within the Zn toxicity experiment; however, no significant differences wre observed between the treatments of Zn or Si (Figure 37). Although the total number of fruit and the number of harvested fruit did not differ significantly between treatments, increasing the Zn supply to the plants increased the number of developing fruit until termination of the experiment. The number of aborted, matured or harvested fruit had a tendency to be higher in the adequately supplied Zn solution (Zn and Zn+Si), although not significantly so.
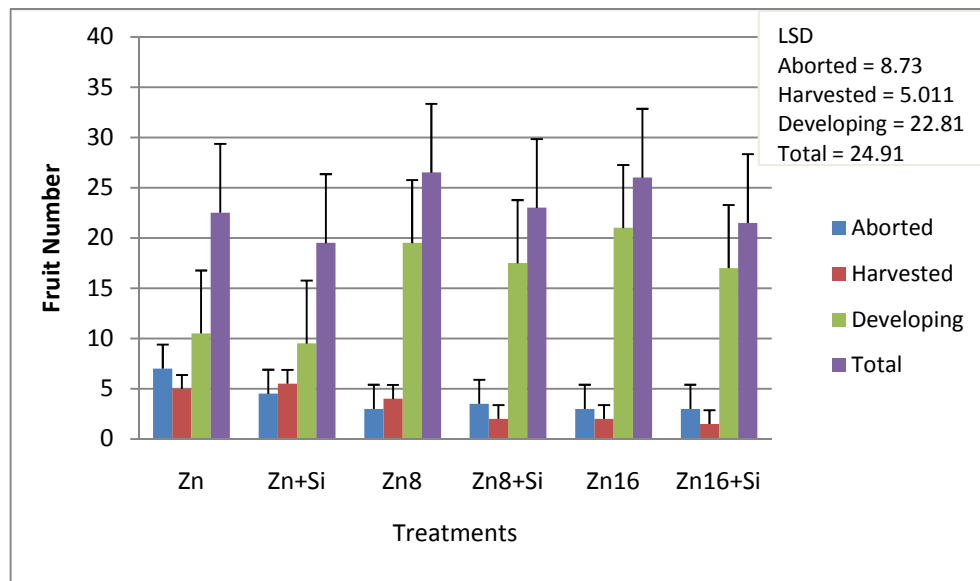
Figure 37. Number of developing, mature and aborted fruit in Zn toxicity experiment of cucumber cv Tyria plants

In the Mn toxicity (Mn Tx 1 and Mn Tx 2) experiments, increasing levels of Mn increased the scores of the plants (Figure 38). In Mn Tx 1, the plants only showed signs of deterioration and were rated from Day 28, whilst in Mn Tx 2, ratings began as early as Day 1. Significant differences were observed between treatments on different days, however, significant differences were more prevalent in the second experiment. In Mn Tx 1, the applications of both, Mn and Si, significantly improved the condition of the plant as an interactive effect, but no significant differences were recorded for just Mn or Si applications. In Mn Tx 2, Mn applications significantly affected the condition of the plant, where increasing levels lead to poorer visual plant condition. Silicon did not have the same impact on the visual condition of the plants in Mn Tx 2. Due to the toxic nature of excessive applications of Mn on the plant, the condition of the plant was negatively impacted, which in turn lead to reduced plant health and plant life longevity.

With increasing plant age, plant condition scores increased (Figure 39). Although increasing Zn applications produced higher scored ratings, the differences between the treatments were not significant ($p=0.05$), where differences were at a level of $p=0.1$, outside of the range required for significance in this experiment. The biggest differences were recorded over the first 4 days of scores (Day 16, Day 19, Day 22, and Day 25). Increasing Zn applications did not significantly impact on the visual condition of the cucumber plants.

52
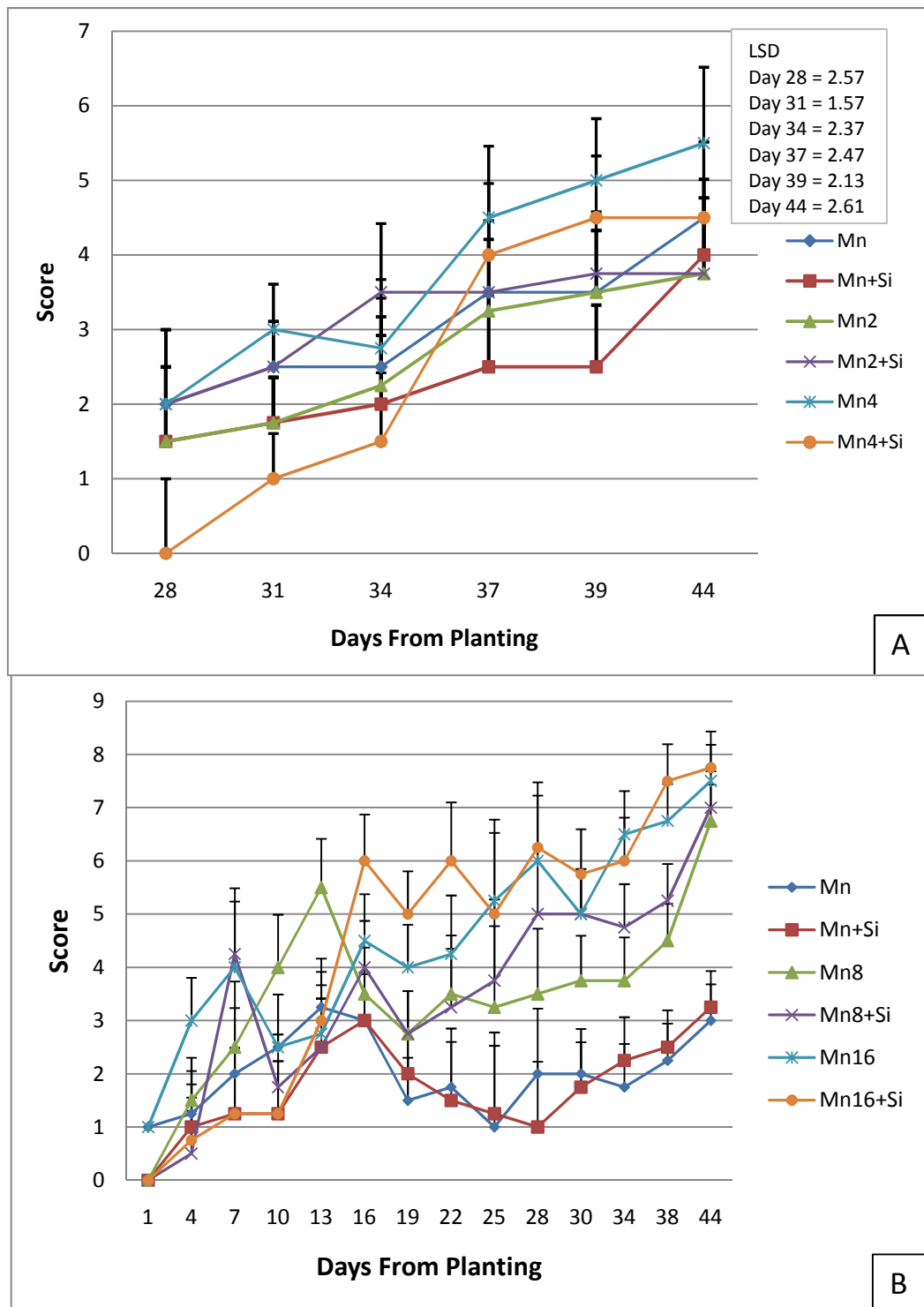
Figure 38. Visual (objective) scored ratings of plant condition for Mn Tx 1 (A) and Mn Tx 2 (B) experiments (0 = healthy and 10 = dead plants) of cucumber cv Tyria plants
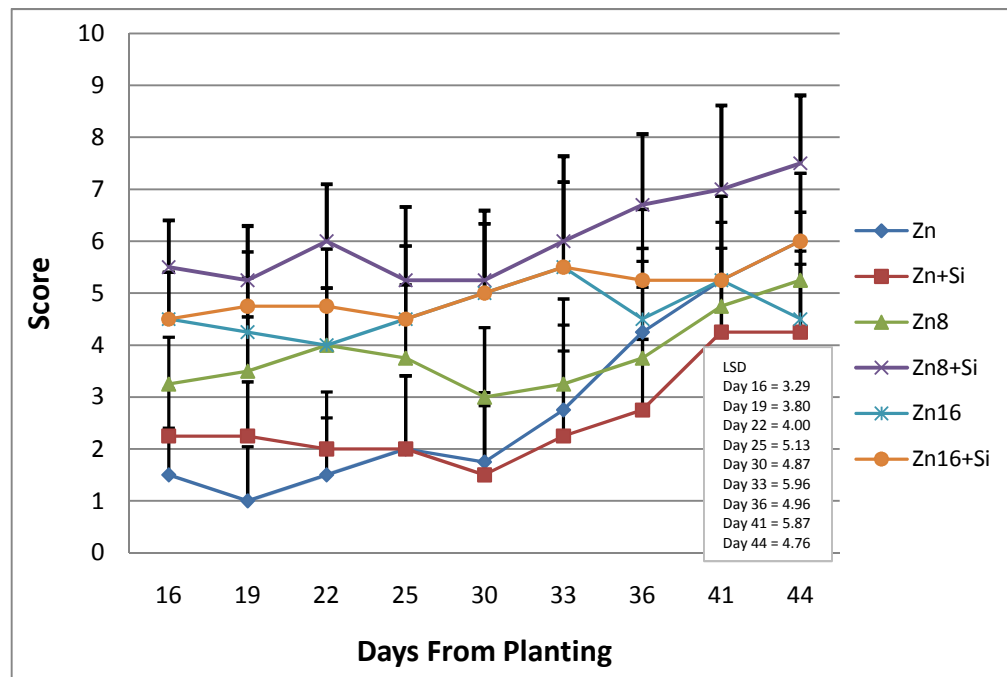
Figure 39. Scored rating of plant condition for Zn toxicity experiment (0 = healthy and 10 = dead plants) of cucumber cv Tyria plants

Mn Tx 1 showed some significant differences in the alteration of the solution pH with time, but this was also not consistent and appeared to be random (Figure 40). There was more variation between treatments in Mn Tx 2, particularly, as plants got older. Only two significant differences were recorded for changes 4 and 10, again suggesting that these changes are random and not part of a trend, (graph B, Figure 40). Water and nutrient solution use by the plants also did not have any impact on the pH change of the nutrient solution over time.

