INVESTIGATING THE EFFECT AND EFFICIENCY OF STEEL SLAG AS A SOURCE OF SILICON FOR PLANT UPTAKE

By

Adeel Dadabhay

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School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Sciences

University of KwaZulu-Natal

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Dissertation summary

Silicon (Si) is the second most abundant element on the planet, after oxygen, making about 25% of the Earth's crust. Since it exists in the Earth's crust, many plants can accumulate it in large concentrations, in amounts similar to macronutrients. Si enhances growth and yield of some annual and vegetable crops, promotes upright growth (stronger and thicker stems, shorter internodes), prevents lodging, promotes favourable exposure of leaves to light, provides resistance to bacterial and fungal diseases and decreases the effects from abiotic stresses such as: high or low temperatures, salinity, heavy metal and aluminium toxicity and water deficiency.

Si treatment effects on plant growth under disease and drought stress were analysed to determine the effectiveness of steel slag as a source of Si for plant uptake. Four Si treatments: slag products (SP1.7 and SP5.0); Agri-sil granular (AGS); and potassium silicate (Pots) were tested on rye grass, maize, zucchini, green pepper, broccoli and beans under disease and drought stress. Energy Dispersive X-ray (EDX) microanalysis was performed to determine the Si content within the leaves resulting from the different treatments over time. All treatments provided a positive Si uptake into the plant leaves. Maize had the highest rate of Si levels taken up into the leaves over a period of 180 days for all Si treatments, when compared to the other crops under disease stress. Si-treated plants accumulated Si into their leaves at a higher rate under disease stress than drought stress. Si treatments improved the growth of all test crops. Steel slag was an effective treatment for providing Si for the uptake in plants and to improve plant growth.

The effect of pre-harvest Si application to inhibit *Colletotrichum capsici* on post-harvest pepper fruit (*Capsicum annuum* L.) was analysed. Pepper fruit were harvested from pepper plants (*Capsicum annum* L. cv. Revelation) that were Si treated to provide Si for plant uptake. Si treatments used were: Pots, which was used as a positive control; SP1.7; SP5.0; and AGS. The area covered by infection (%) on the fruit was recorded every seven days for a period of 21 days, to determine the disease progress. All Si treatments significantly reduced the rate of infection by the pathogen. By day seven, the disease progress was inhibited, with a recorded area of infection being below 3.5% compared to the control, which was at 8.4%. By day 14, it was inhibited from

33.6% (control) to below 16% and by day 21, it was inhibited from 57.4% (control) to below 31% for all Si treatments. Area under the disease-progress curve (AUDPC) value (%days) was the lowest for the SP1.7 treatment, which means it enhanced post-harvest disease resistance by the greatest amount. SP5.0 had the highest AUDPC value from all Si treatments. Pre-harvest application of Si reduced post-harvest anthracnose disease in green pepper fruit.

The efficiency of steel slag as a source of Si for citrus and avocado uptake was analysed. Three different species: *Citrus sinensis* (Orange cultivars: Valencia and Navel); *Citrus limon* L. (lemon); and *Persea americana* L. (avocado) were used for this study. Five Si treatments were tested: Pots; AGS; Agri-sil liquid (ASL); SP5.0; and SP1.7. EDX was performed to determine the Si content within the leaves resulting from the different treatments over time. All Si treatments provided a positive uptake of Si into the citrus leaves. Valencia trees treated with the SP1.7 had the highest rate of Si taken up into the leaves, with an area under curve (AUC) value of 210.24%days, followed by SP5.0 with an AUC value of 195.48. SP1.7 and AGS provided the highest rates of Si uptake into navel orange leaves with AUC values of 187.02 and 187.92, respectively. Lemon trees treated with SP1.7 and AGS had the highest rates of Si taken up into the leaves. Citrus trees treated with SP1.7 had higher rates of Si taken up into the tree leaves, with the exception of the AGS treatment having the highest rate of Si taken up in lemon. Avocado trees treated with SP1.7 had the highest rate of Si taken up into the leaves, with an AUC value of 28.29. Steel slag was an efficient and effective source of Si for the uptake in citrus and avocado leaves.

Declaration

I, **Adeel Dadabhay**, declare that:

i. The research reported in this thesis, except where otherwise indicated, is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

iii. This thesis does not contain other persons' data, pictures, graphs or information, unless acknowledged as being sourced from other persons.

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v. Where I have produced a publication of which I am an author, co-author or editor, I have indicated in detail which part of the publication was actually written by myself and have fully referenced such publications.

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Adeel Dadabhay (Candidate)

Prof. M.D. Laing (Supervisor) ...  .....

Dr I. Basdew (Co-supervisor) 

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Dedication

I dedicate my work towards my loving wife, Aadila Dadabhay, for her support throughout my studies.

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Dissertation introduction

Silicon (Si) is the second most abundant element in the earth's crust after oxygen (Rodrigues and Datnoff, 2015). The earth's crust is largely composed of Si that is found as silicate minerals, aluminosilicates and various forms of silicon dioxide (Jones and Handreck, 1967). Despite the abundance of Si in soils, it is not an indication that there are sufficient quantities of soluble Si available for plant uptake (Jones and Handreck, 1967). Plants take up Si in the form of monosilicic acid (H_4SiO_4), which is present as a liquid and as an adsorbed phase of Si in soils (Jones and Handreck, 1967). The soil pH, clay content, minerals, organic matter, and Iron (Fe) and Aluminium (Al) oxides/hydroxides are components that have an influence on the concentration of monosilicic acid (Jones and Handreck, 1967). The fertilization process can rapidly increase the concentration of monosilicic acid in the soil solution, so Si fertilization had become a pivotal practice in areas with an intensive cropping system for those soils which inherently lack sufficient soluble Si quantities (Jones and Handreck, 1967).

The effects of Si on fungal pathogens, including the properties, spectrum of efficacy, and mode of action of Si, are still under intensive research. Under controlled hydroponic conditions, plant growth and development are affected by Si (Assis *et al*., 2013). When plants are exposed to various stresses, Si is evidently pivotal to plant health (Keeping *et al.*, 2013). Primarily, resistance to plant diseases through the uptake of Si is considered to be due to both an accumulation of absorbed Si in the epidermal tissue, and the stimulation of metabolic or pathogenesis-mediated host defence responses (Keeping *et al.*, 2013).

Drought stress results in a restriction in the uptake of water; Research shows that plants with Si maintained water uptake better than Si-deficient plants (Pereira *et al*., 2013). Si enhances yields under drought stress (Pereira *et al*., 2013). Wheat crops have an increased amount of hydrogen peroxide under drought conditions, when grown without Si applications (Pereira *et al*., 2013). Si enhanced the activities of superoxide dismutase and peroxidase in the wheat plant, creating a detoxifying mechanism (Pereira *et al*., 2013).

In agriculture, slags can be used as fertilizers and as a corrective of soil acidity (Ning *et al*., 2014). Slags are calcium and magnesium silicates, which show neutralizing action due to $Si0_3^{2-}$ base (Ning *et al*., 2014). Additionally, steel slags have been used as low-cost source to supply Si to rice plants (Ning *et al*., 2014).

The objectives of this study were to:

1. Investigate the efficiency of steel slag as a source of Si for uptake into plants.
2. Investigate the effect steel slag has on plant growth under disease and drought stress.
3. Investigate the effectiveness of steel slag as a pre-harvest Si treatment to control post-harvest anthracnose disease on green pepper fruit.
4. Investigate the efficiency of steel slag as a source of Si for uptake in citrus and avocado.

This thesis is presented in the form of five chapters. Individual chapters cover specific objectives of the study conducted. This dissertation follows a standard format that has been adopted by the University of KwaZulu-Natal because it facilitates the publishing of research out of the dissertation far more readily than the older monograph form of dissertation. As such, there is some unavoidable repetition of references, methods and some introductory information.

This research was undertaken in the Discipline of Plant Pathology, at the University of KwaZulu-Natal, Pietermaritzburg Campus, under the supervision of Prof. M.D. Laing and Dr. I. Basdew.

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Chapter One

Literature review

1.1 Introduction

The use of silicon (Si) in agriculture began in China 2000 years ago, where farmers incorporated rice straw with manure as a fertilizer to enhance plant performance and yield (Matichenkov *et al*., 2001). Si used to reduce blast on rice was first reported in 1917 by a plant chemist from Japan (Matichenkov *et al*., 2001). From the 1980s, Si's potential to decrease the intensity of many diseases has been researched for a wide variety of plant species (Matichenkov *et al.*, 2001). Si is known to play a major role in reducing plant diseases and improving drought tolerance (Kvedaras *et al*., 2010). Supplementing plants with Si is a simple, sustainable way to help maintain and enhance plant health in agriculture (Kvedaras *et al.*, 2010).

The earth's crust is largely composed of Si that is found as silicate minerals, aluminosilicates and various forms of silicon dioxide (Jones and Handreck, 1967). Despite the abundance of Si in soils, it is not an indication of the presence of sufficient quantities of soluble Si available for plant uptake (Jones and Handreck, 1967). Plants take up Si in the form of monosilicic acid (H_4SiO_4), which is present as a liquid and as an adsorbed phase of Si in soils (Jones and Handreck, 1967). The soil pH, clay content, minerals, organic matter, and iron (Fe) and aluminium (Al) oxides/hydroxides are components that have an influence on the concentration of monosilicic acid (Jones and Handreck, 1967). The fertilization process can rapidly increase the concentration of monosilicic acid in the soil solution, so Si fertilization had become a pivotal practice in areas with an intensive cropping system for those soils that inherently lack sufficient quantities of soluble Si (Jones and Handreck, 1967). The procedures established to determine the Si available for plants, the critical soil Si levels and soluble Si fraction in solid fertilizers were pivotal components contributing to the evolution in research on Si in agriculture in the twenty-first century (Teixeira *et al.*, 2017). These measurements were the key components required for the development and implementation of effective Si fertilizer management in crop production (Teixeira *et al*., 2017).

The disease intensity of numerous plant diseases caused by both soil-borne and seed-borne pathogens has been effectively decreased in agronomic and horticultural crops when soluble Si fertilizers are applied (Keeping *et al.*, 2013). Diseases such as wilts, root rots, and galling, caused by plant pathogens such as *Fusarium*, *Pythium*, *Rhizoctonia* and *Meloidogyne*, are less severe when Si is made available, resulting in a reduction in the rate of disease progress and a reduction in disease severity (Goussain *et al*., 2005). *Bipolaris oryzae*, which causes brown spot in rice and produces symptoms of severe grain discoloration, can be suppressed using Si (Han *et al.*, 2015).