The Zn toxicity experiment illustrated the same trends as the other experiments with no consistent significant differences observed between treatments over time. Random significant differences such as in change 8 and 10 do not portray trends or translate to meaningful or inferable results. Again, water or nutrient solution uptake and use did not have any impact on the change in solution pH (Figure 41).
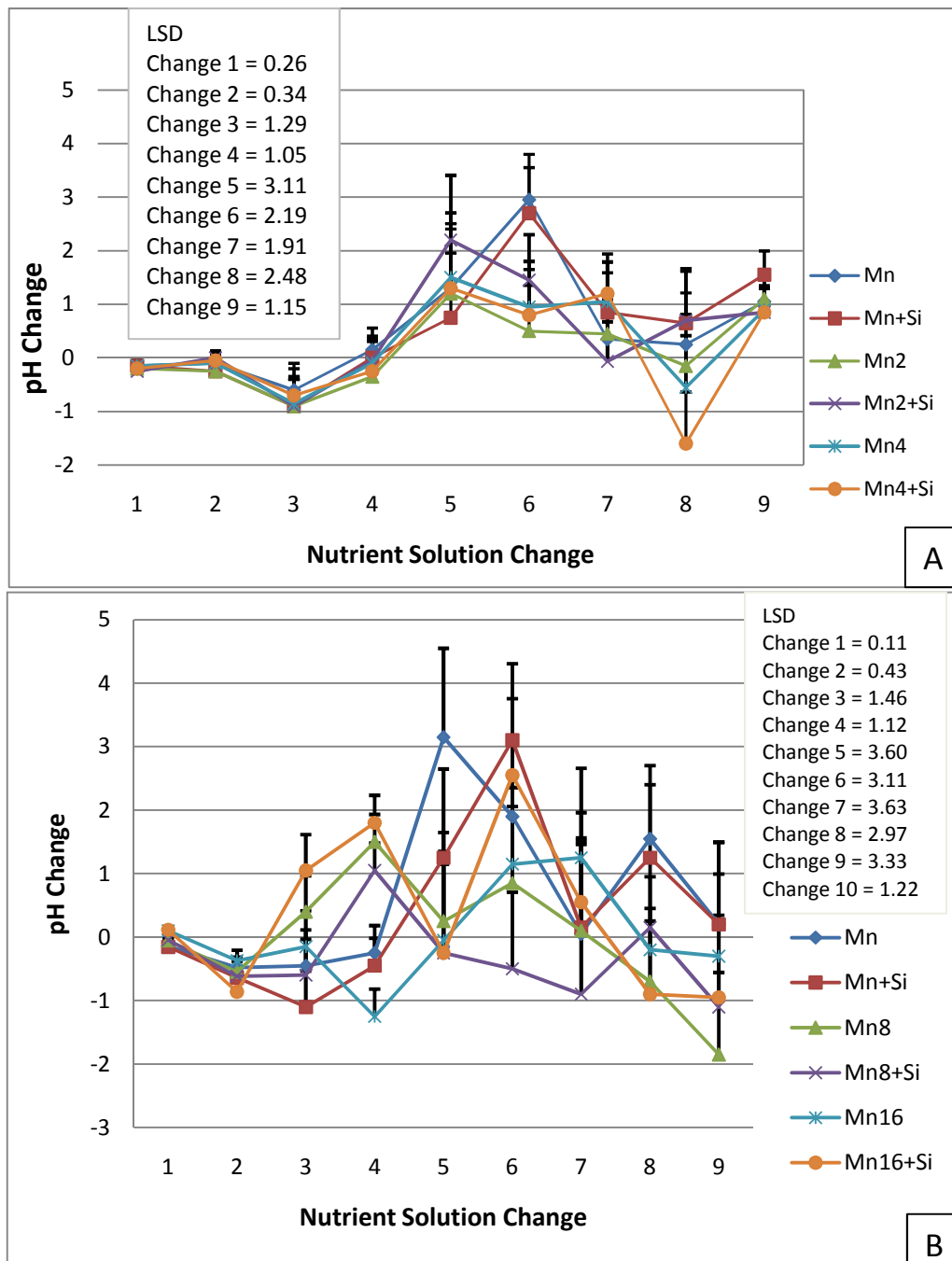
Figure 40. Changes in pH between each solution change recorded for Mn Tx 1 (A) and Mn Tx 2 (B) experiments
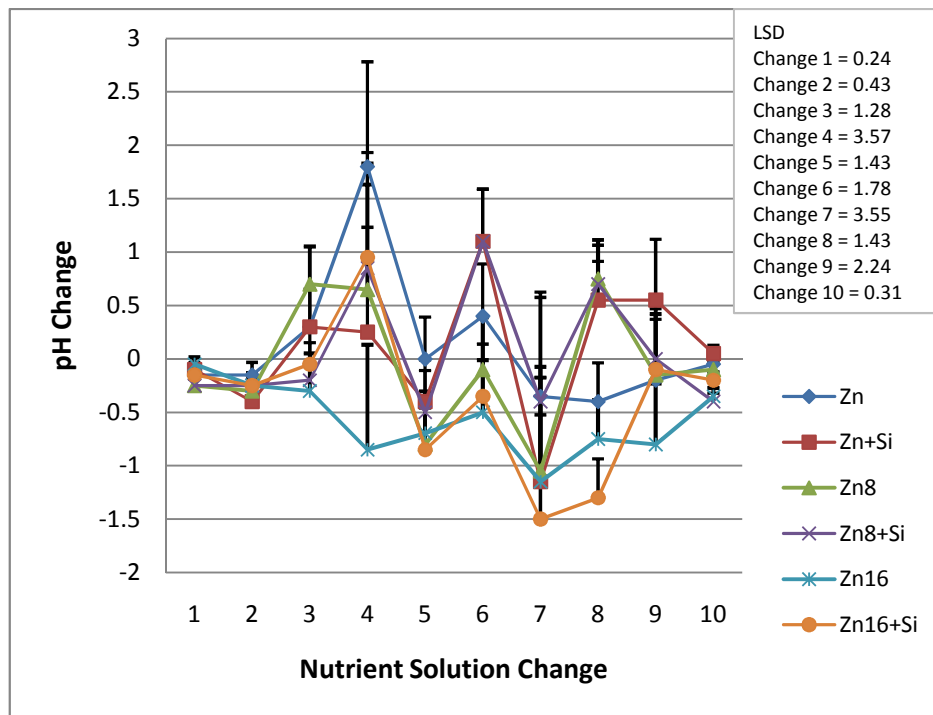
Figure 41. Changes in pH between each solution change recorded for the Zn toxicity experiment

Since the application of excessive levels of Mn in Mn Tx 1 did not show many significant effects on the growth of the plants, very few significant changes were expected in the change of EC within the same experiment. Although large EC variations were observed in this experiment (Figure 42), the only significant differences between treatments were observed at Change 7. The general trend observed for the first Mn experiment was a gradual increase in solution EC with time and between changes coinciding with increasing size of the plants. Very few significant differences were observed between the treatments.
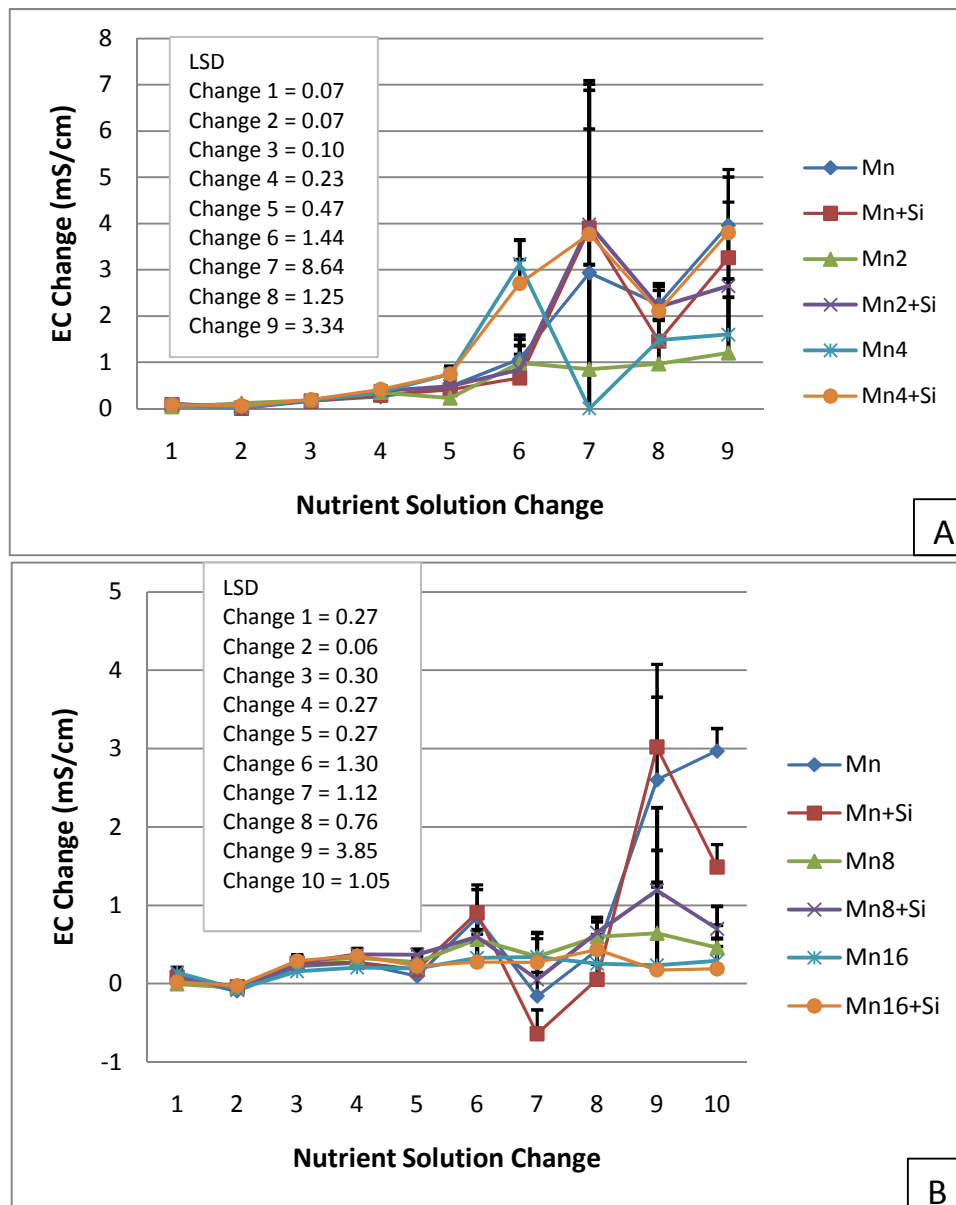
Figure 42. Changes in EC between solution changes for Mn Tx 1 (A) and Mn Tx 2 (B)

The Mn Tx 2 experiment showed significant differences between the treatments on EC measurement (Figure 42, graph B), for changes 5, 8 and 10. Unlike Mn Tx 1 and the deficiency experiments, the amount of water used by the plants impacted the EC of the solution significantly. Water use was, therefore, used as a covariate in the analysis to allow for direct comparison between the treatments without the impact of water loss from the solution affecting the result. The high levels of Mn applied in Mn Tx 2 significantly, and negatively, impacted on the growth of the plants resulting in reduced solution uptake and consequently limited the effect on solution EC.

Unlike Mn Tx 2, water use did not significantly affect EC changes in the Zn toxicity trial and, therefore, was not used as a covariate in the analysis. As with the other experiments, with increasing

plant size, their need for more solution (nutrients and water) increased, subsequently increasing changes in EC were observed for this developmental period. Silicon did not effect any significant change in EC, whether through increased nutrient or water uptake from the nutrient solution (Figure 43).
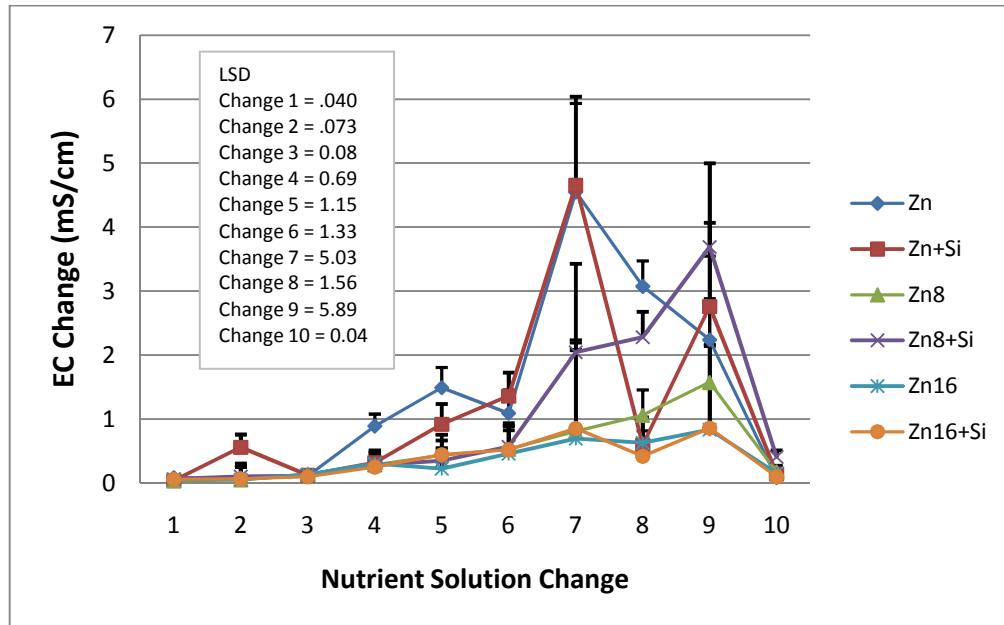
Figure 43. Changes in EC between solution changes for the Zn toxicity experiment.

The presence of Mn in the nutrient solution significantly increased the uptake of Si from the nutrient solution, although Si in the solution was purely a contaminant. This indicates the requirement of the cucumbers for Si as a nutrient which is absorbed from the nutrient solution. The presence of Si as a contaminant, even in very low quantities could reduce the efficacy of the experiments. However, the Si treated plants showed increased responses over that of the untreated plants indicating that the contaminant Si was still insufficient to meet the needs of the plants. The only nutrient to exhibit daily uptake differences in Mn Tx 1 was Mn (Table 5). This increased daily Mn uptake indicates, to some extent, the luxury consumption of the plants, whereby the presence of extra Mn does in turn result in increased uptake by the plant. The uptake is in proportion to the increase in application rate, where Mn2 values are double the Mn values for daily uptake by the plant and Mn4 are 4 times the level for the standard Mn solutions. At this stage, no relationship with Si or Zn is exhibited. The interactive effect with Zn may only be present under deficient conditions. As with the deficiency experiment, water uptake played a large, significant role in nutrient uptake. It was, hence, necessary to use water uptake as a covariate in the analysis.

Table 5. Daily nutrient uptake of Mn Tx 1 plants (recommended, 2 and 4 x the recommended application rates for Mn plus Si application) (* p≤0.05)

Treatment	Daily Mn Uptake (ppm)
Mn	53.6
Mn+Si	50.6
Mn2	104.4 *
Mn2+Si	110.9 *
Mn4	199 *
Mn4+Si	199.1 *

Mn Tx 2 exhibited more differences in the daily uptake of nutrients, of Ca, Mn and Zn (Table 6). However, no effect on Fe was apparent. With increasing Mn applications, the uptake of Ca and Mn by the plants significantly increased, whilst there was a negative effect on the uptake of Zn resulting in reduced Zn uptake at high Mn applications. The uptake of both, Ca and Zn, was significantly affected by the amount of water taken up by the plant, hence, water use was again used as a covariate in the analyses. The uptake of Mn by the plants, on the other hand, was not affected by the amount of water taken up by the plant.

Table 6. Daily nutrient uptake of Mn Tx 2 plants (recommended, 8 and 16 x the recommended application rates for Mn plus Si application) (* p≤0.05, ** p≤0.01)

Treatment	Daily Uptake (ppm)		
	Calcium	Manganese	Zinc
Mn	114.8	360	151.4
Mn+Si	110.4	360	153.1
Mn8	127**	2265**	133.7*
Mn8+Si	119.8*	2133**	133.7*
Mn16	131.1**	4076**	124.8**
Mn16+Si	131.9**	4167**	121.2**

Magnesium, Zn and K showed significant changes in their uptake by the plants with increasing levels of Zn (Table 7). As with the toxic applications of Mn, excess of Zn in the nutrient solution increased Zn uptake, independent of the uptake of water by the plant. High levels of Zn appeared to negatively impact Mg and K uptake by the plant. At higher rates, the uptake of Mg and K by the plant was significantly reduced. Zinc did not significantly affect the uptake of Mn by the plant when applied at high levels, unlike the high levels of Mn limiting the uptake of Zn. The relationship between these two nutrients is more complex, particularly in the way their uptake interacts with each other.