Reductions in the intensities of plant diseases result in healthier plants, which has an overall effect in terms of enhanced growth. In the *Pyricularia oryzae* (rice) model pathosystem, the mechanical barrier formed from Si polymerization underneath the cuticle layer and in the cell walls was the initial hypothesis used to investigate the role Si played in plants, in order to result in a reduction in the number and size of blast lesions (Hou and Han, 2010). Later research has provided evidence that disease resistance effect due to soluble Si may occur through priming of the host plant resistance mechanisms. The presence of Si results in a potentiated activation of the phenylpropanoid pathway, hence an increase in soluble phenolics and lignin within the plant (Camargo *et al*., 2008). Chitinases, β -1,3-glucanases, and defence enzymes are maintained at higher levels during the infection phases, and the transcription of defence-related genes occur at higher rates (Camargo *et al*., 2008). When Si-treated plants are exposed to a pathogen, this results in an activation of the antioxidant metabolism, which results in a suppression of the cytotoxic effect of the reactive oxygen species that causes lipid peroxidation in the cell membrane (Camargo *et al*., 2008). Leaf gas exchange parameters of Si-treated plants are higher upon pathogen infection for crops at the physiological stages, which indicates the ameliorating effect of Si on photosynthesis (Camargo *et al*., 2008). Despite the advances in research on the roles Si plays in disease resistance in plants, the exact mechanisms by which Si influences the plant physiology through the stimulation of host defence mechanisms still requires further investigation at the genomics, proteomics, and metabolomics levels.

1.2 History

The agricultural use of Si began more than 2000 years ago in China (Matichenkov *et al*., 2001). The emperor ruling at the time decreed the incorporation of the use of rice straw and manure as a fertilizer to enhance the performance and yield of the crops (Matichenkov *et al.*, 2001). Around the 1800s, botanists began to measure the elemental composition of several species of plants and discovered Si present in substantial quantities in these plants in relation to other elements (Matichenkov *et al*., 2001).

Despite these discoveries, Si has been regarded as unimportant for plant development for many years. Davy (1819) was one of the initial scientists to investigate the form of Si present in the epidermis of various monocots. He theorized that the plant's epidermis was siliceous and that this played a role in supporting and protecting the plant from biotic stresses. Liebig (1840) hypothesized that Si was involved in cereal stalk rigidity and that a deficiency in Si resulted in wheat lodging. He also investigated the use of sodium silicate as a fertilizer by conducting research with this source of Si on sugar beet development under greenhouse conditions. Kreuzhage and Wolff (1884) initially studied microscopically the distribution and specific location of Si bodies in different leaf tissues of oats. Due to evidence showing that the cell lumen contained a high level of Si, they suggested that this element might be involved in resistance against plant diseases.

1.3 Silicon in soils

After oxygen, Si is the second most abundant element present in the earth's crust (Almeida *et al*., 2009). The concentrations of Si range from 23–46.5% in rocks (Almeida *et al*., 2009). Si in smaller concentrations has also been found in carbonaceous rocks such as limestone and carbonites (Almeida *et al*., 2009). Si concentrations of above 46% have been recorded to be present in silcretes (Almeida *et al*., 2009). Si concentrations in the petrocalcic horizon are found to be lower than 8% when compared to silcretes (Almeida *et al*., 2009). Highly weathered minerals such as Oxisols have concentrations of Si lower than the petrocalcic horizon (Almeida

et al., 2009). Despite a general abundance of Si in soils, certain soils contain low concentrations of Si and especially Si in the form available for plant uptake (Almeida *et al*., 2009). Highly weathered Oxisols and Ultisols soils are acidic, and lack base saturation and mineral content, with extremely low levels of Si (Almeida *et al*., 2009). Soils that have a large component of quartz sand and have been used intensively for the production of crops are typically low in Si concentrations which are in a form for plant uptake (Almeida *et al*., 2009).

Si is grouped into three phases: the liquid phase, the adsorbed phase and the solid phase (Costa and Moraes, 2006). The compositions of these different phases are detailed in the classification of Si compounds in soils that is presented in Fig. 1.1.

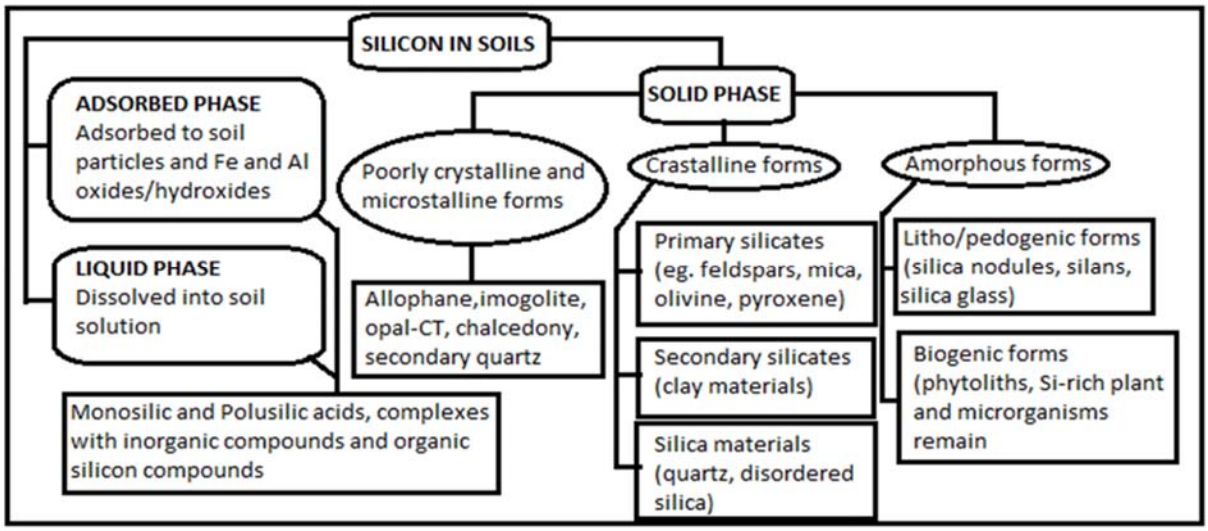

Figure 1.1: Different fractions of Si in soils (Tubana and Heckman, 2015).

1.3.1 Silicon cycle in soil

The solid, liquid, and adsorbed phases of Si are the key components of the Si cycle in soil (Fig. 1.2) (Rodrigues and Datnoff, 2015). The liquid Si phase contains monosilicic acid and is the only form that is absorbed by plants and microorganisms. It is estimated that 60–200 Tmol Si per year is stored in plants (Rodrigues and Datnoff, 2015). Si can be added to soils with applications of manure and compost, and the decomposition of Si-rich manure increases the level of Si (Rodrigues and Datnoff, 2015). The interaction of organic matter and Si is an extremely rare

occurrence but a formation of colloidal aluminium-Si polymers at several soil solution pH levels can occur (Rodrigues and Datnoff, 2015). The liquid phase is regulated by a number of processes which affect the chemical properties of Si; these are as follows: the dissolution of Si that consists of primary and secondary minerals; the absorption of Si in the soil solution by the plants and microorganisms; the desorption from various solid phases; the preservation of the stable Si in the soil; leaching; and additives due to agricultural practices (i.e., fertilization, irrigation, manure application) (Rodrigues and Datnoff, 2015). Irrigation water which is in its natural state may contain various forms of Si. The contribution of Si to the soil solution from the atmosphere is a fraction compared with the other Si inputs to the soil-plant system (Rodrigues and Datnoff, 2015).

1.4 Silicon in plants

1.4.1 Silicon uptake, transport and deposition in plant

Plants take up Si from the soil solution in the form of monosilicic acid (Cherry *et al*., 2012). The mechanisms by which the Si is absorbed by plants are described in three ways: active, passive and rejective (Cherry *et al*., 2012). The quantity of Si taken up by the active mechanism is generally greater than that estimated based on the mass flow and is correlated with the density of Si transporters in the rooting system that facilitate the absorption process across the integrated membranes of the rooting system's cells (Cherry *et al*., 2012). Transporters mediate the radial transport and the xylem loading of Si in rice (Han *et al*., 2016). The transporters have been identified and coded by low-Si genes (*Lsi1* and *Lsi2* in roots and the *Lsi6* in shoots) (Han *et al*., 2016). The *Lsi1* gene may encode a membrane protein similar to the aquaporins proteins (Han *et al*., 2016). The quantity of Si uptake by the plant via the passive mechanism is dependent on mass flow (Cherry *et al*., 2012). In the rejective mechanism, the accumulation of monosilicic acid is a result of the low concentrations of Si that are absorbed by plants (Cherry *et al*., 2012).

Plant species may be categorized based on their levels of Si uptake (Cherry *et al*., 2012). The plants that rely mainly on the active, passive or rejective mechanisms were categorized as high,

intermediate- or non-accumulators, respectively (Cherry *et al*., 2012). High-accumulator plants have Si content in the shoot that ranges from 1.0% to 10% dry weight and are generally monocotyledons (Rodrigues and Datnoff, 2015). Due to the high-accumulators' efficient Si-

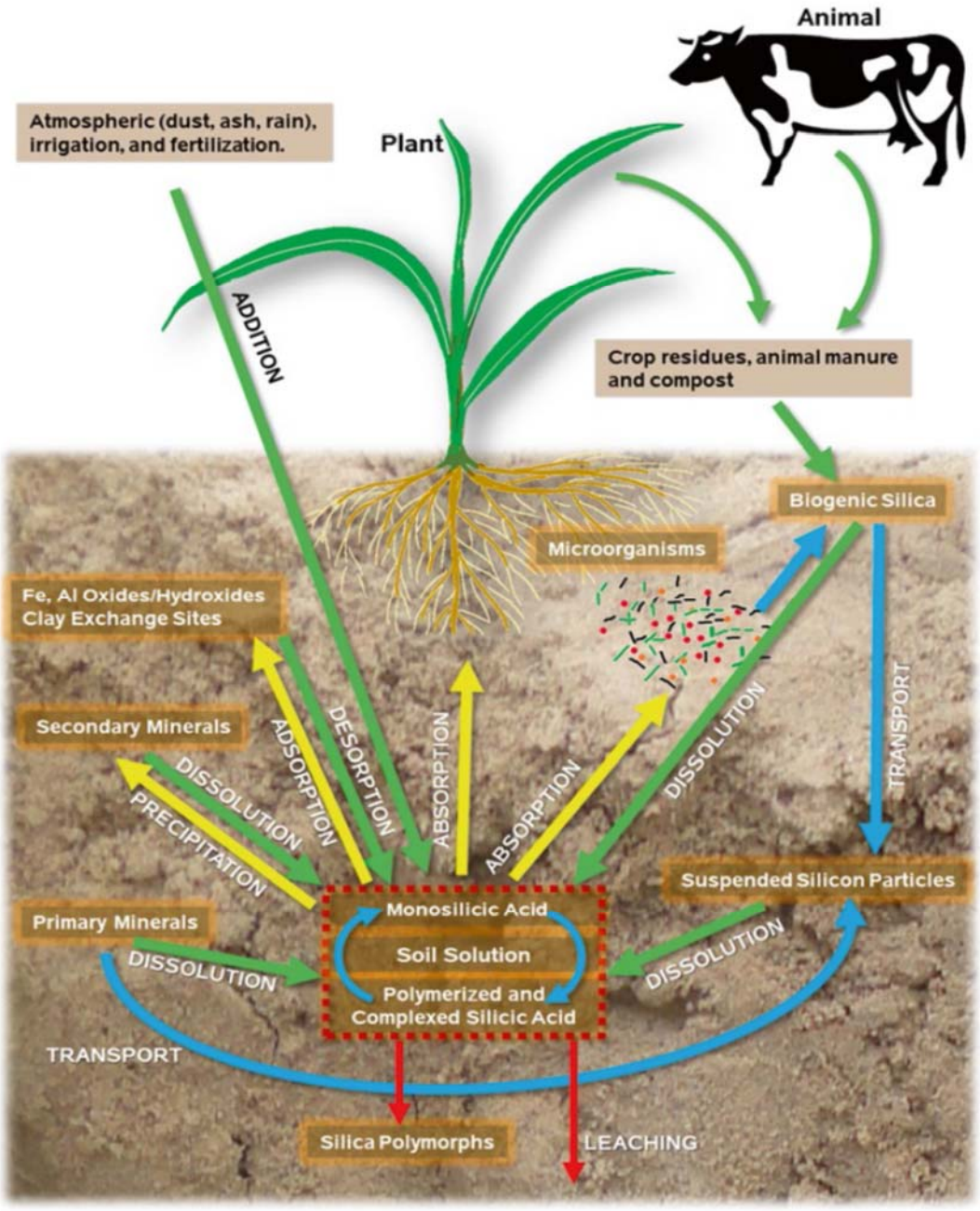

Figure 1.2: Comprehensive cycle of Si in soil (green arrows represent the transformation processes which raise Si concentrations in the soil solution; yellow arrows represent the

transformation processes which reduce Si concentrations in the soil solution; red arrows represent processes that result in Si loss from the soil system or production of a stable, plant-unavailable form of Si; blue arrows represent the transformation processes of Si into a silica pool that contributes this element into the soil solution) (Tubana and Heckman, 2015).

uptake system, the Si levels taken up by the plant from the soil are higher than the uptake of most of the essential macro- or micronutrients (Rodrigues and Datnoff, 2015). Most dry land Gramineae plants are intermediate-accumulators with a shoot Si content that ranges between 0.5% and 1.5% dry weight (Rodrigues and Datnoff, 2015). Dicotyledons typically accumulate less than 0.2% shoot dry weight Si, and these are categorized as the low-accumulator group (Rodrigues and Datnoff, 2015). A lack of specific transporters to facilitate the radial transport and the xylem loading is described to be correlated with the low accumulation of Si in this group (Rodrigues and Datnoff, 2015). A passive diffusion mechanism is responsible for the transport of Si from cell to cell (Liang *et al*., 2005).

Phytoliths are vascular bundles present in silica cells and present in silica bodies in bulliform cells, fusoid cells or pricklehairs in rice, wheat, and bamboo (Rodrigues and Datnoff, 2015). Phytoliths are classified as biogenic opal (Si-O-Si bonding) (Rodrigues and Datnoff, 2015). Si dioxide precipitation occurs generally in the epidermis of the shoots in plants (Rodrigues and Datnoff, 2015). The deposited silica is described as immobile and does not get transported to the meristematic tissues (Rodrigues and Datnoff, 2015). Transpiration is a pivotal process in Si transportation and deposition in plants; hence, the duration of plant growth correlates with the concentration of Si (Camargo *et al*., 2008). Older leaves have been found to contain larger concentrations of Si than younger leaves (Camargo *et al*., 2008).

1.4.2 Effects on plant growth

Much research has reported the benefits of Si on the growth of a wide variety of agronomic and horticultural crops. The beneficial effects of Si are more obvious when plants are under biotic or abiotic stresses. The beneficial effects of Si on plant growth and development are based on a

number of different mechanisms which include: the formation of a protective outer layer composed of silica deposits; the reactivity of the absorbed Si with the heavy metals ions; other compounds within plants; and the metabolic functions of Si in stressed plants (Rodrigues and Datnoff, 2015).

1.5 Disease stress

1.5.1 Mechanisms of silicon-enhanced resistance

The effects of Si on fungal pathogens, including the properties, spectrum of efficacy, and mode of action of Si, are still under intensive research. Under controlled hydroponic conditions, plant growth and development are affected by Si (Assis *et al*., 2013). When plants are exposed to various stresses, Si is evidently pivotal to plant health (Keeping *et al.*, 2013). Primarily, resistance to plant diseases through the uptake of Si is understood to be due to both an accumulation of absorbed Si in the epidermal tissue, and the stimulation of metabolic or pathogenesis-mediated host defence responses (Keeping *et al.*, 2013).

1.5.1.1 Physical defence

Si transported and deposited on the tissue surface serves as a physical barrier that protects plants from fungal infection and pests (Liang *et al*., 2005). The increase in resistance has been correlated with several factors: a dense layer of silicified cells in the epidermis of the leaves; a silica layer below the cuticle; a double cuticle layer; a silicon-cellulose membrane; and papilla formation (Liang *et al*., 2005). Si inhibits physical penetration by pathogenic fungi and pests, strengthens plants' mechanical structure, and decreases plant cells' susceptibility to enzymatic degradation by fungal pathogens (Liang *et al*., 2005).

Beneath the cuticle of leaves, after polymerization of monosilicic acid, a layer of silica forms, which is believed to be pivotal for inhibiting pathogen penetration (Malav and Ramani, 2015). Si forms complexes with organic compounds in epidermal cell walls; hence, this increases the resistance to degradation by enzymes produced by pathogenic fungi (Malav and Ramani, 2015).

Si is associated with lignin-carbohydrate complexes within the epidermal cell walls (Malav and Ramani, 2015).

It was reported that silicified epidermal cell walls correlate with a decrease in severity of the blast disease (*Magnaporthe grisea* L.) in susceptible and partially resistant rice cultivars (Yang *et al*., 2014). The thickness of the epidermal cell wall is not significantly influenced by the presence of Si (Yang *et al*., 2014). Foliar-applied Si on cucumbers were believed to have formed a physical barrier and had an osmotic effect (Cherif *et al*., 1994). It was concluded that Si in the rice leaf epidermis improved resistance against *M. grisea* appressorial penetration (Yang *et al*., 2014). Prophylactic effect against powdery mildew was lost when Si uptake was interrupted in cucumber plants (Cherif *et al*., 1994). These authors concluded that silicified epidermal cell walls in leaves are a pivotal factor for the decrease in severity of fungal plant diseases. However, resistance to fungal pathogens in plants treated with Si is much more complex than simply a physical resistance, and root-applied Si was shown to result in enhanced systemic acquired resistance when crops were infected by powdery mildew (Christalcatalani *et al*., 2017).

1.5.1.2 Biochemical defence

The enhancement of biochemical resistance is due to increased activity of defence-related enzymes in leaves (polyphenoloxidase, peroxidase, phenylalanine ammonia-lyase, and glucanase); increased production of antifungal compounds (lignin, flavonoids, phytoalexins pathogenesis-related proteins); and an activation of various plant defence-related genes (Correa *et al*., 2005). When necrotizing pathogens infect a plant, many plants develop an enhanced resistance against further pathogen attack, which is referred to as systemic acquired resistance (SAR) (Rodrigues and Datnoff, 2015). Two mechanisms, which play a role in increasing the enzyme activity and antifungal compounds due to Si treatments on plants, induce defence responses similar to SAR (Rodrigues and Datnoff, 2015).

There are possibly other biochemical and physiological mechanisms which play a role in the Si-mediated resistance of plants to diseases. Higher levels of salicylic acid, jasmonic acid, and ethylene have been shown to be induced by Si enhancements in a number of host-pathogen interaction (Yang *et al*., 2014). These include powdery mildew of *Arabidopsis* caused by *Golovinomyces cichoracearum* and rice-brown spot caused by *Cochliobolus miyabeanus* (Yang *et al*., 2014).

1.5.1.3 Defence-related enzymes

Defence-related enzymes are pivotal to disease resistance. Several studies have shown that reduced disease intensity in the Si-treated plants was correlated with increased levels of defensive enzymes activity. Si drives a priming process for the plant to accumulate defence-related enzymes in plant leaves, which are then induced by fungal infection, resulting in a faster, stronger defence reaction by treated plants.

1.5.1.4 Molecular mechanism

Si serves as a modulator of host resistance to pathogens (Debnath *et al*., 2010). The biochemical and physiological mechanisms that are potentiated by Si are complex phenomena (Debnath *et al*., 2010). Resistance to infection is acquired during the infection process by the expression of a protein rich in proline integrated with silica at the site of pathogen penetration (Debnath *et al*., 2010).

Silicon triggers metabolite biosynthetic pathways such as the phenylpropanoid and terpenoid pathways for the accumulation of antimicrobial compounds or phytoalexins (Debnath *et al*., 2010). Silicic acid is involved in local and systemic resistance (Debnath *et al*., 2010). Genome-wide research on tomato, rice, *Arabidopsis* spp. and wheat grown in soil treated with Si and in relation to non-treated control plants, has demonstrated a differential expression of numerous genes involved in the host resistance (Almeida *et al*., 2009; Filgueiras *et al*, 2011; Nascimento *et al*., 2018).

1.5.1.5 Silicon effect on plant diseases

Plant pathogens, such as those causing damping-off, crown and root rot in horticultural crops, cause major reductions in crop quality and yield (Rodrigues and Datnoff, 2015). Several pathogens are known to have disease intensities reduced when plants have obtained adequate tissue level of Si (Rodrigues and Datnoff, 2015). Si is also known to decrease the intensity of numerous plant diseases caused by biotrophic, hemibiotrophic and necrotrophic pathogens in many crops of great economic importance (Rodrigues and Datnoff, 2015). Table 1.1 provides a list of plant diseases in horticultural and agronomic crops that were affected by Si treatments.

Table 1.1: Effect of silicon on plant diseases that affect various crops (Rodrigues and Datnoff, 2015).

Crop	Pathogen	Disease	Effect
Asparagus	*Phomopsis asparagi*	Stem blight	Positive
Avocado	*Phytophthora cinnamomi*	Phytophthora root rot	Positive
Banana	*Mycosphaerella fijiensis*	Black sigatoka	Positive
	Cylindrocladium spathiphylli	Root rot	Positive
	Meloidogyne javanica	Root-knot nematode	Positive
Barley	*Alternaria* spp.	Black point	Positive
	Blumeria graminis	Powdery mildew	Positive
Bean	*Colletotrichum lindemuthianum*	Anthracnose	Positive
	Pseudocercospora griseola	Angular leaf spot	Positive
Bermuda grass	*Bipolaris cynodontis*	Leaf spot	Positive
Coffee	*Meloidogyne exigua*	Root-knot nematode	Positive
	Hemileia vastatrix	Coffee leaf rust	No effect
	Cercospora coffeicola	Brown eye spot	No effect
Cotton	*Ramularia areola*	Ramularia leaf spot	No effect
	Ramularia gossypii	Areolate mildew	Positive
Cucumber	*Podosphaera xanthii*	Powdery mildew	Positive
	Colletotrichum orbiculare	Anthracnose	Positive
	Corynespora citrullina	Leaf spot	Positive
	Botrytis cinerea	Gray mold rot	Positive
	Didymella bryoniae	Black rot	Positive
	Pythium ultimum	Crown and root rot	Positive