Table 7. Daily nutrient uptake of the Zn toxicity experiment plants (recommended, 8 and 16 x recommended rates of Zn application plus Si application, * p≤0.05, ** p≤0.01)

Treatment	Daily Uptake (ppm)		
	Magnesium **	Zinc *	Potassium **
Zn	6.231	15.44	38.89
Zn+Si	6.243	16.64	37.5
Zn8	3.806	64.84	24.27
Zn8+Si	3.651	67.14	22.47
Zn16	3.13	61.6	19.52
Zn16+Si	2.436	76.56	16.35

The application of Si to the plants did not show a significant difference between treatments (Figure 44). With increasing levels of Mn, up to toxic levels (Mn16), the concentration of Mn within the plant tissue increased significantly, corresponding to the increased uptake of Mn by the plant. With increasing levels of Mn applied, plant tissue contained significantly ($p≤0.01$) higher Fe levels; this is contradictory to visual symptoms of Fe deficiency. Despite the reduced uptake of Zn by the plants with increasing levels of applied Mn, the Zn concentration of such plants was significantly higher ($p≤0.05$).
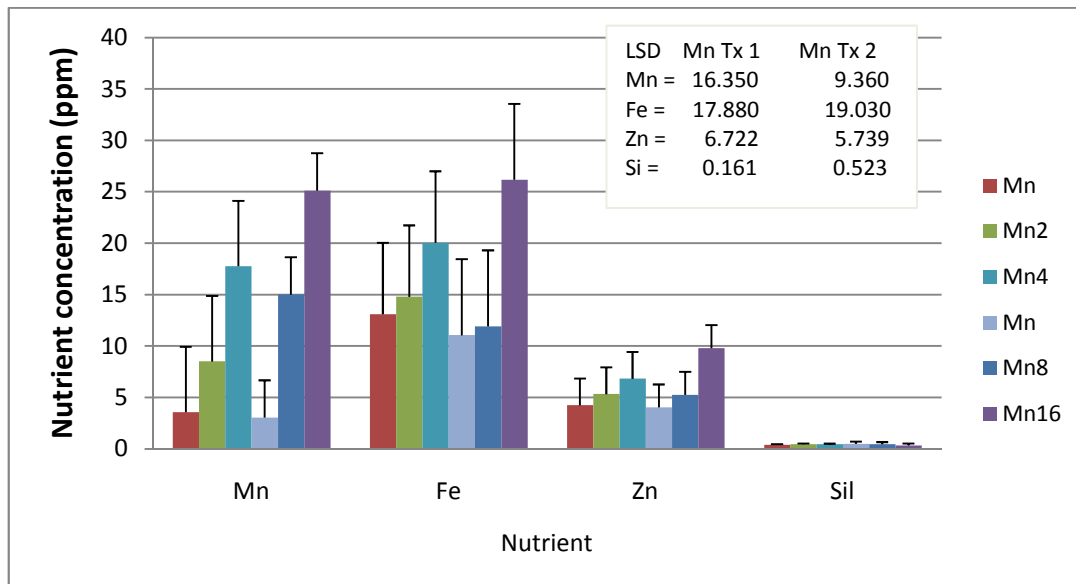
Figure 44. Nutrient concentration of cucumber plants cv Tyria of both Mn toxicity experiment (only nutrients which differed significantly between treatments are presented)

Only in the Zn toxicity experiment was significantly higher Si levels were observed in the plant tissue (Figure 45) following application. The only other nutrient to exhibit significant differences between Zn treatments was the Zn application ($p≤0.05$). With increasing levels of Zn in the nutrient

solution, significantly higher levels of Zn were recorded in the plant tissue. Although some differences were observed in Mn and Fe, these were not significant ($p>0.05$).
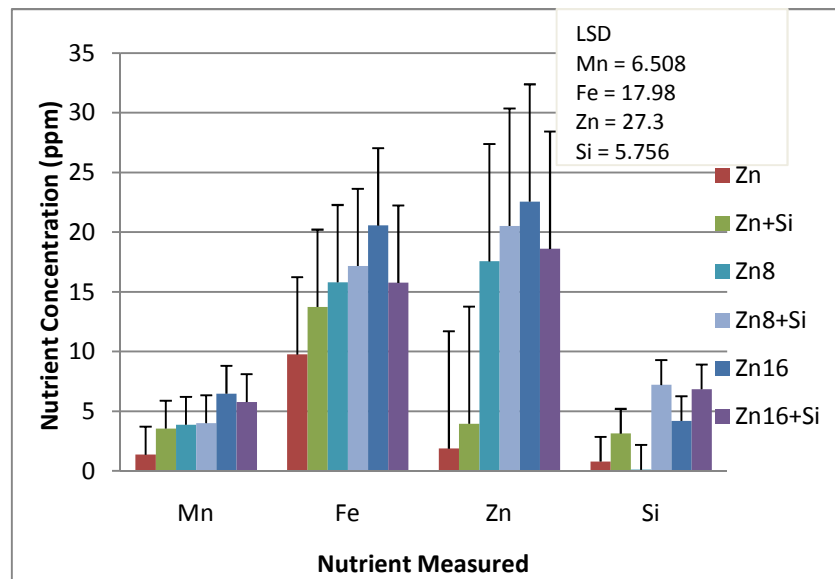
Figure 45. Nutrient concentration of cucumber plants cv Tyria of the Zn toxicity experiment (only nutrients which differed significantly between treatments are presented)

Although Mn Tx 1 was supposed to be applications of excessive or toxic amounts, it seemingly resulted in luxury consumption and had no significant effect on the make-up of the chlorophylls or carotenoids in this experiment. Although the trends showed an increasing level of chlorophylls and total carotenoids with increasing the Mn application to Mn2, further doubling of the Mn (Mn4) resulted in a tendency of a reduction in these pigments (Figure 46). Individual evaluations between treatments and comparisons with least significant difference tests (LSD's) (Steel & Torrie, 1980) (Table 8), showed only total carotenoids (C_{x+c}) to be significantly different between treatments ($p \le 0.05$). The only 2 treatments exhibiting significant differences between each other were Mn and Mn2, where Mn2 had significantly higher levels of total carotenoids (C_{x+c}).
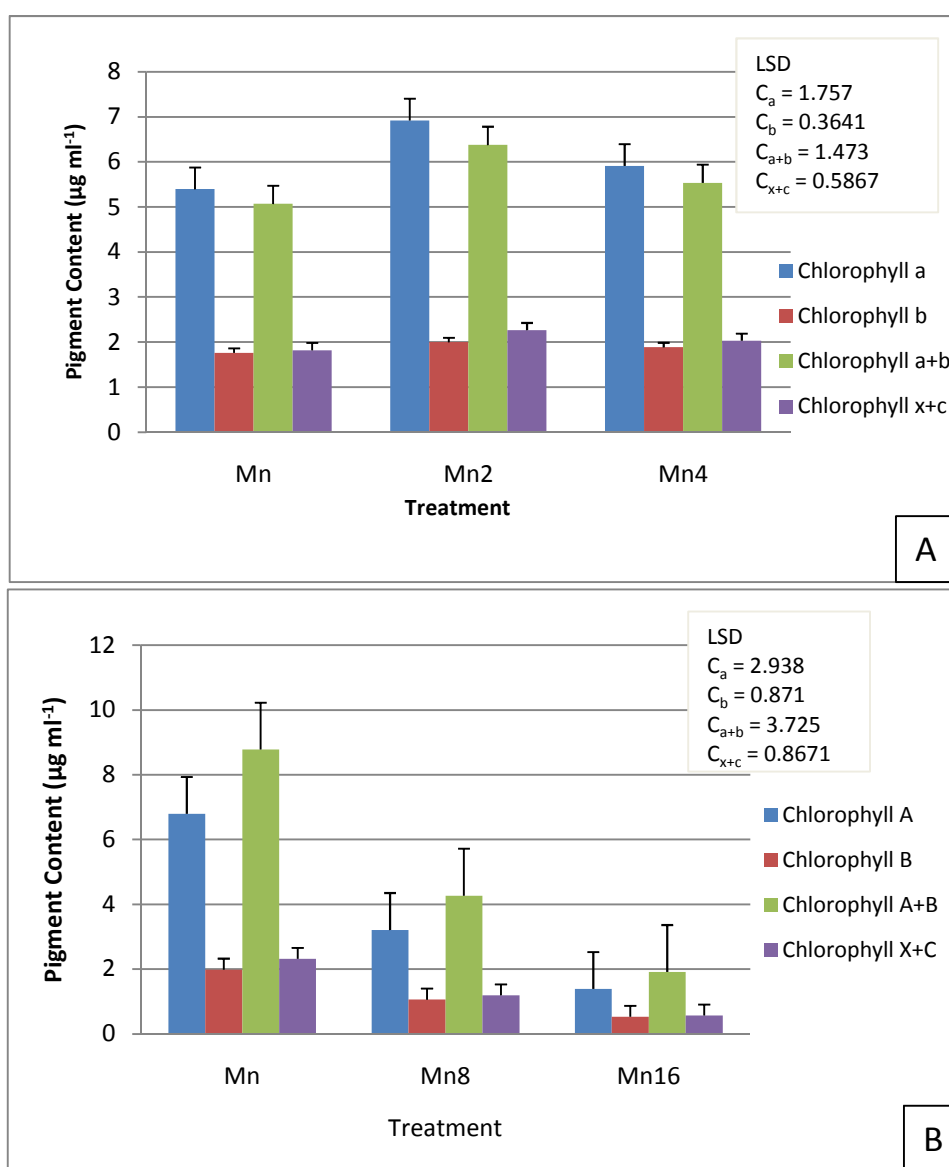
Figure 46. Chlorophyll and carotenoid concentration as impacted by increasing Mn levels from recommended (Mn) to toxic amounts (Mn2, Mn4, Mn8 and Mn16) on cucumber plants cv Tyria (Mn Tx 1 and Mn Tx 2, A and B respectively)

Table 8. Least Significant Difference (LSD) test on total carotenoids in cucumber plants of Mn Tx 1

Treatment	Chlorophyll $x + c$ ($\mu g \, ml^{-1}$)	LSD (p=0.05)
Mn	1.820	a
Mn x4	2.029	ab
Mn x2	2.265	b

As the levels of Mn were increased (Figure 46, graph B), the concentration of chlorophylls and total carotenoids (C_{x+c}) in the leaves significantly reduced ($p<0.01$). Excessive levels of Mn significantly reduced the concentration of chlorophyll and carotenoid in the plant tissue. As with the

deficiency experiment, both Zn and Si had no significant effect on the chlorophyll and total carotenoid (C_{x+c}) concentration (Figure 47). Although large variations between the treatments can be observed in Figure 45, none of these treatments exhibited any significant differences.
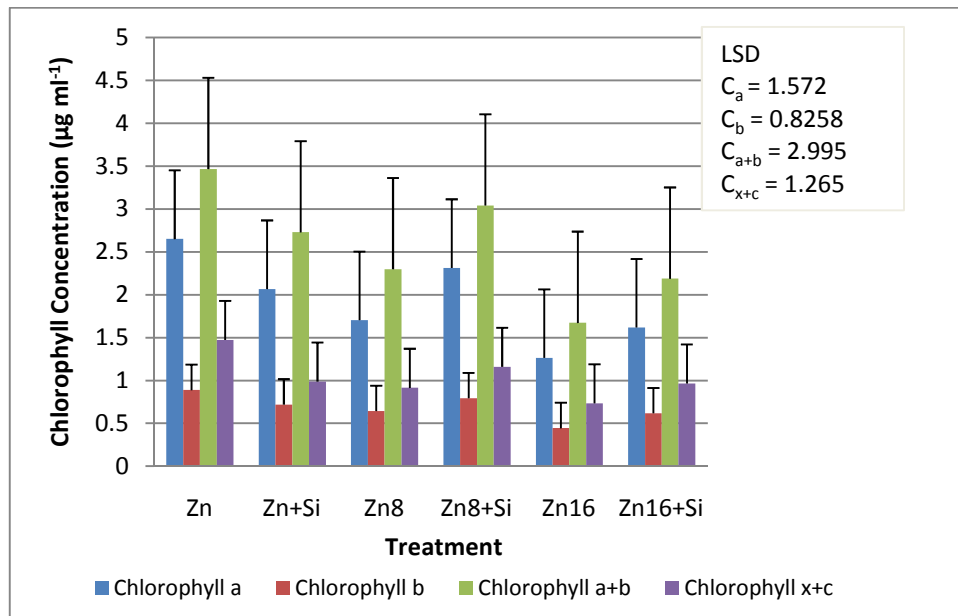
Figure 47. Chlorophyll and carotenoid concentration of cucumber plants cv Tyria in the Zn toxicity experiment (recommended, 8 and 16 x recommended rates of Zn)

2.4   DISCUSSION

Stiles (1916) argued that simple hydroponic systems (such as the ones used in these experiments) have a limitation in the study of the nutrition of plants due to the variability of nutrients within the solution over time. Nutrient concentrations in nutrient solutions vary due to the time delay between nutrient solution changes, whereby sufficient applications become deficient as time progresses between applications. This can compound or confuse results. To limit this more frequent solution changes, as well as frequent pH and EC adjustments were carried out. Nevertheless there were variations in pH and EC within the experiments. The recorded and observed results, however, gave the expected trends for Mn, Zn and Si concentrations. It is recommended, though, that further studies should investigate the use of continuous flow solutions not requiring replenishment of nutrients over time.