	Pythium aphanidermatum	Crown and root rot	Positive
Eucalyptus	*Oidium eucalypti*	Powdery mildew	Positive
Grape	*Uncinula necator*	Powdery mildew	No effect
Kentucky bluegrass	*Sphaerotheca fuliginea*	Powdery mildew	Positive
Lettuce	*Bremia lactucae*	Downy mildew	Positive
Melon	*Podosphaera xanthii*	Powdery mildew	Positive
	Trichothecium roseum	Pink rot	Positive
	Fusarium semitectum	Fusarium	Positive
Maize	*Ustilago maydis*	Corn smut	Positive
	Pythium aphanidermatum	Pythium root rot	Positive
	Fusarium moniliforme	Stalk rot	Positive
Pea	*Mycosphaerella pinodes*	Leaf spot	Positive
Peach	*Monilinia fructicola*	Brown rot	Positive
Pearl millet	*Sclerospora graminicola*	Downy mildew	Positive
Pepper	*Phytophthora capsici*	Phytophthora blight	Positive
Perennial ryegrass	*Microdochium nivale*	Fusarium patch	Positive
	Pyricularia oryzae	Gray leaf spot	Positive
Potato	*Phytophthora infestans*	Late blight	No effect
Pumpkin	*Sphaerotheca xanthii*	Powdery mildew	Positive
Rice	*Meloidogyne* spp.	Root knot nematodes	Positive
	Pyricularia oryzae	Leaf and panicle blast	Positive
	Bipolaris oryzae	Brown spot	Positive
	Rhizoctonia solani	Sheath blight	Positive
	Monographela albescens	Leaf scald	Positive
	Magnaporthe salvinii	Stem rot	Positive
	Xanthomonas oryzae	Bacterial leaf blight	Positive
Rose	*Sphaerotheca pannosa*	Powdery mildew	Positive
Rye	*Erysiphe graminis*	Powdery mildew	Positive
Sorghum	*Colletotrichum graminicola*	Anthracnose	Positive
Soya bean	*Phakopsora pachyrhizi*	Asian soya bean rust	No effect
	Phytophthora sojae	Phytophthora root rot	Positive
Strawberry	*Pestalotia longisetula*	Pestalotia leaf spot	Positive
	Botrytis cinerea	Gray mold	No effect
	Colletotrichum acutatum	Anthracnose fruit rot	Positive
Sugar cane	*Puccinia melanocephala*	Rusts	No effect

	Leptosphaeria sacchari	Ring spot	Positive
Tobacco	*Belladonna mottle virus*	BdMV	Negative
	Tobacco mosaic virus	TMV	No effect
	Tobacco ringspot virus	TRSV	Positive
Tomato	*Oidiopsis sicula*	Powdery mildew	Positive
	Phytophthora infestans	Late blight	No effect
	Pythium aphanidermatum	Pythium root rot	Positive
	Ralstonia solanacearum	Bacterial wilt	Positive
Wheat	*Fusarium* spp.	Foot rot	Positive
	Blumeria graminis	Powdery mildew	Positive
	Septoria nodorum	Septoria leaf blotch	Positive
	Pyricularia oryzae	Leaf blast	Positive
	Puccinia triticina	Leaf rust	Negative
	Drechslera tritici-repentis	Yellow spot	Negative
	Oculimacula yallundae	Eye spot	Positive
	Puccinia spp.	Rusts	No effect
	Bipolaris sorokiniana	Spot blotch	Positive
Zucchini	*Podosphaera xanthii*	Powdery mildew	Positive

1.6 Drought stress

1.6.1 Drought is an abiotic stress

Drought causes losses in agriculture, reducing growth and yields of agricultural crops. Drought is an abiotic stress which results when precipitation is below the minimum requirement for plant productivity.

1.6.2 Drought limits plant growth

Drought has a direct effect on the activity of photosynthesis of plants, negatively impacting the photosynthetic pigments and their components (Masoumi *et al*., 2010). Drought lowers the activity of carboxylation of Rubisco, the pivotal enzyme involved in the photosynthetic mechanisms (Masoumi *et al*., 2010). Drought in plants causes: an accumulation of reactive oxygen species in plants which disrupts the cell membrane, protein, nucleic acid, and lipids of plant cells, resulting in the death of cells; a diminution in stomatal conductance and an

escalation in the foliar temperature, which results in reduction of the photosynthetic rate due to the denaturing of different enzymes; a reduction in plant growth rate, osmotic potential, and leaf water potential; and a decrease in seed yield and seed quality (Masoumi *et al*., 2010).

1.6.3 Role of silicon on plant growth under drought

Si increases the activity of antioxidants, including superoxide dismutase, peroxidase, ascorbate peroxidase, glutathione reductase and non-enzymatic antioxidants (cysteine, glutathione and ascorbic acid (Sonobe *et al.*, 2010). Superoxide dismutase detoxifies the reactive oxygen species by converting them into hydrogen peroxide. Si enhances water availability to plants and enhances the dry matter contents under drought conditions (Pereira *et al*., 2013).

Drought stress results in a restriction in the uptake of water. However, plants with Si augmentation maintain water uptake better than Si-deficient plants (Pereira *et al*., 2013). Si enhances yields under drought stress (Pereira *et al*., 2013). Wheat crops have an increased amount of hydrogen peroxide under drought conditions when grown without Si applications (Pereira *et al*., 2013). Si enhanced the activities of superoxide dismutase and peroxidase in the wheat plant, creating a detoxifying mechanism (Pereira *et al*., 2013).

1.6.4 Mechanisms of silicon-mediated alleviation of drought stress in plants

1.6.4.1 Increased uptake of essential nutrients

All plants need essential nutrients in adequate amounts to provide mechanical, biochemical, and physical strength to the plants (Masoumi *et al*., 2010). Nutrients in plants become imbalanced under abiotic stresses (Masoumi *et al*., 2010). Silicon plays a pivotal role in the uptake of nutrients that are restricted due to drought (Masoumi *et al*., 2010).

1.6.4.2 Regulation of gas exchange attributes

Stomata regulate the water level inside the plant and conserve water by controlling the transpiration rate (Ahmed *et al*., 2011). Si application enhances the net stomatal conductance in plants (Ahmed *et al*., 2011). Enhancement of Si accumulation in plants increases their

tolerance against drought by increasing the carbon dioxide accumulation, reducing the transpiration rate, and maintaining the leaf water potential by leaf adjustment (Sonobe *et al.*, 2010). Si affects the stomata of plants; therefore, it improves the water use efficiency of drought-stressed plants by lowering the transpiration rate of the plant leaf (Sonobe *et al.*, 2010).

1.6.4.3 Osmotic adjustments

Plants can maintain their optimum water potential under drought stress by osmotic adjustment (Romero-Arnada *et al*., 2006). Si causes the water potential inside the leaves to increase, due to enhancements of leaf thickness (Romero-Arnada *et al*., 2006). Si deposition inside the leaf inhibits the water molecules leaving the leaf surface, so these plants maintain their water contents under drought conditions (Romero-Arnada *et al.*, 2006).

1.6.4.4 Modification in plant structure

Modification in certain attributes of plants, like regulating leaf size, enhancing root length, or rolling of leaves, aids in maintaining the osmotic adjustment to counter water deficiency (Moustakas *et al*., 2011). Application of Si partially alleviates the drought stress by altering the plant structure (Moustakas *et al*., 2011). Si application of calcium and magnesium silicates in plants increases the plant's height, resulting in improved yields, and reducing the stem lodging under drought conditions (Abdalla and El-Khoshiban, 2007). Si application enhances the water potential of plants under drought stress through the formation of a silica-cuticle double layer on the leaves' epidermal cells (Abdalla and El-Khoshiban, 2007).

1.7 Introduction to sources of plant-available silicon

Si sources for agricultural processes must demonstrate a number of features: high soluble Si content; low cost; balanced ratios and quantities of calcium and magnesium; enhanced phosphate mobility; suitable physical properties; simple application requirements; and the absence of heavy metals (Cha *et al*., 2006). For effective use of Si fertilizer in agricultural

management, an adequate knowledge of physical and chemical characteristics of Si sources, rates, and methodologies for application is required (Cha *et al*., 2006).

1.7.1 Steel slag as a source of silicon for plants

Si-rich, metallurgical slags have been evaluated; due to the high temperatures used in iron-making and steelmaking processes, Si is released from a crystalline form to a reactive form (Ning *et al.*, 2016). This results in larger quantities of soluble Si forming, which can hence be absorbed by plants (Ning *et al.*, 2016).

The slag produced in the hot metal desiliconization process contains primarily silica, and it has been used to develop potassium silicate fertilizers (Deus *et al*., 2018; Ning *et al.*, 2016). Development through the addition of potassium helps the desiliconization slag dissolve in water and in the organic acids released by plant roots (Ning *et al.*, 2014). The potassium in the fertilizer is released at a very low rate and is efficiently absorbed by the plant (Ning *et al.*, 2014). In the desiliconisation process of the molten metal, the potassium carbonate (K_2CO_3) is continuously added into a ladle containing the hot metal (Ning *et al.*, 2014). The uniformly melted slag that is recovered from the hot metal ladle is solidified by a process that involves cooling and granulizing the slag (Deus *et al*., 2018; Ning *et al.*, 2014). This fertilizer has been demonstrated to be as effective as other commercial potassium silicate fertilizers and integrated potassium chloride-calcium silicate fertilizers (Deus *et al*., 2018; Ning *et al.*, 2014).

1.8 Conclusion

Steel slags have been applied as a calcium silicate fertilizer where plant-available Si is deficient in the soil and is recognized as a cost-effective amendment to improve plant growth (Ning *et al*., 2016). Despite the recognition of its uses, the opportunity to research the full potential of steel slag and its role in plant growth is open.

Si has numerous amounts of benefits for plants and steel slag as a source of Si paves the way to a possibility of ensuring high economic value to the agricultural industry (Deus *et* al., 2018).

Only 10% of steel slag produced as a byproduct is recycled hence using steel slag in the agricultural industry is a benefit to the environment (Ning et al., 2016).

Steel slag provides an opportunity to damage to the environment while simultaneously increasing food security by improving plant growth.

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Chapter Two

Determining the effectiveness of steel slag as a source of silicon using Energy Dispersive X-ray (EDX) microanalysis

Reductions in water availability required for agriculture has been stimulated by global climatic changes. Drought and disease stresses are two factors influencing crop growth, development, and yield processes. An application of silicon (Si) is known to improve plant growth and health under abiotic and biotic stresses. Si taken up into plant leaves over time, and Si treatment effects on plant growth under disease and drought stress, were analysed in this study to determine the effectiveness of steel slag as a source of Si for plant uptake. Four Si treatments: slag products (SP1.7 and SP5.0); Agri-sil granular (AGS); and potassium silicate (Pots) were tested on rye grass, maize, zucchini, green pepper, broccoli, and beans under disease and drought stress. Energy Dispersive X-ray (EDX) microanalysis was performed to determine the Si content within the leaves resulting from the different treatments over time. All treatments provided a positive Si uptake into the plant leaves. Maize had the highest rate of Si levels (>250%days) taken up into the leaves over a period of 180 days for all Si treatments, when compared to the other crops under disease stress (<250%days). Si-treated plants accumulated Si into their leaves at a higher rate under disease stress than drought stress. Si treatments improved the growth of all test crops. Steel slag was an effective treatment for providing Si for the uptake in plants and to improve plant growth.

2.1 Introduction

The greatest reduction in water availability required for agriculture has been stimulated by global climatic changes (Sonobe *et al.*, 2010). Drought and disease stresses are two factors influencing crop growth, development, and yield processes (Masoumi *et al*., 2010). Drought stress is responsible for about a 20% decrease in crop yield worldwide (Masoumi *et al*., 2010). A decrease in leaf growth and an increase in root and shoot ratio as a consequence of drought stress have been observed in many plant species (Sonobe *et al.*, 2010).

In plants, drought stress leads to a loss of turgor as well as stomatal closure, which affects the photosynthetic apparatus (Pereira *et al*., 2013). Stomatal and nonstomatal limitations are considered to be the component reducing photosynthetic rates under drought stress (Pereira *et al*., 2013).

Plant diseases are known to reduce yield and crop quality by impeding the physiological functions of plants (Rodrigues and Datnoff, 2015). Improving and enhancing productivity under various stress conditions is of prime importance in ensuring future food security (Rodrigues and Datnoff, 2015). It is also necessary to control and manage the available water supply and to seek suitable alleviators to achieve water and food security (Rodrigues and Datnoff, 2015).

Silicon (Si) has not been regarded as an essential nutrient for plants, despite an increasing volume of research evidence proving that Si is beneficial for plant growth, development, and resistance against abiotic and biotic stress. Si is taken up by plants in the form of silicic acid (H_4SiO_4) from the soil solution (Almeida *et al*., 2009). Si availability is low in highly-weathered soil owing to heavy desilication-aluminization (Almeida *et al*., 2009). Mono-cropping reduces plant-available Si in soil. Hence, a suitable and efficient source of Si to increase plant yields is of great importance (Almeida *et al*., 2009).

Steel slags contain several mineral elements, such as CaO, SiO_2, MgO, Fe and MnO (Ning *et al.*, 2014). Steel slag applications on agricultural land have an economic and environmental benefit (Cha *et al*., 2006). Steel slags have been applied as calcium silicate fertilizer in fields where plant-available Si is deficient in the soil (Cha *et al*., 2006). Application of a slag-based Si fertilizer not only enhances the fertility of degraded soils, it also improves the growth, yield, and resistance to plant diseases of crops (Cha *et al*., 2006).

This study analysed the effectiveness of steel slag as a source of Si to enhance plant growth under disease and drought stress, using Energy Dispersive X-ray (EDX) microanalysis to determine the Si content taken up by plants from the slag products being tested.

2.2 Materials and methods

2.2.1 Plant material

Rye grass (*Lolium perenne* L.), zucchini (*Cucurbita pepo* L. cv. Star 8021), broccoli (*Brassica oleracea* L. cv. Parthenon), bean (*Phaseolus vulgaris* L. cv. Red speckled), maize (*Zea mays* L. cv. Revelation), and green pepper (*Capsicum annuum* L. cv. Sc701), provided by Sunshine Seedlings from Pietermaritzburg, were used as test crops in disease and drought trials which were carried out in greenhouse tunnels at the University of KwaZulu-Natal (UKZN). Seedlings were prepared in trays and transplanted into 2.5L pots (one plant per pot). Rye grass seeds were sprinkled into a 2.5L pots (no set number of seeds per pot). These trials were conducted once.

2.2.2 Treatment application

Four Si treatments were tested: Agrisil-granular (AGS); slag products (SP1.7 and SP5.0); and potassium silicate (Pots). Slag products were provided by Phoenix services LLC. Slag products were applied at a rate of 1t ha^{-1}, Pots was applied at a rate of 500ppm per pot (5ml diluted into 50ml of water), and AGS was applied at 9.24g per pot. Each product was applied immediately after transplant as a once-off per growing season. Pots was applied once every four weeks, to maintain the Si concentrations in the growing media.

2.2.3 Drought and disease trials

Drought and disease trials were run simultaneously in separate greenhouse tunnels, with the same protocol described in 2.2.2. The disease trial was misted, regularly, with water via a misting irrigation system, and the drought trial was water restricted, in a separate tunnels from the disease trial, with no irrigation systems present. Temperature was kept consistent at 23˚C, in the tunnels throughout the experiment for both trials.

Four Si treatments (AGS, SP1.7, SP5.0 and Pots) were applied to each crop with five replications. Water only (no Si treatment) was used as a negative control and Pots as a positive control. Five Leaf samples were collected from each plant every 30 days from transplant for a period of 180 days. Plant heights for four of the crops (maize, green pepper, zucchini, and broccoli) in both trials were recorded at the end of the experiment to determine the effectiveness of Si on plant growth under abiotic and biotic stress.

For the disease trials, maize plants were inoculated mechanically by stapling an infected leaf onto the experimental plants. Maize was infected with Gray leaf spot (*Cercospora zeina* L.), provided by Pannar seeds (PTY) LTD, Greytown. Bean plants were mechanically inoculated with rusts (*Phragmidium* spp.) by rubbing infected leaves onto the experimental plants taken from disease plots in UKZN, broccoli was inoculated with blackrot (*Xanthomonas campestris* L.),taken from disease plots in UKZN, by adding infected soil into the experimental plants' growing media. Green pepper and zucchini leaves were kept under humid conditions until Powdery mildew infected the plants. Rye grass acquired a rust (*Puccinia graminis* f.) infection, naturally, two months after planting.

2.2.4 Energy Dispersive X-ray microanalysis

Energy dispersive X-ray (EDX) microanalysis was performed to determine the Si content within the leaves resulting from the different treatments, at the Microscopy and Microanalysis Unit (MMU) at UKZN. Leaf samples were collected from each treatment and freeze dried. Si was then detected with an EDX detecting unit connected to a Philips XL 30 environmental scanning electron microscope (ESEM), operating at a voltage of 15kv.

2.2.5 Data analysis

Area under curve (AUC) over time was calculated on Microsoft Excel, using a trapezoid calculation, as described by Simko and Piepho (2012), to calculate the Si uptake rate (%days) in the plant leaves (fig. 2.1). AUC and plant height data were analysed by ANOVA, using the GenStat computer package at a 5% significance level.

$$\text{AUC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Figure 2.1*:* AUC equation*. (yi* is an assessment of the data (percentage, proportion, ordinal score, etc.) at the *i*th observation, *ti* is time (in days, hours, etc.) at the *i*th observation, and *n* is the total number of observations.) (Simko and Piepho, 2012).

2.3 Results

In Table 2.1, all Si treatments caused a positive uptake of Si into rye grass leaves which were significantly different from the control (water). There were no significant differences between the rates at which maize had taken up Si from the Si treatments. AGS provided the highest rate of Si that was taken up by the rye grass with a rate of 132.36%days followed by SP1.7 with a rate of 132.24%days. SP5.0 provided the lowest rate (95.10%days) at which Si was taken up into the rye grass leaves. The Si content in the green pepper leaves exceeded 1% for SP1.7, AGS and Pots after 180 days.

Table 2.1: Mean Si content (%) in rye grass leaves (disease trials).

Rye grass	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.12	0.17	0.18	0.14	0.18	0.16	0.29	30.96a
Pots	0.18	0.36	0.52	0.71	0.88	0.90	1.13	121.32b
SP1.7	0.20	0.45	0.58	0.71	0.89	1.05	1.25	132.24b
SP5.0	0.15	0.32	0.37	0.51	0.67	0.79	0.88	95.10b
AGS	0.24	0.45	0.63	0.65	0.88	1.05	1.26	132.36b
f=	2.8661		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.2601			52.87	21.67	51.45	64.78	44.23
SD=	71.2667							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

In Table 2.2, Si was taken up into maize leaves at the highest rates from AGS (350.01%days) and SP1.7 (333.63%) products, these rates were significantly different from the control (167.58%). The rates at which Si was taken up from these treatments (AGS and SP1.7) were not significantly different from Pots (259.23%days) and SP5.0 (271.92%days).

Table 2.2: Mean Si content (%) in maize leaves (disease trials).

Maize	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.97	0.94	1.00	0.88	0.85	0.93	0.97	167.58a
Pots	0.81	1.13	1.40	1.55	1.54	1.65	1.86	259.23ab
SP1.7	1.11	1.61	1.92	1.93	1.88	2.12	2.18	333.63b
SP5.0	0.54	0.90	1.72	1.6	`.79	`.86	1.80	271.92ab
AGS	1.11	1.42	2.05	2.17	2.26	2.16	2.06	350.01b
f=	2.6911		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.0607			55.21	25.21	47.01	17.24	27.01
SD=	129.83							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

Table 2.3: Mean Si content (%) in green pepper leaves (disease trials).

Green pepper	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.23	0.26	0.34	0.20	0.23	0.31	0.24	47.94a
Pots	0.27	0.53	0.62	0.86	1.04	0.89	1.04	138.51ab
SP1.7	0.55	0.59	0.99	1.33	1.61	1.24	1.11	198.45b
SP5.0	0.38	0.68	0.80	0.73	0.93	0.99	1.16	146.88ab
AGS	0.53	0.63	0.76	1.29	1.27	1.22	1.42	184.95b
f=	2.8661		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.0892			23.54	41.21	66.54	39.8	59.21
SD=	113.4800							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

In Table 2.3, all Si treatments caused a positive uptake of Si into green pepper leaves. There were no significant differences in the rate at which green pepper took up Si from the Si treatments into the leaves. SP1.7% provided the highest AUC value of 198.45% followed by AGS at 184.95%. The Si content in the green pepper leaves exceeded 1% for all treatments after 180 days from treatment application.

In Table 2.4, the Si content in zucchini leaves exceeded 0.90% for all Si treatments after 180days from treatment application. The rate of Si uptake into zucchini leaves was significantly different between SP1.7 (159.17%days) and the control (81.15%days). There were no significant

differences between the rates at which Si was taken up by zucchini into the leaves for all Si treatments.

Table 2.4: Mean Si content (%) in zucchini leaves (disease trials).

Zucchini	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.41	0.58	0.50	0.38	0.39	0.42	0.41	81.15a
Pots	0.33	0.55	0.61	0.76	0.79	1.17	1.27	141.27ab
SP1.7	0.49	0.64	0.64	0.75	0.90	1.11	1.05	159.17b
SP5.0	0.24	0.61	0.61	0.61	1.03	1.01	0.99	135.24ab
AGS	0.29	0.46	0.49	0.70	0.91	0.93	1.00	125.13ab
f=	1.0005		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.4305			60.23	28.23	30.33	61.88	42.56
SD=	76.5200							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

Table 2.5: Mean Si content (%) in broccoli leaves (disease trials).

Broccoli	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.56	0.66	0.66	0.64	0.61	0.53	0.47	108.93a
Pots	0.69	0.82	1.11	1.31	1.12	1.28	1.43	208.03b
SP1.7	0.42	0.52	0.68	0.71	0.81	0.73	0.97	124.41ab
SP5.0	0.51	0.82	0.51	0.54	0.67	0.77	0.81	118.98ab
AGS	0.54	0.57	0.58	0.70	0.63	0.80	0.89	120.93ab
f=	1.2651		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.3165			45.67	46.76	52.45	68.23	50.12
SD=	98.8900							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

In Table 2.5, the rate of Si uptake into Broccoli leaves for Pots was 208.03%days, which was significantly different from the control (108.93%). SP1.7, SP5.0 and AGS were not significantly different from the control. All Si treatments did provide a positive uptake of Si into broccoli leaves.

Table 2.6: Mean Si content (%) in beans (disease trials).