The nutrient uptake curve for Mn and Zn was demonstrated in these different experiments from deficient to sufficient to luxury consumption and finally through to excessive application of the different nutrients. The results have shown the impact of these different rates of application on the

physiology of cucumber plants. The essentiality of Mn and Zn to plant growth was also clearly shown within these experiments, more particularly the deficiency experiment. The key roles (such as chlorophyll production in Mn nutrition, or plant growth in Zn nutrition and leaf positioning in Si nutrition (Marschner, 1993)) which each nutrient plays in the growth of the plants were identified through the various measurements and recordings conducted during the growth of the plants. Visual evaluation of plants is a qualitative feature, and it is possible that there was a lack of consistency in this evaluation due to the limit placed by criteria used for such an evaluation.

During early growth, Mn shortages were not evident in the development of plant leaves, but as growth continued, the impact of Mn shortages became clearly visible with the recently emerged leaves becoming increasingly smaller. It is possible that sufficient Mn was available in the seed of the plant to sustain the plant in its early stages of growth, but not to supply the plant's entire needs. Unlike Mn, Zn shortages in the nutrient solution were very quickly realised, showing either a higher sensitivity to Zn shortages or seeds contain very limited quantities of Zn, indicating a complete reliance on the plant's environment for its supply of Zn (Marschner, 1993). The very small leaves of the Zn deficient plants, typical of Zn deficiency (Marschner, 1993), tended not to have much longevity and became senescent much earlier than those of sufficiently supplied plants. The expression of Zn deficiency symptoms in these trials was poor due, perhaps, to a very low concentration of Zn present in the water that could not be removed through purifying. Such a very low Zn level may have been sufficient to mask some of the expression of the nutrient shortage.

Application of either Mn or Zn significantly improved the growth and development of leaves, confirming the essentiality of these nutrients to plant growth and development. However, the addition of both, Zn and Mn, to the plants produced an interactive effect, further increasing the growth of the leaves of the plants. Mn and Zn both contribute to chlorophyll and auxin production respectively (Mengel & Kirkby, 2001), and both components are needed by plants to effectively photosynthesize and grow.

As levels of Mn were increased, it was found that cucumber plants are relatively tolerant to increased levels of Mn, where impact on the growth of leaves was only achieved at levels of 8x and 16x of that required for normal growth (Horst, 1988). Initially, this may also indicate not just a high tolerance, but a reportedly large range for luxury consumption before the nutrient negatively impacts growth. Increasing levels of Mn retarded leaf emergence, and thus the rate of growth of the plant. Reduced leaf size with supra-optimal Mn application also has the effect of reducing the photosynthetically active leaf area, which can therefore be expected to reduce photosynthate

production, which, in turn, would reduce the plant's growth rate. Early leaf senescence was seen at high rates of application of Mn, thereby substantially reducing effective leaf area even further. Significantly lower chlorophyll concentrations at such high levels of application of Mn also reduced the photosynthetic competence of the plant. This toxicity resulted in significantly smaller and thinner stems. The yellowing of the youngest leaves is symptomatic of Fe deficiency in cucumber plants, often also found in Mn toxic applications or conditions (Marschner, 1993).

A lack of significance in Mn Tx 1 is attributed to the wide range of luxury consumption that can appear to be found with Mn plant nutrition, particularly in cucumbers. Observed differences in leaf measurements are attributed more to rates of plant growth and leaf emergence than directly to Mn nutrition and application. Mn Tx 2 was conducted to ascertain the impact of excessive levels of Mn on plant growth and development, since such symptoms were not observed at the lower concentrations of Mn Tx 1. Expected toxicity symptoms were observed in Mn Tx 2, and from the results of these experiments, the deficiency experiment showed both deficiency and sufficiency, the first toxicity experiment illustrated luxury consumption whilst the second toxicity experiment illustrated true toxicity.

The studies have shown that both, leaf length and width, are affected by increasing Zn applications. The importance of Zn in auxin production is evident where leaf extension is increased when Zn applications are increased from deficient to sufficient (Zhang & Wu, 1989). Although Zn deficiency symptoms were observed, they were not as prominent as expected, leading to the suspicion that some Zn remained in the water as a contaminant following purification. It is exceedingly difficult and expensive to completely remove all nutrients from water, despite the use of distilled water. Possibly a double distillation could avoid the presence of such contamination.

Where the Zn applications were increased to excessive and toxic levels, the oversupply of Zn inhibited the natural ability of the plant to undergo natural cell extension thereby reducing the length of the internodes and thus the height of the plant. This combined with the reduction of leaf surface area and ability to photosynthesize, reduces the likelihood of the plant reaching the expected plant height under these conditions. Smaller, less vigorous plants result under toxic conditions (Figure 32).

The understanding of Si nutrition in the plant is still in its infancy and insufficient information is available about this nutrient. Under deficient conditions, it was noted in these trials that plants did not grow particularly well, and leaves exhibited a cupped shape. Application of Si, even as a foliar

treatment, reduced the "cupping" of the leaves (Miyake & Takahashi, 1983). Commercially, Si is applied as a component of cucumber nutrient solutions. Whilst foliar sprays are not completely effective, as even after Si application symptoms persist, even if under a reduced expression. A possible explanation is that the effectiveness of foliar application is dependent on the ability of the plant to absorb the nutrient into the leaves (Adatia & Besford, 1986).

The application of Si did not have any impact on the growth of the leaves, whether under deficient or sufficient levels, and limited interactive effects were identified with either Zn or Mn. Si therefore does not have a significant role in the growth and extension of leaves in the cucumber plant. Visually however, when applied with Mn, Si-applied plants were stronger, leaves were more open and plants grew taller and produced more fruit. The symptoms observed in Si-deficient plants were described by Miyake and Takahashi (1983) as typical Si-deficiency in cucumbers. The typical leaf curl was very much dependent on leaf size, where larger leaves had a greater degree of curl, and they also tended to be older leaves (Figure 28). Application of Mn or Zn increased leaf size which in turn resulted in increased curl where Si was not applied. Where curl was still observed in Si applied treatments, it is suspected that either the curl was caused by other factors or insufficient or inefficient application of Si occurred. This does question whether the application rate of Si was sufficient for the plants, although there were a few recorded variables that did respond to Si applications. Further experiments should investigate application methods, rates and sources of Si to apply to ascertain the ideal application method and the necessary level required by the plants. Some leaf curl was observed in the youngest leaves of the plants, but this is probably more attributable to age and size of leaf as opposed to Si deficiency.

It has been found that Si applications in cucumbers help prevent the impact of excessive amounts of Mn in the leaves by preventing localised build-up of toxic levels of Mn across the leaf (Jones et al., 1967). Under low Si conditions, toxic levels of Mn concentrate in specific areas across the leaf resulting in the development of necrotic spots. In older leaves though, the addition of Si does not appear to alleviate the impact of the highest Mn application, but younger leaves appeared to cope much better with these applications with the additional effect of the Si being advantageous. In each application, leaf growth was improved with the addition of Si, significantly so in some of the treatments. This improvement in growth is consistent with research conducted by other scientists (Miyake & Takahashi, 1983). In the Mn Tx 2 experiment, even the application of Si to the toxic treatments did not have a sufficiently alleviating impact of the excessive levels of Mn. The older leaves, however, had fewer necrotic spots occurring on the leaves. These leaves instead tended to

suffer from necrotic margins that died back towards the stem of the leaf, indicating that when high levels of both, Mn and Si, are applied toxicity symptoms will develop, not only in the form of necrotic spots, but in the commonly observed toxicity symptom of marginal necrosis.

The greener and healthier leaves produced by the plants adequately supplied with Mn and Zn were better able to support the development of fruit by the plant. The significant interactive effect of the combination of the two nutrients illustrates the need for all nutrients to be sufficiently available for the plant to grow and produce adequately. However, whilst the number of fruit produced between treatments in the deficiency experiment was the same, when adequately supplied with nutrients, fruit reached harvestable maturity quicker.

Due to the rapid growth of cucumber plants, however, only a limited yield could be evaluated because of the limited height of the growing chambers used for the experiments. Fruit development is dependent on the availability of Mn and Zn, where increasing applications from deficient to sufficient significantly increased the total number of fruit (including harvested, developing and aborted fruit) (Ruano *et al.*, 1988). It was noted however, that with increasing levels of Mn and Zn into toxic levels fewer fruit were harvested and an increasing number of fruit aborted. It would be worth further studies to determine whether this trend continues for the life of a crop and not just over the short period that these experiments were conducted. If this is the case, fruit would be harvestable earlier and at higher numbers than if toxic levels are applied. This would be of commercial significance.

With increased availability of Mn or Zn to the plant, there was an improved visual appearance of the plants and thus the subjective plant ratings which were conducted regularly. Only in the Mn Tox 2 and Zn toxicity trial did the excessive applications significantly affected the visual appearance of the plants. Of note though, in Mn Tox 2, were the ratings which varied greatly from one measurement to the next, improving and then deteriorating. This results in a visual improvement of the condition of the plant from the emergence of new leaves which improved the overall appearance of the plant. The condition deteriorated once again as the health of the new leaves was impacted by the excessive applications of Mn.

Whilst examining the effect of Zn and Mn on the growth and development of the cucumber plant, it was necessary to confirm that other than the nutrient concentration of the solutions, no other physical characteristic of the solutions were affected. In the case of solution pH, nutrient uptake by the plants, and nutrient solution composition with regards to Zn and Mn in this

experiment, did not affect the solution pH and its changes for the duration of this experiment. Only a once-off significant change in solution pH was observed and this was not part of a trend; hence, it can be concluded that this micronutrient does not impact on solution pH. Could pH changes in the solution be a function of the stage of growth or age of the plant, as it appears not to be related to nutrient solution composition? Changes in pH were not affected by the amount of water/nutrient solution taken up by the plant.

Solution EC, a measure of the nutrient concentration of the solution, is a good measure of the uptake of nutrient and water by the plant. At a young age, there were no significant differences between the treatments, but significance developed as plants grew older. This indicates that plant age and size play an important role in the uptake of nutrients by the plant. Hence, changes occurred in the EC of the solution from one solution change to the next (Figure 42). Any change to the solution that affects either the concentration of the solution, by removal of water or nutrients or both together, but at differing rates of removal, will in turn alter the EC of the solution. The demand for water and nutrients of the plant increases, although not at the directly proportional to the plant's growth rate. Plants with sufficient nutrients tended to grow faster and had a higher uptake of water resulting in the need for more frequent solution changes. Plants under nutrient stress (deficiency or toxicity) grew slower; consequently their demand for water and nutrients was lower, and, thus had less of an impact on solution EC. Should the duration of the experiment have been extended, these changes could have been expected to continue due to the growing demand from the sufficiently supplied plants. Extension of the duration of the experiment will be necessary to confirm this observation. Perhaps the number of replications was too small to be able to establish significant trends between treatments, particularly as there was large variation between measurements in the experiments.

Both deficient and excessive treatments produced smaller plants and with this reduction in plants size compared with the sufficiently applied treatments, their rate of uptake of nutrients and water from the solution was significantly lower and, therefore, smaller changes in solution EC were recorded. The larger adequately applied plants grew quicker, requiring larger quantities of nutrients and water, causing larger changes in EC. With the adequately applied treatment having significantly larger leaves than the other treatments, the movement of water through the plant can be expected to be larger due to the increased surface area of the leaves; therefore, a larger uptake of water by the plants was observed over time (Figure 42 and 43).

The large reduction in change between change 6 and change 7 for the Mn4 treatment was possibly caused from stress inflicted on the plant (Figure 42, graph A). Uptake of water by this plant for the duration of the course of this particular solution resulted in almost all of the solution being removed from the container prior to solution change. Some root damage occurred and a small amount of wilting of one of the plants was observed. Time was required for the plant to recover from this stress and to resume the normal or expected growth again, hence the continued change in EC with growth at Change 8 (Figure 42, graph A). A similar occurrence in the Zn toxicity trial was observed and more frequent solution changes were required to prevent this from occurring again.

Variances in change in EC can be explained in general by the amount of nutrients and water taken up by the plant. A large change in EC, from 1.8 mS/cm to 3.2 mS/cm would be a result of a large amount of water being taken up by the plant, and correspondingly fewer nutrients. Small changes in positive EC would be caused by equal or comparatively similar uptake of nutrient and water from the solution. Negative changes in EC (from 1.8 mS/cm to 1.7 mS/cm) are caused by the uptake of comparatively more nutrients than water from the solution. Often this can be compared with the growth of the plants and their differing growth phases. When cucumber fruit expand rapidly, a large amount of water is taken up by the plant over a short period of time (Robinson & Decker-Walters, 1997), this would cause a large positive change in EC as water is removed from the solution at rates in excess of the uptake of nutrients by the plant.

Related to the EC of a nutrient solution is the composition of the nutrient solution. Where EC is a measure only of the amount of salts in the nutrient solution, changes to the composition of the solution due to the differential uptake of nutrients from the solution by the plant should affect the EC of the solution. Measurements therefore to detect the changes in nutrient composition were conducted using Inductively Coupled Plasma Emission Spectroscopy (ICP) and the differences between the composition of the solution from the fresh nutrient solution and prior to solution change at the end of the week or when necessary was determined.