Beans	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.62	0.48	0.64	0.63	0.50	0.60	0.60	104.16a
Pots	0.40	0.54	0.64	0.72	0.78	0.77	0.91	123.24ab
SP1.7	0.64	0.71	0.89	1.12	1.01	1.09	1.08	199.28b
SP5.0	0.66	0.75	0.79	0.74	0.75	0.94	1.07	145.17ab
AGS	0.40	0.57	0.58	0.71	0.83	0.87	0.89	126.21ab
f=	1.0005		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.4306			49.89	31.87	48.23	40.12	65.41
SD=	94.6200							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

In table 2.6, all treatments provided a positive uptake of Si into bean leaves. There were no significant differences between the rates which SI was taken up into the bean leaves for between the Si treatments. SP1.7 provided a significantly different rate (199.28%) at which Si was taken up by the bean plant into the leaves compared to the control 104.16%days.

Table 2.7: Mean Si content (%) in rye grass leaves (drought trials).

Rye grass	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.18	0.25	0.27	0.22	0.24	0.21	0.14	40.12a
Pots	0.24	0.36	0.46	0.56	0.63	0.62	0.89	95.55ab
SP1.7	0.43	0.57	0.79	0.89	1.01	1.14	1.30	157.89c
SP5.0	0.19	0.29	0.44	0.55	0.70	0.74	0.84	97.24ab
AGS	0.31	0.48	0.63	0.78	0.84	1.06	1.15	135.75bc
f=	5.0646		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.0055			46.44	21.23	46.12	9.38	42.21
SD=	58.9900							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

In table 2.7, the AUC values were not significantly different between SP1.7 (157.89%days) and AGS (135.75%days). The Si uptake rates into the rye grass leaves were significantly different between SP1.7, SP5.0 (97.24%days) and Pots (95.55%days). All Si treatments provided a positive uptake of Si into the rye grass leaves. The rate of Si uptake was not significantly different between SP5.0 and the control (40.12%days).

In table 2.8, the rates (AUC values) of Si uptake into the maize leaves were not significantly different between Pots (136.05%days), SP5.0 (106.53%days), AGS (175.14%days) and the control (81.21%days). SP5.0 (190.44%days) provided the highest rate of Si uptake into the maize leaves. There were no significant differences between the AUC values between the Si treatments.

In table 2.9, the rates of Si-uptake were not significantly different between Si treatments. The Si uptake rate into the green pepper leaves was the highest for AGS (141.72%days) and SP.17 (110.95%days). The AUC values of SP1.7 and AGS were significantly different from the control (65.25%days).

Table 2.8: Mean Si content (%) in maize leaves (drought trials).

Maize	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.50	0.47	0.45	0.47	0.45	0.38	0.47	81.21a
Pots	0.52	0.64	0.69	0.75	0.84	0.88	0.95	136.05ab
SP1.7	0.78	0.86	0.96	1.03	1.16	1.26	1.38	190.44b
SP5.0	0.51	0.53	0.48	0.58	0.65	0.67	0.77	106.53ab
AGS	0.70	0.83	0.90	0.99	1.10	1.08	1.18	175.14ab
f=	2.3546		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.0886			70.65	24.45	37.83	42.35	57.43
SD=	87.8300							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

Table 2.9: Mean Si content (%) in green pepper leaves (drought trials).

Green pepper	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.45	0.33	0.40	0.31	0.36	0.35	0.36	65.25a
Pots	0.26	0.38	0.44	0.56	0.67	0.76	0.78	100.56ab
SP1.7	0.28	0.49	0.63	0.63	0.88	0.91	1.01	140.95b
SP5.0	0.40	0.44	0.49	0.64	0.71	0.71	0.82	108.78ab
AGS	0.39	0.53	0.59	0.62	0.81	0.88	0.94	141.72b
f=	0.9222		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.4706			82.23	79.45	38.65	49.34	25.67
SD=	75.0300							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

In table 2.10, all Si treatments provided a positive uptake of Si into the zucchini leaves. The highest rate of Si taken up was 136.20%days from SP1.7. There were no significant differences between the rates at which Si was taken up into the zucchini leaves from all Si treatments. SP1.7 AUC value was significantly different to the control (64.05%days).

In table 2.11, the rates Si was taken up into the broccoli leaves were not significantly different between all Si treatments. Pots had the highest rate of 207.05%days, which was significantly different to the control which had a rate of 93.47%days. The lowest rate Si was taken up into the plant leaves from the Si treatment was from SP5.0 with a rate of 120.12%days.

Table 2.10: Mean Si content (%) in zucchini leaves (drought trials).

Zucchini	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.36	0.41	0.33	0.38	0.32	0.31	0.36	64.05a
Pots	0.24	0.34	0.32	0.41	0.52	0.78	0.91	88.11ab
SP1.7	0.53	0.44	0.67	0.83	0.87	0.95	0.99	136.20b
SP5.0	0.27	0.42	0.53	0.55	0.63	0.66	0.71	99.33ab
AGS	0.23	0.45	0.64	0.65	0.79	0.96	1.08	125.1ab
f=	2.8661		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.2601			53.67	21.23	51.45	64.34	44.24
SD=	71.2600							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

Table 2.11: Mean Si content (%) in broccoli leaves (drought trials).

Broccoli	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.68	0.79	0.68	0.69	0.65	0.58	0.68	93.47a
Pots	0.64	0.72	0.88	1.01	1.14	1.20	1.23	207.05b
SP1.7	0.57	0.78	0.85	0.98	1.12	1.20	1.33	176.28ab
SP5.0	0.49	0.54	0.56	0.67	0.76	0.80	0.85	120.12ab
AGS	0.43	0.59	0.68	0.85	0.77	0.85	1.05	134.52ab
f=	0.5494		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.7016			36.34	43.45	63.25	62.89	63.99
SD=	113.0680							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

Table 2.12: Mean Si content (%) in bean leaves (drought trials).

Beans	Days from Treatment Application							
Treatment	0	30	60	90	120	150	180	AUC (%days)
Water	0.62	0.56	0.54	0.58	0.50	0.55	0.59	86.70a
Pots	0.48	0.56	0.60	0.72	0.73	0.80	0.79	121.47ab
SP1.7	0.41	0.64	0.77	0.77	0.88	1.02	1.06	144.39ab
SP5.0	0.44	0.54	0.77	0.73	0.78	0.83	0.98	177.77b
AGS	0.44	0.63	0.74	0.84	0.90	0.88	1.08	142.47ab
f=	0.5695		CV%	Water	Pots	SP1.7	SP5.0	AGS
P-value=	0.6877			67.56	35.32	46.34	44.24	45.43
SD=	88.4464							

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

In table 2.12, there were no significant treatments between the AUC values of the Si treatments. The highest rate of Si taken up by the bean leaves from an SI treatment, was SP5.0 at a rate of 177.77%days. The rate Si was taken up the plant leaves was significantly different between SP5.0 and the control (86.70%days).

In Fig. 2.2, all Si treatments improved plant growth under disease and drought stresses. For all crops, plant heights were lower in drought trials when compared to the plant heights in the disease trials. SP.7 enhanced the height of the maize plant the most under disease and drought stress. Plant heights of maize were significantly different between SP1.7 and the control (water) under both stresses. There were no significant differences between plant heights for green pepper treated with the Si treatment in both trials. Plant height of green pepper treated with pots was significantly different compared to the control. All plant heights on green pepper treated with Si products exceeded 0.3m compared to the control which had a plant height >0.3m under disease stress. Plant heights of zucchini treated with Pots and AGS were significantly different to the plant height of the control under disease stress. Plant height of zucchini treated with SP1.7 was significantly different to the plant height of the control under drought stress. Plant height of broccoli treated with AGS was significantly different to the plant height of the control under disease and drought stress. There were no significant differences in bean plant heights treated with Si under disease and drought stress.

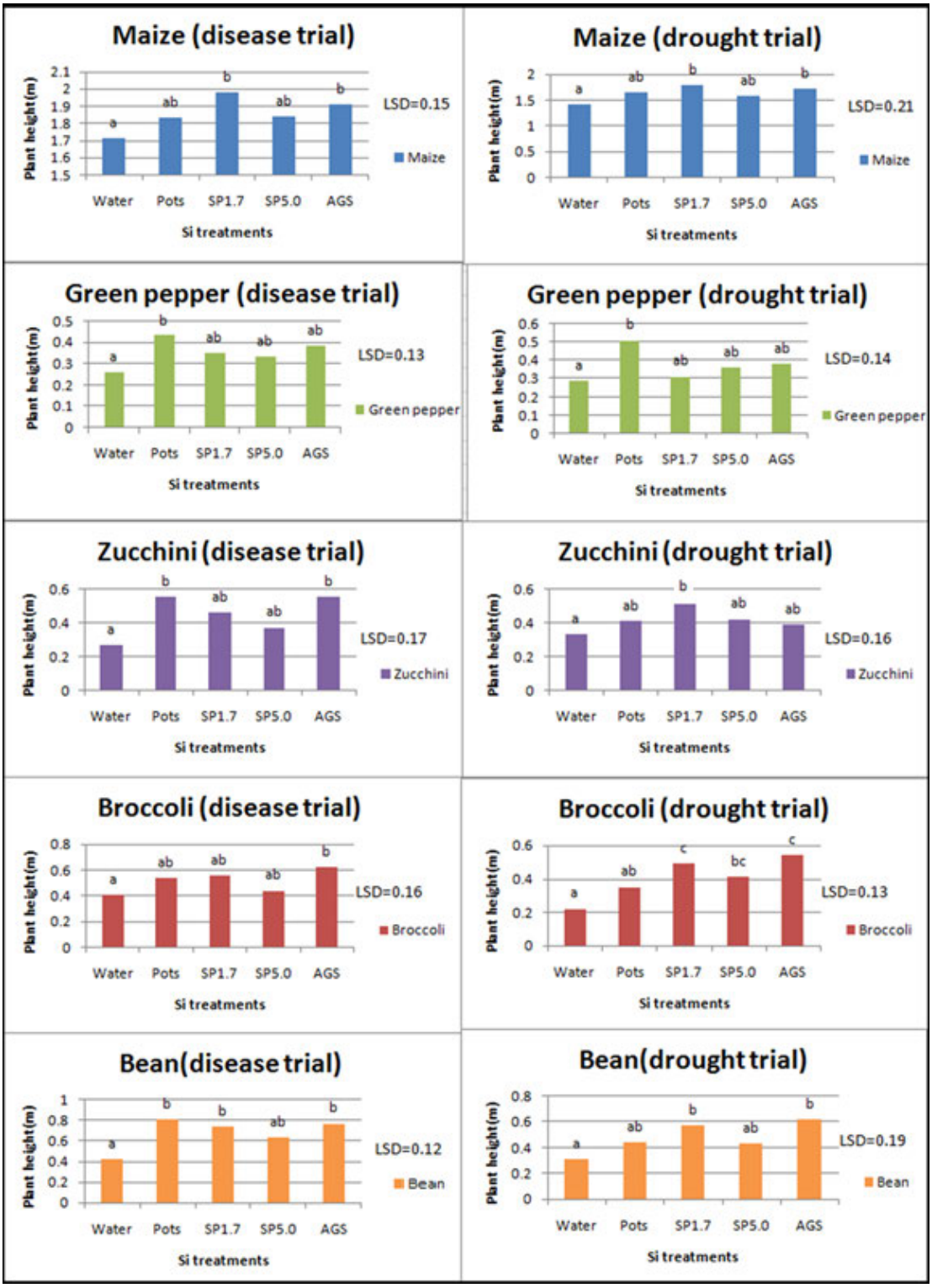

Figure 2.2: Mean plant height (m) for different crops treated with Si products under disease and drought stress.

2.4 Discussion

All treatments provided a positive Si uptake into the plant leaves, but there was a variation in the rates at which Si was taken up. The rates at which Si was taken up by the plants may have been dependent on the particle sizes of the treatments and the state of matter (liquid or granular) in which the treatments were applied. This conforms to the general rule that smaller-sized particles have a relatively larger surface area than larger-sized particles, which results in higher rates of absorption and chemical reactions. This would explain why SP1.7 and AGS were so effective at providing Si for plant uptake, due to the finer granular sizes of the particle compared to the other treatment in both trials. SP1.7 and AGS particle sizes were smaller than SP5.0. An exception to this rule, seemed to be that despite Pots being in a liquid form, the crops did not take up Si as well as the other treatments. A possible factor contributing to this could have been the Pots treatment seeped out from the growing media, which of lowered the time Si was available for plant uptake.

The rates at which Si was taken up varied among Si treatments and among the different crops. This was expected due to the variation in the genetic composition among crops, related to the genes required for Si uptake. The final Si contents would have been dependent on the amount of Si that was made available through the treatments. This conforms to the report from Kaur and Greger (2019), whereby the rate of Si uptake in plants depends primarily on the phylogenetic position of the plant, compared with the environmental effects that encompass Si concentrations in the soil. Although various amounts of Si uptake are reported for a given plant species, and Si accumulation is primarily a phylogenetic feature, the amount of plant-available Si in the soil affects the final amount of Si that is absorbed by the plant (Kaur and Greger, 2019).

All Si treatments improved plant growth for all crops under drought and disease stress. Suppression of diseases and improved drought tolerance would result in better growth and yield in plants. When plants are supplied with Si and then challenged with a pathogen, there is an enhanced activation in antioxidant metabolism, which, in turn, suppresses the damaging cytotoxic effect of the reactive oxygen species that causes lipid peroxidation in the cell

membrane (Rodrigues and Datnoff, 2015). Hamayun (2010) concluded that Si improves the physio-hormonal attributes of plants and mitigates adverse effects of drought stress. The Si treatments increased the plant growth for all crops under biotic and abiotic stresses in this study.

Si was taken up at higher rates in disease trials as compared to the drought trials could have been due to the presence of water playing a role in the chemical composition of the Si treatments. Plants take up Si in a soluble form hence the drought trials with inhibited water supply would have slowed the rate at which Si was taken up into the leaves.

Slag products SP1.7 and SP5.0 were efficient sources of Si for the uptake in plants. The applications of these products are convenient, time efficient and economical. They are applied once-off during the growing season, which results in reduction in labour and time compared to the traditional liquid fertilizers, which have to be applied regularly.

In conclusion, the tested slag products were efficient and effective sources of Si for uptake into plants, which results in enhanced plant growth under disease and drought stress.

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Chapter Three

Effect of pre-harvest silicon application to inhibit *Colletotrichum capsici* on post-harvest pepper fruit (*Capsicum annuum* L.)

Anthracnose disease, caused by *Colletotrichum* spp., is a threat in peppers (*Capsicum annuum* L.) production in tropical and sub-tropical countries. Disease resistance could be achieved with silicon (Si) treatment, in a wide range of tropical and sub-tropical fruits and vegetables. This study analysed the effect of pre-harvest Si application to inhibit *Colletotrichum capsici* on post-harvest pepper fruit (*Capsicum annuum* L.). Pepper fruit were harvested from pepper plants (*Capsicum annum* L. cv. Revelation) that were Si treated to provide Si for plant uptake. Si treatments used were: potassium silicate (Pots), which was used as a positive control; slag products (SP1.7 and SP5.0); and Agri-sil granular (AGS). The area covered by infection (%) on the fruit was recorded every seven days for a period of 21 days, to determine the disease progress. All Si treatments significantly reduced the rate of infection by the pathogen. By day 7, the disease progress was inhibited, with a recorded area of infection being below 3.5%, compared to the control which was at 8.4%. By day 14, it was inhibited to below 16%, relative to 33.6% in the control and by day 21, it was inhibited to below 31%, from 57.4% (control), for all Si treatments. Area under the disease-progress curve (AUDPC) values (%days) were the lowest for the SP1.7 treatment, which means it enhanced post-harvest disease resistance to the greatest degree. SP5.0 had the highest AUDPC value from all SI treatments. Pre-harvest application of Si reduces post-harvest anthracnose disease in green pepper.

3.1 Introduction

Anthracnose disease, caused by *Colletotrichum* spp., especially *C. capsici*, is a threat in sweet pepper (*Capsicum annuum* L.) production in tropical and sub-tropical countries (Rodrigues and Datnoff, 2015). Alternative control methods to control anthracnose have become essential due to environmental pollution, health concerns of the consumers and development of fungicide-resistant pathogen populations (Rodrigues and Datnoff, 2015).General management are exercised in an attempt to control the disease such as: using of fungicides (eg. Azoxystrobin,

Mancozeb, etc), water heating treatments and managing of irrigation to reduce moisture on the fruits which contribute to the spread of the disease before harvests (Rodrigues and Datnoff, 2015). The enhancement of plant disease resistance plays a vital role in minimizing the post-harvest losses and adjusting crop production to meet global population (Christalcatalini *et al*., 2017). It also satisfies the requirements for low toxicity products to enhance plant growth, development and yield, and provide a broad spectrum of defence, and a long-lasting effect (Christalcatalini *et al*., 2017).

Several studies in the past proved that enhanced natural disease resistance could be achieved with silicon (Si) treatment, in a wide range of tropical and sub-tropical fruits and vegetables (Assis *et al.*, 2013). This has been achieved through the range of effects of Si treatment, including: reduced mineral toxicity; increased photosynthetic activity; superior nutrient uptake; and enhanced drought and frost tolerance (Assis *et al*., 2013).

Si transported and deposited on the tissue surface serves as a physical barrier which protects plants from fungal infection and pests (Liang *et al*., 2005). The increase in resistance has been correlated with several factors: a dense layer of silicified cells in the epidermis of the leaves; a silica layer below the cuticle; the double cuticle layer; a silicon-cellulose membrane; and the papilla formation (Liang *et al*., 2005). Si inhibits physical penetration by pathogenic fungi and pests, strengthens the plant's mechanical structure, and decreases plant cells' susceptibility to enzymatic degradation by fungal pathogens (Liang *et al*., 2005).

This study analysed the effect of pre-harvest Si application to inhibit *C. capsici* on post-harvest pepper fruit (*C. annuum* L.).

3.2 Materials and methods

3.2.1 Cultivar and sample material

Pepper fruit were harvested from pepper plants (*C. annum* L. cv. Revelation) that were treated with steel slag products (provided by Phoenix services LLC) to provide Si for plant uptake.

Treatments used were: potassium silicate (Pots), which was used as a positive control; slag products (SP1.7 and SP5.0); and Agri-sil granular (AGS). Water without any Si treatments was used as a negative control. This study was conducted once at the University of KwaZulu-Natal (UKZN).

3.2.2 Disease inoculation

A pin was used to create a small hole into which a suspension containing an isolate of *C. capsici* was rubbed (Fig. 3.2). There were five replications for each treatment and each fruit was kept at room temperature (23^{o}C) for three weeks after inoculation.

3.2.3 Data analysis

Anthracnose disease produces symptoms of lesions forming on the surface of the fruit (Fig. 3.3). The lesions grow in size and number over time as the disease spreads; hence, the lesions cover a large area. The area covered by infection on the fruit was recorded every seven days from inoculation for a total period of 21 days by estimation through eyesight. The area under the disease-progress curve (AUDPC) over time was calculated on Microsoft Excel using a trapezoid calculation (Fig. 3.1), as described by Simko and Piepho (2012), and used to determine which product was the most effective in enhancing post-harvest disease resistance. Results were analysed using an ANOVA procedure, with the GenStat computer package at a 5% significance level.

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Figure 3.1: AUC equati*on. (yi* is an assessment of the data (percentage, proportion, ordinal score, etc.) at the *i*th observation, *ti* is time (in days, hours, etc.) at the *i*th observation, and *n* is the total number of observations.) (Simko and Piepho, 2012).

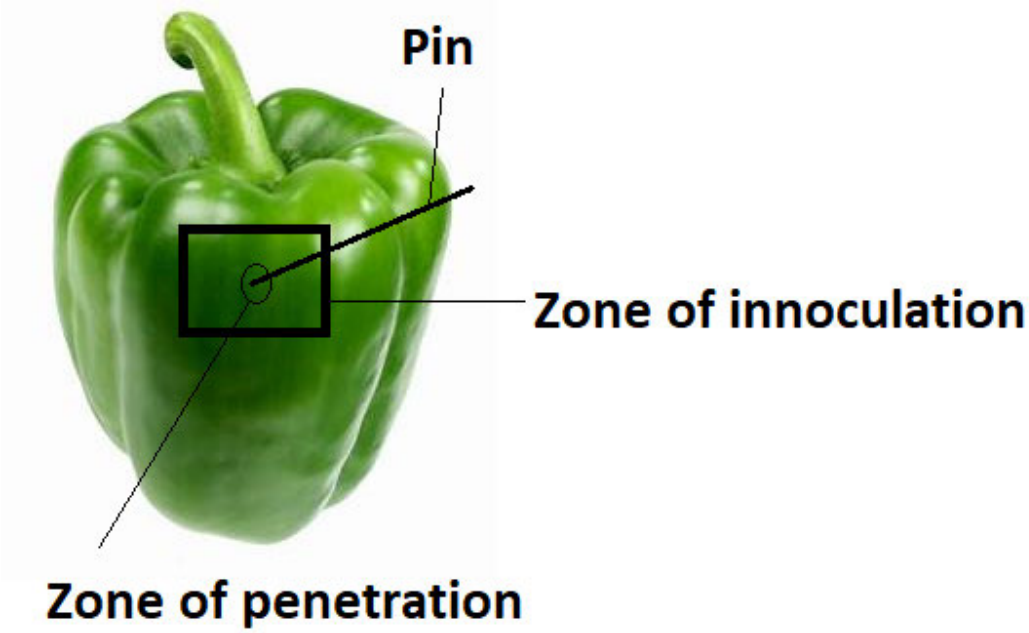

Figure 3.2: Method used to inoculate the pepper fruit with the anthracnose disease.

Figure 3.3: Lesions formed as a symptom of anthracnose disease in green pepper.

3.3 Results

In Table 3.1, the highest areas of infection were observed with water (negative control). All other treatments performed relatively similarly and showed positive results in reducing the rate of infection. By day 7, the infection was inhibited from a mean of 8.4% (control) to below 3.5% for all Si treatments. By day 14, it was inhibited from 33.6% to below 16% and by 21 days, it was inhibited from 57.4% to below 31% for all Si treatments, when compared to the control. AUDPC

values (%days) were the lowest for the SP1.7 treatment, which means it enhanced post-harvest disease resistance to the greatest degree. SP5.0 had the highest AUDPC value compared to the other Si treatments. Rate of disease progress was not significantly different from each other.