Whilst rapid uptake of water by the plant will raise the EC of the solution, uptake of nutrients by the plants is affected by the mechanism of uptake. Where some nutrients are taken up in relation to water, their levels may be expected to be comparatively lower in the solution, or at least the daily uptake may be significantly higher for that nutrient. This could imply the relationship of the nutrient to water availability or the method by which the nutrient is taken up by the plant, either actively or passively. Further studies would be necessary to elucidate and confirm these comparisons. Under very high levels of Mn, it would appear that simply the presence of Mn was sufficient to stimulate its

own uptake, since plants do not selectively take up nutrients (Marschner, 1993). The negative relationship with Zn at high Mn levels was unexpected and could perhaps indicate that these nutrients may be taken up by the plant using similar mechanisms. Under high levels of Mn, Mn out-competes Zn for uptake, resulting in lower Zn uptake. The daily uptake of all other nutrients measured did not show any significant differences with increasing Mn application levels. The addition of Si to the treatments did not have any effect on the uptake of any nutrients by the plants.

Due to the high concentration of Zn in the nutrient solution, its uptake is forced on to the plant by saturation of the uptake sites for Zn. Whilst increasing from eight times the standard rate for Zn to sixteen times the standard rate for Zn, there was not a further doubling of the rate of uptake of Zn by the plant, but rather a small increase. This could indicate that the uptake sites for Zn into the plant are saturated and further uptake is inhibited.

Although Si was not applied to the solution, exceptionally low levels must have been present in the purified water that was used to make up the solutions, or, small contaminations of Si must have occurred to result in the difference in Si concentration in the solutions during the course of a week, between solution changes. This contamination may have affected results, but the significant impact shown when Si was applied does indicate that Si deficiency conditions did prevail.

Excessive levels of Zn inhibit the uptake of Mg by the plant, explaining the typical Mn deficiency symptoms (Marschner, 1993) observed in the plants, with interveinal chlorosis observed on the older leaves of the plants. This can be the expression of an Mg shortage by the plants as well when applied with excessive levels of Zn (Mengel & Kirkby, 2001). Although K shortages do also cause yellowing in cucumber leaves, the yellowing is normally characterised by chlorosis of the leaf margins, a symptom that was not observed in excessively applied Zn plants. Since low levels of K uptake were recorded, it is believed that the levels were sufficient to have prevented the expression of deficiency symptoms for K by the plants. Although the uptake of Mg and K was significantly reduced with increasing levels of application of Zn to the plants, there were no observed differences in these nutrients in the plants.

Increased concentrations of some nutrients in excessively applied treatments may simply be due to a higher concentration because plants were significantly smaller. The higher nutrient concentration in the plants did not correspond with the reduced rate of uptake of the same nutrient. So, although plant uptake was lower, the proportionate nutrient concentration was higher due to the plants being significantly smaller in size.

Reports have shown that Fe deficiency may be observed under toxic levels of Mn application (Horst, 1988). However, due to the similarity of the symptoms, it is possible that Fe deficiency occurred, but was masked by other toxicity symptoms. Iron uptake by the plants was not consistent or significantly affected by the treatments of Mn or Zn through deficient to excessive applications (Figure 15, Figure 44, Figure 46).

It should be noted that only iron sulphate was used as the source of Fe in the nutrient solution. Iron has limited availability when in solution and is particularly pH sensitive (Marschner, 1993); perhaps a chelated Fe product such as Fe-EDTA should have been used to ensure adequate availability of applied Fe at all times (Harris, 1966). Fe-EDTA or Fe-DTPA or other such chelated products are used as a preferential source of Fe in commercial hydroponic systems (Derek Askew, *pers*. *comm*.). This might also explain the varying results that were observed with the Fe measurements made by the ICP. Whilst it is expected that insignificant changes in rate of uptake due to the treatments applied will result in no significant difference in Fe concentration in the plants, it was found that under increasing applications of Mn or Zn, plant Fe concentration significantly increased. This result is surprising, since it has been found that under toxic levels of Mn application, Fe deficiency can be observed (Horst, 1988), but this deficiency is not as a result of a shortage of the Fe in the tissue, but possibly the suppression of the use of Fe due to the excessive presence of Mn. Further experiments are needed to fully understand this relationship. Despite the significant differences found in the Fe concentration, some of the observed variation in the nutrient concentration may be as a result of the source of Fe used not being adequate enough for hydroponic growth and production. In the presence of Zn, there was a significant increase in the uptake of iron by the plants, perhaps indicating a synergistic effect on plant growth and development by the two nutrients.

Due to the extensive yellowing observed in the different treatments, and knowing the impact these nutrients could have on chlorophyll development, measurement of actual chlorophyll and carotenoid (C_{x+c}) concentration of the leaves was conducted to determine the response of the plants to the application of Mn, Zn or Si. Both, Zn and Si, did not play a significant role in the production of chlorophylls and carotenoids in the plants and the presence of these pigments is due to other causes (such as Mg) (Table 7) and not related to the application of Zn and Si. The chlorosis of leaves is, hence, due to other causes (nutritional or physiological) and not a result of the presence or absence of these two nutrients.

Reduced chlorophyll and total carotenoid (C_{x+c}) concentrations reduce the ability of the plant to adequately photosynthesise and produce sufficient sugars for growth and development, thereby reducing the ability of the plant to grow. This, in part, would have contributed to the small size of plants observed at these high levels of Mn. Additionally, the lack of Zn in Zn deficient plants would have also affected the auxin concentration of such plants, as Zn is a cofactor for enzymes in IAA biosynthesis (Zhang & Wu, 1989).

2.5   CONCLUSIONS

Whilst it is acknowledged that there are many factors that affect nutrient uptake and plant nutrition, these experiments were designed to minimise the variables and to provide an adequately controlled environment in which to study Zn, Mn and Si nutrition of cucumber plants. A fault in the design of the equipment used in the experiments did lead to stunted growth and deformities of the leaf shape and size. The growing points were wet by the nutrient solution either through capillary action up the outer stem surface or through the air bubbles exploding at the surface of the solution. Adjustment of the position of the air pipes or the use of filter paper around the hole may have reduced this occurrence. Although infrequent, necrosis of the growing point in Zn toxicity trial was observed and was attributed to prolonged wetting of the growing point.

The necessity of micronutrients Zn and Mn is clearly apparent from the deficiency experiments conducted. A lack of Zn leads to stunted growth or plants, both in the extension of the internodes as well as the size of the leaves of the plants. The stunted growth is probably in part a cause of a lack of the generation of auxin by the plant due to the limited availability of Zn to the plant. Combining this shortage of auxin in the plants with the yellowing of the leaf tissue, inefficient photosynthetic processes could have also limited the growth and development of the plants. The cause of the yellowing was not identified, but it was not due to a lack of chlorophyll, since chlorophyll production was not affected by Zn applications to the cucumber plants. Deficiency symptoms were identifiable, determined and recorded for both deficiency and toxicity. As with Zn deficient conditions, toxic Zn applications did not significantly affect the development and production of chlorophyll and carotenoids within the leaves. Toxic levels of Zn also resulted in chlorosis and stunted plants, the nutrient solution of such toxic Zn levels damaged the growing point of plants if remaining wet for any length of time.

Zinc applications affected the number of fruit produced by the plant compared with the no Zn treatment, implying Zn is required for fruit production, but it is also important to note that under deficient conditions, the number of internodes measured was fewer as the plants were growing more slowly. Since flowers and fruit are produced at the nodes, the plants produced fewer fruit as well. Under toxic conditions, however, Zn did not impact on the number of fruit produced or even the number of fruit aborted. The role of Zn may be purely in the growth and development of the plant and not directly impact on flowering and fruiting.

Fast growing plants tend also to affect the condition of the nutrient solution quicker and to a greater extent. Due to the rapid uptake of nutrients and water from the solution, Zn sufficient plants tended to use up more water than nutrient from the solutions, resulting in higher solution ECs. These solutions needed more regular refreshing and changing, particularly as these plants grew bigger. Despite the changes to the EC of the solution, pH of the solution was not significantly affected until the plants were much older and larger. These changes occurred as a result of the development of an oil-like substance that coated the roots, the identity of which is unknown but could have been exudates from the roots to assist them in nutrient uptake.

Under deficient conditions, the mere addition of Zn will significantly increase the uptake of this nutrient by the plant. Manganese appeared to increase the uptake of Zn providing proof of a relationship between the nutrients. Uptake of Zn was also related to the uptake of water by the plant, however, under excessive conditions, uptake of Zn was no longer related to water uptake. When an element is present in excessive quantities, due to the high level of abundance of the nutrient in the solution, its uptake is perhaps continuous and at rates unaffected by plant water uptake.

Manganese, on the other hand, appears to affect many processes in the cucumber plant. It has been shown to influence chlorophyll production, photosynthesis and protein production (Horst, 1988). Manganese directly affects plant leaf colour, where it significantly increases the development of chlorophyll and carotenoids until excessive levels are reached. Impact on the development of these pigments does limit growth of the plant; hence, under both, deficient and toxic, levels of application, chlorosis of the leaves was directly linked to a reduction in chlorophyll and carotenoid production. Under both, deficient and toxic, levels of Mn application, smaller plants resulted. Of importance was the need to increase the application of Mn to the plants to force a toxic response. Cucumber plants have a relatively high tolerance to Mn and up to 4 x the standard application did

not significantly impact the growth of the plants. This range from sufficient to 4 x the standard application could be termed luxury consumption (Mengel & Kirkby, 1987).

Manganese, moving from deficient to sufficient levels of application significantly increased the uptake of a variety of nutrients, including Ca, K, Zn and Mg, as well as Mn. Moving into toxic conditions Mn continued to influence the uptake of these nutrients, except Mg and K. Under toxic levels of Mn, Zn uptake is negatively impacted, indicating a possibility of a related uptake mechanism by the plants where, due to the high levels of Mn in the nutrient solution, there is an inability to take up sufficient quantities of Zn, which was confirmed in the reduced Zn levels in the tissue analyses of the plants. Such reduced levels of Zn could, therefore, result in a reduction in the production of auxins, thereby reducing the growth of the plants (Zhang & Wu, 1989). Plants supplied with toxic levels were also much smaller than sufficiently supplied plants.

Although plant growth and development was affected to varying degrees by increasingly toxic levels of Mn, fruit growth, development and maturation was not affected significantly. Fruit development was delayed, but not significantly so. Increasing levels of Mn could produce more differentiating results. Due to the limited space of the growth chambers, inclusion of varying levels of Zn were not possible and further experiments need to investigate the interactive nature of the relationship between these two nutrients and plant growth and development.

Silicon, although not reported as an essential element, has been found to affect and improve plant growth and development under certain conditions. Silicon does not affect the development of chlorophyll or carotenoids in the leaf of cucumber plants, it does not affect the normal development and growth of a cucumber plant, but it does affect the visual appearance of the plant. Visual ratings of the cucumber plants did show significant improvements with the addition of Si in the deficient experiments that were conducted. These differences were not recorded in any of the toxic experiments.

Since very few measurements that were conducted appeared to influence the growth and development of the cucumber plants, it can be questioned as to whether Si is an essential nutrient. Further studies were conducted on this nutrient and are reported further in Chapter 4 of this thesis.

CHAPTER 3

MANGANESE, ZINC AND SILICON STUDIES OF CUCUMBER (*Cucumis sativus*) USING A MINIATURE HYDROPONIC SYSTEM

Dominy, A.P. and Bertling, I.
Horticultural Science
School of Agricultural Sciences and Agribusiness
University of KwaZulu-Natal
Private Bag X01
Scottsville 3209
Pietermaritzburg
South Africa

1. Introduction

Man depends on plants and plant products, in some form or other, for his survival (Glass, 1988). As a result, there is a strong requirement to have a better understanding of plant production, particularly plant nutrition and plant nutrient interactions to supply the large amounts of plant resources required for the rapidly increasing population. Plant nutrition involves more than the determination of nutrient requirements, it also investigates the nature and properties of the medium in which the plant grows, as well as the reaction of the plant to those properties. In order to understand plant nutrient interactions, requirements and processes in which these nutrients are involved, it is necessary to eliminate the variation due to media and focus solely on the plant by using hydroponic methods such as water culture.

The importance of nutrient balances plays a major role in nutrient uptake and plant growth and development. The interaction of macro- and micronutrients (such as manganese, zinc and silicon) has been studied for an extended period of time. It has been found that zinc (Zn) uptake is inhibited by high concentrations of copper (Cu), manganese (Mn) and iron (Fe) (Giordano *et al.*, 1974), whilst high levels of phosphorus (P) are known to induce Zn deficiencies (Mendel and Kirkby, 1987). Fe and Mn are also found to interact with Zn, and more particularly under P induced Zn deficiencies (Warnock, 1970). Mn uptake is

depressed by magnesium (Mg), calcium (Ca), Cu, Fe and Zn (Maas *et al.*, 1969), whilst Mn reduces Fe uptake (Havlin *et al.*, 1999). Silicon (Si) availability is affected by Fe and aluminium (Al) oxides in the soil, and Si uptake is reduced by boron (B), Mn and Fe in the plant (Kabata-Pendias and Pendias, 1984).

The micronutrients Mn and Zn are essential for plant growth and development. The essentialities of other micronutrients, such as Si, are still under debate (Kabata-Pendias & Pendias, 1984). The uses of and requirements for Si are still under determination and its contribution to 'normal' growth and development needs further investigation. The relationship between Zn, Mn and Si and other plant nutrients is not fully understood. Therefore, a miniature hydroponic water culture system was developed to obtain an insight into these interactions by studying cucumber development from a deficient to a sufficient Mn, Zn and Si application level.

2. Materials and Methods

A miniature hydroponic system consisting of 5,5 L plastic containers with sealable lids was used for the experiments. A hole was made in the lid through which a single cucumber plant could grow. Four litres of the nutrient solution were filled into each container which was continually aerated by a small fish tank airpump. Plants were grown in a modified Shives solution (Veltrup, 1976), consisting of 195.64 mg/l KH_2PO_4, 54.44 mg/L K_2SO_4, 530.73 mg/L KNO_3, 462.19 mg/L $MgSO_4$, 974.33 mg/L $Ca(NO_3)_2$, 11.21 mg/L $FeSO_4$, 3.14 mg/L H_3BO_3, 174 µg/l $CuSO_4$, 125 µg/L Na_2MoO_4, and 193 µg/L $CoSO_4$. Mn was applied at 2 levels, 0 mg/L and 6.12 mg/L as $MnCl_2$, whilst Zn was applied at 0 mg/L and 1.16 mg/L as $ZnSO_4$. In a separate experiment, Si was applied as a foliar spray every 3 days at a rate of 498.75 mg/l as $Na_2O_3Si.9H_2O$, with 1 mL/L spray of Tween 20 surfactant.

A 2x2x2 factorial design was used with 2 replications. The experiments were conducted in a controlled environment of 24^0C, RH of 30 - 60%, and 12 h daylength. Light (307.02 µmol m^{-2} s^{-1}) was supplied by 120 "Growlamps®", Osram, and 12 100 W incandescent lights.

Two cucumber seeds, (*Cucumis sativus* cv Tyria), were placed on a piece of moist filter paper above the hole in the lid of the container filled with distilled water. A glass container was placed over this to maintain a consistently high humidity. Upon germination, the roots were allowed to grow down into the water. The weaker of the two plants or the ungerminated seed was then removed. Once the primary leaves began to emerge, the distilled water was replaced with nutrient solution. The nutrient solutions were exchanged on a weekly basis for the first 6 weeks and thereafter every 3 to 4 days. Samples of the solutions were taken at the start of the week and prior to replacing of the solution. Electrical conductivity and pH were measured on a daily basis using a Hanna® pH/EC probe, and leaf measurements were taken of every second leaf every 3 days using a digital calliper. Nutrient solution pH was maintained between 5.3 and 6.5 with 0.5 M KOH.

Nutrient solution samples were analysed using inductively coupled plasma emission spectroscopy (ICP). K, Ca, Fe, Mn, Zn, Mg and Si were analysed on the following wavelengths 769.896 nm, 318.128 nm. 259.940 nm, 259.373 nm, 206.200 nm, 293.654 nm and 251.611 nm respectively. Plant condition was also evaluated and plant height, leaf measurements, fruit measurements, fresh mass and dry mass were also recorded. The experiment was terminated after 8 weeks, upon which leaf measurements, plant heights, fruit measurements, fresh mass and dry mass were recorded. Data was analysed by ANOVA and regression using GENSTAT®.

Results

Increasing Mn applications from a deficient to a sufficient level promoted growth of the plants, resulting in significant increases in the uptake of all elements examined (Mg, Ca, Fe, K, Si and Zn) (Figure 1). At termination of the experiment, Mn sufficient plants were taller, had a larger fresh mass and dry mass, and an increase in fruit production as well as earlier fruit production (Tables 1 & 2). Leaves were significantly larger throughout the growth of the plant (from mature to immature leaves). Quicker growth resulted in basal leaves maturing earlier and thus being more susceptible to leaf curl, a symptom observed due to silicon deficiency.

Increasing Zn application from a deficient to a sufficient level produced a similar effect to Mn applications, (Figure 2), except that there was an increase in total number of fruit produced as well as total number of harvestable fruits. No effect was found on the number of fruit aborting. Zn applications increased plant height, plant fresh mass and dry mass, but had no significant effect on root growth and development (Table 2). The more mature leaves on the Zn sufficient plants were also more susceptible to leaf curl and necrotic spot development. Combined increases of Mn and Zn to a sufficient level of application produced significant interactive effects. Nutrient uptake was increased substantially, however, no significant effect was observed on plant growth except for the production of larger leaves. The number of aborted fruit increased significantly (Table 1). The mature leaves were more susceptible to silicon deficiency symptoms.

Application of Si had no effect on nutrient uptake or any aspect of plant growth and development except for reducing Si deficiency symptoms (leaf curl and necrotic leaf spots) in mature leaves. Applying Si to a Zn sufficient treated plant resulted in significantly reduced Mn uptake (Figure 3), improved plant condition as well as reduced deficiency symptoms in mature leaves.

Discussion

The importance and essentiality of Mn and Zn to plant nutrition and cucumber nutrition, in particular, is borne out by the significant increase in uptake of all nutrients examined as the application of Mn and Zn was increased to a sufficient level (Figures 1 and 2). The increase in plant growth and development (Table 2) is seemingly a response to the increased uptake of nutrients by the plant, which in turn was responsible for earlier fruit production and harvest. However, due to the quicker growth, the more mature leaves were more susceptible to degradation/necrosis by Si deficiency. This possible loss of efficiency of the plants was not evident in the fruit production. Despite improving the condition of the more mature leaves of the plants, Si applications did not have any effect on the fruit production. It is suggested that the effects of a foliar spray would only be observable later during the growth of the plant and were not evident up to termination of the experiment.

Zn deficient versus Zn sufficient plants did not show the anticipated difference in growth and development. Zn, known for its importance in auxin production (Shkolnik, 1984), was expected to have a stronger response than observed. Recognised deficiency symptoms such as shortened internodes, rosetting of terminal leaves, and interveinal chlorosis were not observed either (Mengel & Kirkby, 1987). Zn is a ubiquitous element and it is extremely difficult to remove all traces of Zn from water. Since Zn is only required in small amounts, the levels remaining in the water (0.75 mg Zn/L) must have been sufficient for the plants to obtain and prevent the formation of deficiency symptoms. However, the application of a combination of 1.16 mg Zn/L and 6.12 mg Mn/L resulted in an increase in uptake of all other nutrients from the hydroponic solution. Since the levels of application were only in the region of a deficient to a sufficient range, and growth was enhanced significantly by both these elements, the lack of observed interaction is not surprising at this stage of growth and could be expected to be more evident later in the life of the plant. Zn and Si display a significant increase in Mn uptake (Figure 3). This could be attributed to the reduced damage

and lack of Si deficiency symptoms of the more mature leaves of the plants as well as increased leaf expansion due to the Zn essentiality for auxin production.

Silicon had a positive effect in improving the quality of the more mature leaves of the plant, and in combination with Zn, it had a positive effect on the uptake of Mn (Figure 3). This illustrates the need for all nutrients to be in balance and available to meet plant requirements. Mn and Zn, although producing similar responses as their application was increased from a deficient to a sufficient level, are unable to substitute for one another since together they improved fruit mass (Table 2). Extended culture periods need to be studied, and the responses and nutrient interactions of the cucumber when Mn and Zn applications are increased from a sufficient to a toxic level need to be ascertained.

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Table 1. Fruit harvest data of cucumber obtained at termination of experiment expressed as mean per treatment. (Significance level * = p(0.05), ** = p(<0.001), and ns = non significant). Comparisons made between the levels of nutrient applied.

Treatment	Fruit Number	Aborted Fruit	Fresh Fruit Mass (g)
0 mg Mn/L	15.6 *	2.00 ns	10 **
6.12 mg Mn/L	22.0 *	3.62 ns	252 **
0 mg Zn/L	15.5 *	2.37 ns	64 *
1.16 mg Zn/L	22.1 *	3.25 ns	188 *
No Si spray	18.3 ns	3.00 ns	144 ns
49.8 mg Si/l spray	19.4 ns	2.62 ns	109 ns
0 mg Zn/L + 0 mg Mn/L	11.3 ns	2.75 *	10 ns
1.16 mg Zn/L + 6.12 mg Mn/L	21.8 ns	5.25 *	376 ns

Table 2. Plant measurements of cucumber obtained at termination of experiment expressed as mean per treatment. (Significance level * = p(0.05), ** = p(<0.001), and ns = non significant). Comparisons made between the levels of nutrient applied.

Treatment	Plant Height (mm)	Fresh Plant Mass (g)	Fresh Root Mass (g)
0 mg Mn/L	820 **	108 **	11.7 **
6.12 mg Mn/L	1847 **	403 **	84.4 **
0 mg Zn/L	1020 *	186 ns	42.3 ns
1.16 mg Zn/L	1648 *	324 ns	53.8 ns
No Si spray	1272 ns	246 ns	47.5 ns
49.8 mg Si/l spray	1396 ns	265 ns	48.6 ns
0 mg Zn/L + 0 mg Mn/L	618 ns	78 ns	11.2 ns
1.16 mg Zn/L + 6.12 mg Mn/L	2273 ns	512 ns	95.3 ns

Fig. 1. Effect of Mn application on nutrient uptake of cucumber. Values represent the mean concentration remaining in the solution prior to solution exchange.
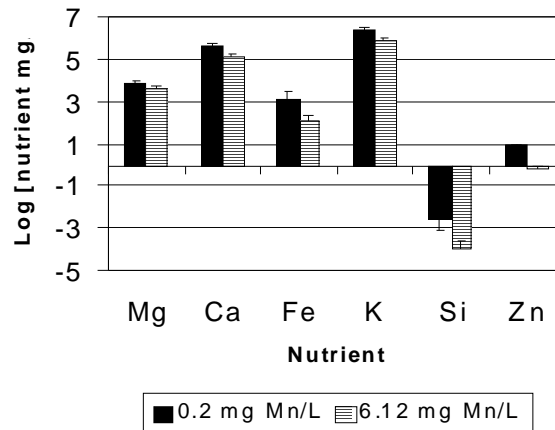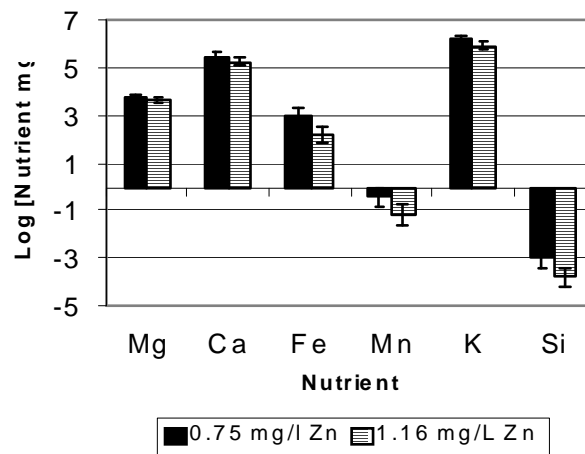

Fig. 2. Effect of Zn application on nutrient uptake of cucumber. Values represent the mean concentration remaining in the solution prior to solution exchange.
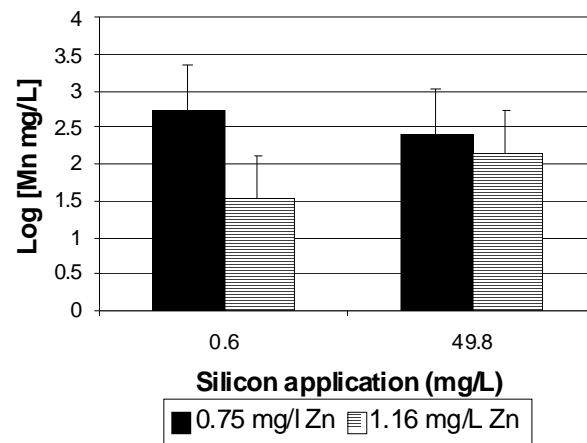
Fig. 3. Interactive effect of Si and Zn on Mn uptake. Values represent the mean concentration remaining in the solution prior to solution exchange.

CHAPTER 4

IMPACT OF SILICON DEFICIENCY ON CELL STRUCTURE IN CUCUMBER (*Cucumis sativus*)

4.1   INTRODUCTION

The importance of understanding the impact of nutrients on the growth and development of plants cannot be understated. Plants are the 'basic foundation for food', acting as a primary source of nutrients and nutrition to all levels of "life" (Glass, 1988). Plant production is therefore primarily impacted by the availability of nutrients and resources required for adequate plant growth and development. The concept of essentiality of certain elements for plant growth and development was first proposed by Arnon & Stout (1939) who stated that non-essential elements positively affect growth but are not essential for the completion of the life cycle of plants, but they may be essential for the completion of the life cycle for certain species. Therefore, it is important to understand the impact of shortages of particular nutrients on growth and development of a particular species.

Beneficial effects of Si application on plant growth and development have been reported by many researchers (Ma & Takahashi (1990), Epstein (1993), Belanger *et al.* (1995)). The causes for the improvement of plant growth following Si application, and the biochemical reactions Si imparts, have not been completely elucidated yet; however, many of the effects of Si, from the improved nutritional value of the plants to its ability to withstand pathogen attack, have been studied (Ma & Takahashi*,* 1990; Menzies *et al.,* 1991). In understanding the extent of the essentiality of Si to plants, Takahashi & Miyake (1977) identified plants as either "silicon accumulators" where Si uptake is greater than uptake or "non-accumulators" where Si uptake occurs at a rate similar to that of water uptake by the plants. Miyake & Takahashi (1983) have suggested that cucumber is a non-accumulator, taking up silica "passively".

It is apparent that SiO_2 is taken up passively and transported in the plant in the transpiration stream through apoplastic movement resulting in the deposition of SiO_2 within the cell walls. Readily available silicate is found in the soil solution as silicic acid which is absorbed easily by plants along with the uptake of water (Epstein, 1994). Once deposited, the SiO_2 is unavailable and has not been reported to be translocated. Jones *et al.* (1963) provided evidence that Si is included into xylem conducting vessels and into the epidermis of cells. In these positions, the Si adds structural strength and support to tissues. In higher plants, it has been found that Si plays a role in the protection of

plants against fungal attack whereby silicate crystals are deposited on the surface of the leaf reducing the susceptibility of the leaf to hyphal attack from fungal growth (Marschner, 1993).

Several researchers (Jones *et al.,* 1967; Samuels *et al.,* 1993; Belanger *et al*., 1995), have studied the deposition of silica in plant tissue and its impact in disease prevention. Such research has focussed on the beneficial effects brought about by the addition of Si, with little description of the aspect of Si shortages on plant growth and development. The aim of this research was to confirm the symptoms of Si deficiency in cucumbers as well as to identify the impact of Si on cell structure and tissue integrity using electron microscopy.

4.2   MATERIALS AND METHODS

All chemicals were supplied by Sigma (St Louis, Missouri, U.S.A.). A miniature hydroponic system consisting of 5.5 L plastic containers with sealable lids into which one hole was made in the centre was used for the experiments. Aeration was provided by a fish tank pump. A single cucumber seed (cv Tyria) was placed onto moist filter paper above the hole in the lid. In order to maintain high humidity around the germinating seed a glass container was placed over the filter paper. This container was removed once the primary root had reached the distilled water in the container. Upon emergence of the primary leaves the distilled water was replaced with nutrient solution. Four L modified Shive's (Franco & Loomis, 1947) solution (consisting of 195.64 mg/l KH_2PO_4, 54.44 mg/L K_2SO_4, 530.73 mg/L KNO_3, 462.19 mg/L $MgSO_4$, 974.33 mg/L $Ca(NO_3)_2$, 11.21 mg/L $FeSO_4$, 6.12 mg/L as $MnCl_2$, 1.16 mg/L as $ZnSO_4$, 3.14 mg/L H_3BO_3, 174 µg/l $CuSO_4$, 125 µg/L Na_2MoO_4, and 193 µg/L $CoSO_4$) were placed in each container. The nutrient solutions were replenished on a weekly basis for the first six weeks and thereafter every three to four days.

Two Si treatments were applied as foliar sprays, the first one was an application every three days and the second an application every seven days at a rate of 498.75 mg/L Si as $Na_2O_3Si.5H_2O$. Additionally, to half of the plants 1 mL/L Tween 20 was added as surfactant of the Si sprays. The results were compared with no Si sprays, but water plus surfactant sprays only.

Three replications of six plants each were used in this experiment. Plants were grown in a glasshouse at 18 to 24°C with natural day length (13.5 h), with pH and electrical conductivity measured on a daily basis using a HANNA Instruments® pH/EC probe. Nutrient solution pH was maintained between 5.3 and 6.5 with 0.5 M KOH and HCl, whilst the EC of the solution was

maintained between 1.8 and 2.2 mS/cm with fresh solution and distilled water. Leaf measurements of every second leaf were taken every three days using digital callipers.

Once fruit were ready for harvest (180 to 200 mm in length), fruit length, width, and mass as well as the number of fruit harvested per plant were compared between treatments. The number of harvestable fruit as well as the number of aborted fruit per plant was also recorded. The plants were furthermore rated every three days to provide a measure of the general health of plants on a scale of 1 to 10, with "1″ being "completely healthy" and "10" as "dead". Ratings were based on the general health and appearance of the plant, the percentage leaf curl, leaf die back and necrosis. The experiment was terminated once the plants reached a maximum height of 1.8 m. All measurements taken were analysed using GENSTAT® Release 7.22 Discovery Edition, (VSN International) statistical analysis to determine significant differences at the 5% significance level.

Leaf samples were obtained from the 5th and 7th leaves of the plants once angular necrotic spots had begun to develop on mature leaves accompanied with an inward curling of the leaves prior to complete necrosis (Figure 1). These leaves were analysed by transmission electron microscopy using a Philips CM120 (Biotwin, the Netherlands).

Tissue samples were trimmed and diced and placed into 2-6% glutaraldehyde fixative made up in 0.05 M Na-cacodylate buffer for one hour. The tissue was then washed twice in 0.05 M Na-cacodylate buffer for 1 h and subsequently placed in osmium tetroxide for 2 h before rinsing twice in 0.05 M Na-cacodylate for 30 minutes. The diced samples were then dehydrated in a graded alcohol series (30%, 50%, 70%, 80%, 90% ethanol) for 10 minutes in each solution. The dehydration process was completed with two rinses of absolute ethanol for 15 minutes each. Discs were then embedded and polymerised using an Epon-Araldite mixture following two 30 minute washes in propylene oxide. For adequate infiltration, the discs were first placed into a propylene oxide Epon-Araldite mixture (3:1, v:v) for two hours, followed by a propylene oxide Epon-Araldite solution (1:1, v:v) for 2 h changed into propylene oxide : Epon-Araldite (1:3, v:v) for 8 h and finally pure Epon-Araldite for 24 h. Thereafter tissues were placed in moulds and polymerised at 70°C for 48 h.

Sections of tissue were cut from the polymerised discs using an ultra-microtome and placed onto 400 mesh copper grids and stained using lead citrate. The specimens were then viewed under the transmission electron microscope at varying magnifications and 80 to 100 KV for optimal resolution.

4.3   RESULTS AND DISCUSSION

Leaves of plants grown without Si exhibited the following symptoms: comparatively smaller size, downward curling leaf tips and gradual interveinal necrosis (Figure 1) compared with plants to which Si was applied (Figure 2). Silicon deficient leaves had necrotic spots (Figure 3) which eventually coalesced as the entire leaf senesced. These symptoms were similar to those described by Miyake and Takahashi (1983) as Si-deficiency in cucumbers. However, plants to which Si was applied exhibited stronger growth, larger leaves, and a lack of interveinal necrosis.

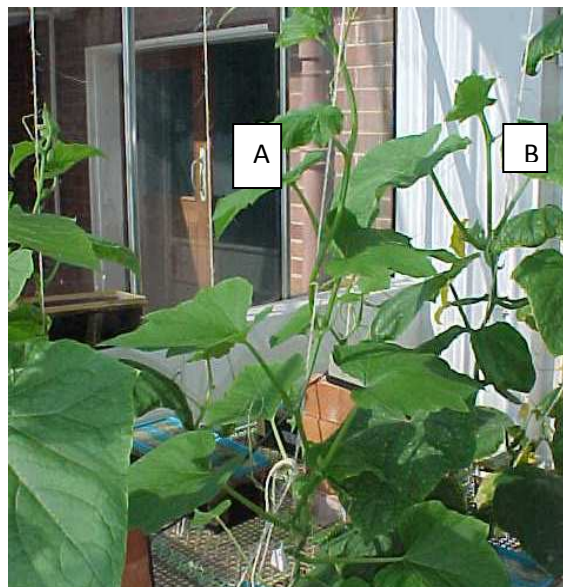Figure 1. Silicon deficient plants - curling of leaves and necrotic spots

Figure 2: Silicon supplied plant without symptoms (plant A) and plant without Si application (plant B)

Electron micrographs of non-Si treated plants showed loss of integrity of cell walls and cellular structures within the necrotic spots (Figure 4). However, tissue surrounding the necrotic region showed "flaccid cell walls", walls without any fixed structure or shape, almost finger-like in shape (Figure 5). Membranes of these cells also had large numbers of plasmodesmata, possibly to allow for the rapid removal of metabolites to other plant parts (Figure 6). Cells contained poorly defined organelles, possibly due to breakdown of organelle membranes. In Si-treated leaves no such plasmodesmata could be found and organelles were clearly distinguishable (Figure 7).

Figure 3: Leaf from Si deficient plant exhibiting necrotic spots.
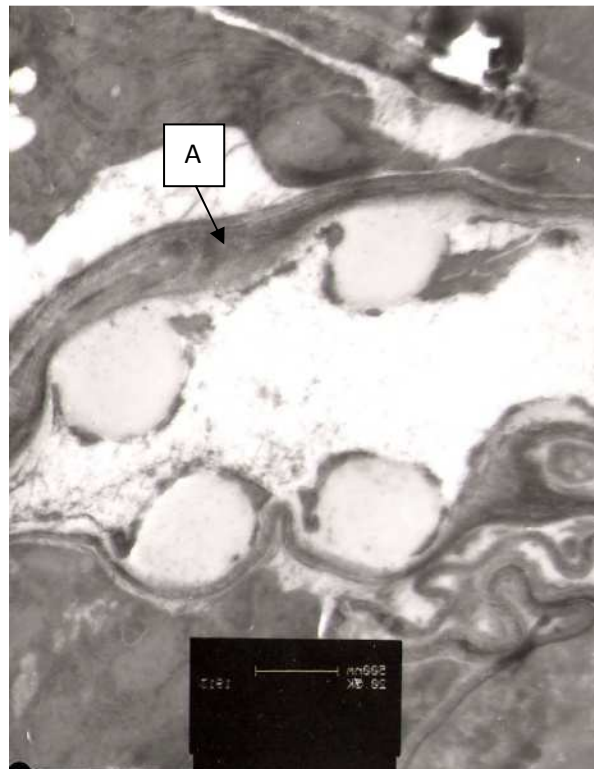

Figure 4: Electron micrograph of a mesophyll cell (A) in Si applied plants (magnification x3300)

As plasmodesmata typically allow rapid transportation of water and solutes between cells via the symplastic pathway (Mengel & Kirkby, 2001), it could be assumed that the increased presence of plasmodesmata in the cells, particularly of the mesophyll, is a response to the reduced lifespan of these cells. There is some suggestion that Si is deposited in the middle lamella along with cellulose providing the cell wall with additional strength. As Si-deficiency can reduce cell wall strength (Jones & Handreck, 1967), the lifespan of these structures is reduced and, therefore, rapid translocation of cell solutes along the symplastic pathway to recover solutes would be required for the removal of re-usable solutes from the cells entering necrosis.

Figure 5: Plasmolysed cells of Si deficient plants (magnification x20500)
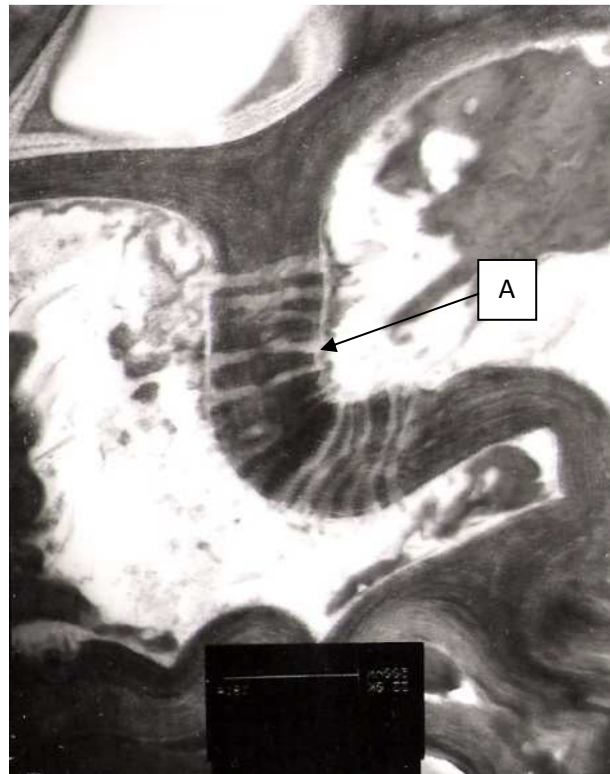
Figure 6: Plasmodesmata (A) in mesophyll cells of Si deficient plants (magnification x33000)
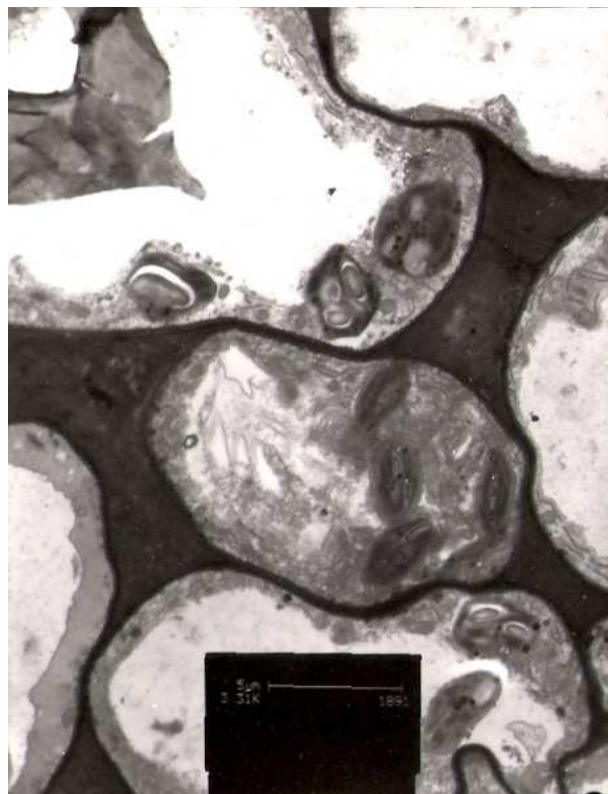

Figure 7. Clearly defined organelles in silicon sufficient cells (magnification x20500)

Similarly, leaf measurements showed the importance of regular Si supply to plant leaves. Foliar sprays applied on a three day interval significantly increased early leaf development (Figure 8 and 9), resulting in larger leaves developing quicker. Leaves of plants without Si application as well as less frequent applications did not exhibit this same trend, although as the leaves developed on these plants, the difference between them and the measured leaves of the frequently applied Si plants decreased, becoming insignificant three to four weeks after germination.

Figure 8: Effect of foliar Si application on leaf growth and development for Leaf 3 (No Si = without Si application, 3 Days = application every 3 days, 7 Days = weekly application of Si)

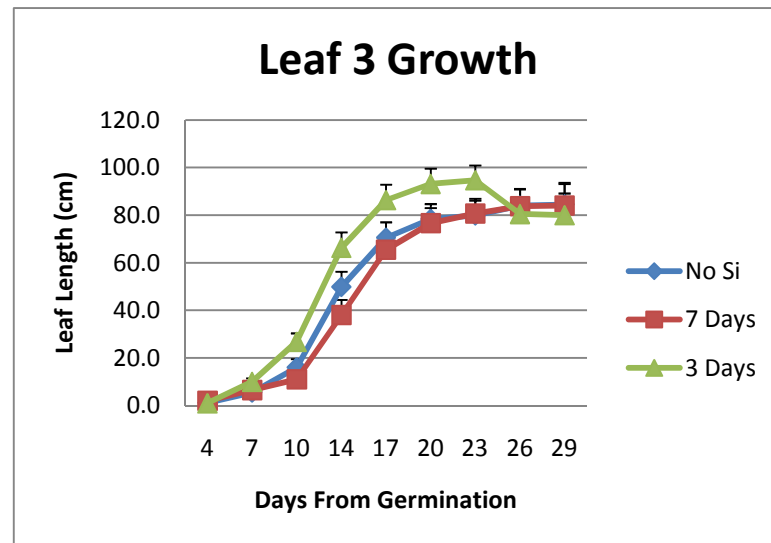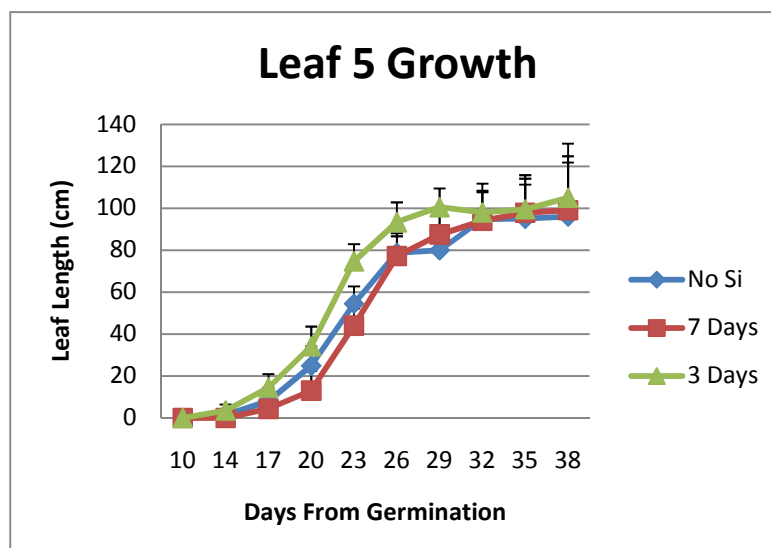
Figure 9: Effect of foliar Si application on leaf growth and development for Leaf 3 (No Si = without Si application, 3 Days = application every 3 days, 7 Days = weekly application of Si)

Plants which did not receive Si showed a significantly slower increase in leaf length of their third leaf than the 3-day treatment or the weekly Si spray (Figure 8 and 9). Surprisingly, although significant differences in leaf length were observed at day 10, 14 and 17, no differences between leaf length of the different treatments were detectable by day 22 (leaf 3 and 5) of leaf growth. These leaves had already matured due to quicker growth rates and had begun to senesce by the time the less frequently applied and nil Si treatment leaves achieved the same size. Since Si-treated leaves reached full expansion earlier and the higher Si treatment resulted in significantly longer leaves, these leaves also started to senesce earlier than the other treatments. Altogether, leaf length of fully expanded leaves in position 3 and 5 were not significantly different at full expansion between plant treatments.

Comparison of the number of aborted fruit by the plants, whether on a daily basis or overall, exhibited no significant differences between treatments. The total number of fruit held on the plant was also evaluated every three days from the start of fruit production at day 14 until the end of the experiment. Initially, the treatments with no Si or less frequently applied Si (every 7 days) showed significantly higher fruit counts on the first three days of counting. Thereafter, no significant differences were observed (Table 1). This impact on yield has also been reported by Woolley (1957) in tomatoes. Hodson & Sangster (2008) noted that, due to the ubiquitous nature of silica, it is very difficult to eliminate it from the nutrient solution and zero applications should be described as "silica minimal" plants.

Table 1. Number of harvested fruit from cucumber plants supplied with either no Si, or Si every 3 (3 days) or 7 (7 days). Treatments with common letters within a column are not significantly different from another.

	Days From Emergence								
Treatment	13	17	20	23	26	29	32	35	38
No Silicon	1.33 a	1.37 ab	2.33 a	2.83 a	3.67 a	5.67 a	5.33 a	7.33 a	9.00 a
7 Days	1.17 a	1.83 a	2.17 ab	1.67 a	3.67 a	5.33 a	6.83 a	9.00 a	8.33 a
3 Days	0.17a	0.17 b	0.67 b	1.00 a	3.50 a	4.50 a	6.00 a	6.67 a	7.33 a
Standard Error	0.59	0.50	0.52	0.67	0.72	0.78	0.94	1.04	1.19

The objective scoring of the plants every three days showed no significant differences until plants neared termination of the experiment. At this point, a final set of leaf measurements and

score ratings were conducted. Several significant differences between treatments were observed (Figure 10).

Silicon appears to play a structural role in plants, and, although there is still much discussion about its essentiality to plant growth and development (Zhu *et al.*, 2004), it appears to play a more essential role in monocotyledonous plants, particularly "wetland grasses" (Jones & Handreck, 1967). Dicotyledonous plants, such as cucumber, could be considered to be suitably evolutionary advanced not to require such levels of Si for optimal plant growth and development, however, the expression of Si-deficiency symptoms (Figure 10) illustrates a certain degree of Si dependence.
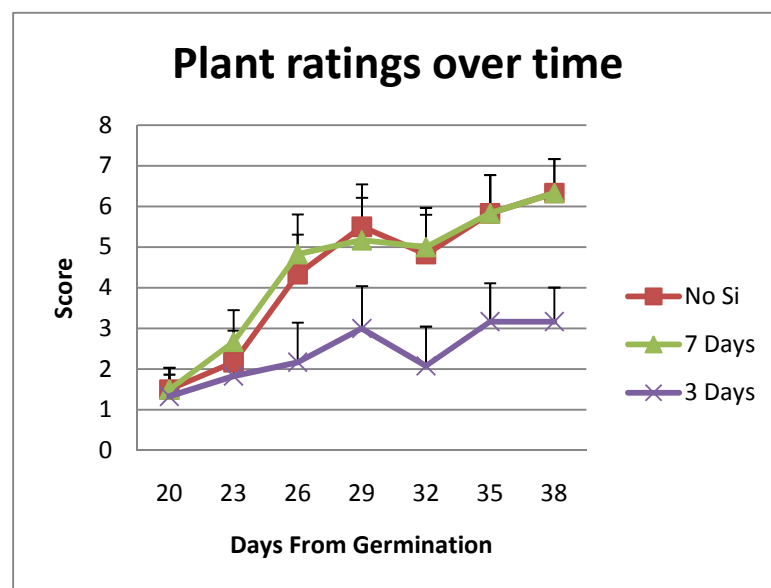

Figure 10: Plant rating over time of cucumber plants supplied with Si or not supplied with Si for 3 (3 days) or 7 (7 days) (Score 0 = healthy and 9 = dead).

Despite observing the symptoms described, it was expected that measurable differences would be realised between the treatments. The first significant difference that was recorded related to leaf length. These differences were measured in the early stages of leaf growth and development only, within the first 10 to 14 days of growth. Towards the end of this period, differences between treatments lost significance (Figures 8 and 9). The observed differences indicate that the added Si increased early leaf growth and development above non-applied treatments allowing for development of longer leaves earlier in the growth of the plants. Frequently applied Si has an effect on the growth and development of cucumber plants.

Average leaf length seemed to reduce towards Day 38 after germination (Figures 8 and 9), at the termination of the experiment. This was due to the senescence of some of the relatively long

leaves 3 and 5 closer to the end of the experiment. Although leaf senescence was observed in all treatments, it appeared to impact more on frequently applied Si more than on the other treatments. This was consistent for both leaves 3 and 5, but due to termination of the experiment, this trend could not be adequately evaluated and further investigation should be conducted to determine whether applied Si stimulates quicker leaf expansion but shortens the longevity of the leaf.

Applied Si did not appear to affect the production of fruit, their growth and development, final yield or whether the plants aborted more fruit. Whether this could be borne out over the long term would require further elucidation.

Objective rating of the condition of the plants on a scale of 1 to 10 showed a general deterioration in the condition of all the plants, but most specifically the treatments where Si was not applied or only applied every 7 days. Despite there being small differences from an early stage, (Figure 8), significant differences were only observed towards the end of the experiment.

Further elucidation is required to identify the role of Si in cell longevity and integrity as either a structural component or an integral part of cell processes. Although Si has been identified as a beneficial nutrient, its function as an essential element for cucumbers should also be investigated in light of its use within the growth and development of cucumber plants.

CHAPTER 5

CONCLUSIONS

This research has shown the importance of hydroponic systems in understanding the nutrition of plants, in particular cucumbers. This is particularly true for micronutrient studies and evaluations. However, design and management of the system are crucial to obtain accurate results from the research. Should this work be repeated, more frequent nutrient changes would assist in improving clarity in the measured results.

Manganese and Zn nutrition are vital to plant growth and development and the research established the impact of deficiencies on the growth and development of the cucumber plant. Whilst excessive levels of these nutrients do impact on the growth of the plants, toxic levels are much higher than adequately applied levels. Whether this is true for all crops will require further work, but it may also be a trait typical of cucumber plants to have a high tolerance to Mn and Zn. This research also illustrates the classic nutrient uptake curve for these nutrients from deficient to sufficient through luxury consumption to toxicity. It can be expected from this research that excessive applications of up to four times the nutrient requirement of Mn or Zn will not negatively affect a cucumber crop, and only when these levels are exceeded will the crop be significantly impacted. Whether this also holds true under soil conditions will require further studies, but no large variation from this theory is expected.

Deficiency and toxicity symptoms were established for both these nutrient elements and can be used as a guide to any grower with respect to micronutrient management. Whilst each nutrient has important roles within the growth and development of the plant, the essentiality of both these nutrients cannot be questioned. Each nutrient is involved in very specific processes or functions within the plant. The numerous measurements conducted during the course of these experiments highlight the intrinsic involvement of these nutrients in the growth and development of the plant. Many of the processes and functions of these nutrients within the plant have already been established. Ensuring adequate supply will result in good growth and development of a cucumber crop.

Whilst there are many essential elements, all elements do not act independently of one another. Each plant nutrient may have clearly defined roles within plant growth and development; however, many of these nutrients do interact with one another. This has been demonstrated to be the case with Mn and Zn where a combination of these elements resulted in a significantly improved

growth of cucumber plants. This further underlines the importance of applying all nutrients in their adequate levels to achieve optimal growth and development of the plant. Deficient or excessive levels of Mn or Zn will impact the ability of the plant to take up or effectively utilise the other nutrient as was exhibited in the deficiency trials. Commercially, therefore, ensuring adequate and consistent supply of these nutrients is essential to healthy crop production. However, more research is required to fully understand the amount of interaction between Mn and Zn in plant growth and development.

Silicon on the other hand, is only recently a more investigated nutrient and thought only to be essential to monocotyledonous plants. The research conducted has shown the importance of the supply of Si to cucumber plants. Si deficient conditions lead to shortened longevity of leaves, reduced leaf development and growth. Many of the functions and processes in which Si is involved have not been established, hence, the Si requirement by plants requires full elucidation. Si is an ubiquitous element and is commonly found as a contaminant of all water sources. For commercial agriculture, such contamination may supply fully the needs of the plants without supplemental application. However, this research has shown more regularly applied Si does result in improved plant growth and development, hence, further investigations are suggested to fully establish nutrient uptake curves, critical levels of application, the full role of the nutrient in the plant, efficiency of methods of application as well as cost-benefit evaluations of applying the nutrient as a standard application under commercial conditions.

Silicon is not classified as an essential element for plant growth and development, meaning that plants can complete their lifecycle without the addition of Si. However, Si is important for growth and development of plants and a full understanding of the nutrient and the roles which it plays will not just benefit the scientific community, but could lead to substantial savings and improved production in the commercial agricultural arena. Whilst foliar sprays were used in these experiments, the efficiency of such applications could be questioned due to the reduced solubility of such an element as well as the ability of the plant to absorb such a nutrient through the leaf cuticle. The benefits of foliar applications with respect to disease management in crops is well documented, its nutritional benefits through foliar application are yet to be elucidated.

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