Table 3.1: Infected area (%) over time and area under the disease-progress curve (AUDPC) in green pepper infected with anthracnose disease and treated with different pre-harvest Si treatments.

		Days from innoculation			
Treatment	0	7	14	90	AUC (%days)
Water	0.00	8.40	33.60	57.40	2982b
Pots	0.00	3.00	13.40	30.60	1410a
SP1.7	0.00	2.80	12.60	28.80	1326a
SP5.0	0.00	3.20	15.40	29.40	1440a
AGS	0.00	2.60	14.60	30.40	1428a
f=	104.5900	Cv%	water	Pots	SP1.7
p-value=	0.0004		6.23	11.34	6.32
SD=	204.35000		SP5.0	AGS	
			14.34	5.66	

AUDPC = Area under the disease progressive curve used to estimate the rate of disease progression as %days

3.4 Discussion

Anthracnose disease progress was significantly slower in pepper fruits that had been harvested from Si-treated plants. This was an indication that Si was taken up by the plant, which was treated with Si products, into the fruits. After 21 days, it was observed that anthracnose disease progressed faster in the control (without Si treatments), compared to the Si treatments. The possible reason for the disease reduction in fruits subjected to Si treatments, compared to the control, may have been due to the physical barrier formation, which resulted in delaying the penetration of germinating fungal spores. A previous study had showed that the percentage numbers of appressoria of *C. gloeosporioides* were higher on tomatoes being treated with Si, compared to controls (Weerahewa and David, 2015). This happened when the fungus penetration process was delayed; the number of appressoria increased on the surface of the

tissue, which implies the presence of a physical barrier over the surface of fruit peel that impeded the process of infection (Weerahewa and David, 2015).

In addition, Si application in post-harvest had been recommended for increasing storage life and maintaining fruit quality, because of more phenylalanine ammonia-lyase and total phenolic activities that enhance the fruits' ability to resist stressful cold conditions (Habibi, 2015). This also suggested that Si may be involved in modulating enzymes when there is an increase of flavonoids and phenolics in response to high concentrations of Si (Habibi, 2015).

Application of Si treatments to plants prior to harvest may be an alternative method of application to control post-harvest diseases, provided a plant is given adequate time to effectively take up the Si into the fruits. This could possibly be a measure to delay the initial post-harvest infection and reduce the disease progress of *C. capsici* in green pepper fruit.

Until now, fungicides have been most commonly used to control post-harvest diseases, but the chemical residues and pathogen resistance to chemicals are critical issues (Rodrigues and Datnoff, 2015). Thus, the uses of Si treatments may replace the application of fungicide or other washing agents in post-harvest treatments to control post-harvest fungal diseases. Although the application of Si treatments and the mechanisms involved in Si resistance of plants to fungus are not yet fully understood, the result in this study showed that Si may be considered a promising component in delaying the growth of fungi and potentially as a decay-control product, as well as a preservative.

In conclusion, pre-harvest application of Si on green pepper reduces post-harvest anthracnose disease progress in sweet pepper fruits.

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Chapter Four

Determining the effectiveness of steel slag as a source of silicon for citrus and avocado uptake using Energy Dispersive X-ray (EDX) microanalysis

Silicon (Si) is the second most abundant element in the earth's crust after oxygen. Plant-available Si can be found in the soil solution in an undissociated form as monosilicic acid. Si fertilisation has been reported to increase fruit yield, accelerate fruit growth by 30–80% and fruit ripening by two to four weeks. Three different species: *Citrus sinensis* (orange cultivars: Valencia and Navel); *Citrus limon* L. cv. Eureka (lemon); and *Persea americana* L. cv. Hass (avocado), were used for the study. Five Si treatments were tested: potassium silicate (Pots); Agri-sil granular (AGS); Agri-sil liquid (ASL); slag products (SP5.0 and SP1.7). Energy Dispersive X-ray (EDX) microanalysis was performed to determine the Si content within the leaves resulting from the different treatments over time. All Si treatments provided a positive uptake of Si into the citrus leaves and avocado. Valencia trees treated with SP1.7 had the highest rate of Si taken up into the leaves, with an area under curve (AUC) value of 210.24%days, followed by SP5.0 with an AUC value of 195.48%days. SP1.7 and AGS provided the highest rates of Si uptake into Navel orange leaves, with AUC values of 187.02%days and 187.92%days, respectively. Lemon trees treated with SP1.7 and AGS had the highest rates of Si uptake into the leaves. Citrus trees treated with SP1.7 had higher rates of Si uptake into the tree leaves, with the exception of AGS treatment, which had the highest rate of Si uptake in lemon. Avocado trees treated with SP1.7 had the highest rate of Si taken up into the leaves, with an AUC value of 28.29%days. Steel slag was an efficient and effective source of Si for the uptake in citrus and avocado leaves.

4.1 Introduction

Silicon (Si) is one most abundant element in the earth's crust (Rodrigues and Datnoff, 2015). Plant-available Si can be found in the soil solution in an undissociated form as monosilicic acid (Almeida *et al*. 2009). In highly weathered soils, Si availability in soil solution reduces considerably because of soil acidification, organic complexes, presence of aluminium, iron and

phosphate ions, temperature, sorption/dissolution reaction and soil moisture (Almeida *et al*. 2009). A supplementation with a Si fertilizer, in order to improve the quality and yield of agricultural crops under abiotic and biotic stress conditions, becomes a need.

The beneficial role of Si in citrus has been demonstrated in only a few studies. Si fertilisation has been reported to increase fruit yield, accelerate growth by 30–80% and fruit ripening by two to four weeks (Mvondo-She and Marais, 2019). A similar study conducted in grapefruit revealed that calcium silicate slag fertilization increased root and shoot mass by 19–40% (Mvondo-She and Marais, 2019). Additionally, an increase of 14–41% in tree height and 31–48% increase in shoot mass for Valencia trees were reported (Mvondo-She and Marais, 2019). In greenhouse studies, potassium silicate application improved fresh shoot mass by 30–40% in one-year-old and two-year-old sweet orange (*Citrus sinensis* L.) trees over a six-month period (Mvondo-She and Marais, 2019).

In agriculture, slags can be used as fertilizers and as a corrective of soil acidity (Ning *et al*., 2014). Slags are calcium and magnesium silicates, which show neutralizing action due to $Si0_3^{2-}$ base (Ning *et al*., 2014). Additionally, steel slags have been used as a low-cost source to supply Si to rice plants (Ning *et al*., 2014).

The main chemical components which slags contain in their composition, and which are of great importance, are CaO, MgO, SiO_2, P_2O_5, FeO and Mn (Ning *et al*., 2014). The composition concentration of these components in each slag is dependent on raw materials, steel type and furnace conditions (Ning *et al*., 2014).

In this study, the efficiency of steel slag as a source of Si uptake in citrus and avocado was analysed.

4.2 Materials and methods

4.2.1 Plant material

Three different citrus species: *Citrus sinensis* (orange cultivars: Valencia and Navel); *Citrus limon* L. cv. Eureka (lemon); and *Persea americana* L. cv Hass (avocado), were used for the study. Trials were conducted on farms in Northwood (orange trials) and Ebenezer (lemon), which were located in the Richmond area of KwaZulu-Natal. Avocado trials were conducted in greenhouse tunnels at the University of KwaZulu-Natal. All trials were conducted once.

4.2.2 Silicon fertilizer application

Application on lemon and oranges was done using five Si treatments (provided by Phoenix services LLC): slag products (SP1.7 and SP5.0); Agri-sil granular (AGS), Agri-sil Liquid (AGL); and potassium silicate (Pots). Pots was used as a positive control and water (no treatment) was used as a negative control. SP1.7 and SP5.0 were applied at a rate of $1tha^{-1}$, as a once-off application during the growing season. AGS was applied at 37g per tree, once during the growing season, and AGL was applied at 1000ppm per tree, once every three months. Phoenix products and AGS in granular form were sprinkled within a 3m radius around the base of the tree for all trees (except avocado). Irrigation and maintenance of trees were done by the owners of the farm

The Si uptake experiment was performed over 12 months. Each treatment consisted of five replications (5 treatments x 5 replications, 1 control x 5 replications); the 30 trees per citrus species were organized in a randomized complete blocks design. Leaf samples were collected for analysis before treatment, six months after treatment, and 12 months after treatment.

Application on avocado was done using the same Si treatments: SP1.7; SP5.0; AGS; AGL; and Pots. Water (no treatment) was used as a control. Six-month-old avocado seedlings were transferred into 2.5L pots (one tree per pot). SP1.7 and SP5.0 were applied at a rate of 1 tha^{-1}. AGS was applied at 9.24g per pot. Pots was applied every four weeks, at 500ppm per pot (5ml dissolved in 50ml of water). AGL was applied every four weeks, at 1000ppm per pot.

The Si uptake experiment for avocado was performed over three months. Each treatment consisted of five replications. Leaf samples were collected monthly over the course of the experiment.

4.2.3 Energy dispersive X-ray microanalysis

Energy dispersive X-ray (EDX) microanalysis was performed to determine the Si content within the leaves resulting from the different treatments at the Microscopy and Microanalysis Unit (MMU) at UKZN. Leaf samples were collected from each treatment and freeze dried. Si was then detected with an EDX detecting unit connected to a Philips XL 30 environmental scanning electron microscope (ESEM), operating at a voltage of 15kv.

4.2.4 Data analysis

Area under curve (AUC) over time was calculated on Microsoft Excel using a trapezoid calculation (Fig. 4.1) , as described by Simko and Piepho (2012), to calculate the Si uptake rate (%days) in the citrus leaves. AUC data were analysed by ANOVA, using the GenStat computer package at a 5% significance level.

AUC $= \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$

Figure 4.1*: AUC equation. (yi* is an assessment of the data (percentage, proportion, ordinal score, etc.) at the *i*th observation, *ti* is time (in days, hours, etc.) at the *i*th observation, and *n* is the total number of observations.) (Simko and Piepho, 2012).

4.3 Results

In table 4.1, The rate of Si taken up into valenica leaves from treatment SP1.7 (210.24%days) was significantly different from Pots (132.26%days), and AGL (108.36%days). The rates of Si uptake into the tree leaves were significantly different from the control (50.22%days). The mean Si content in the tree leaves exceeded 0.40% for all Si treatments after 12 months.

In Table 4.2, all Si treatments had a positive uptake of Si into the tree leaves. SP1.7 and AGS provided the highest rates of Si uptake into the leaves, with AUC values of 187.02%days and 187.92%days, respectively. Navel trees treated with SP5.0 had a higher rate of Si uptake into the leaves than the AGL. There were significant differences in the rate of Si taken up into the tree leaves for all Si treatments compared to the control 72.90%days.

Table 4.1: Mean Si content (%) in Valencia leaves.

Valencia	Months from treatment application			
Treatment	0	6	12	AUC (%days)
Water	0.10	0.13	0.10	50.22a
Pots	0.10	0.28	0.43	132.26bc
SP1.7	0.14	0.40	0.70	210.24 d
AGL	0.06	0.21	0.36	108.36b
SP5.0	0.14	0.34	0.60	195.48cd
AGS	0.07	0.23	0.51	140.58bc
f=	7.8707	Cv%	water	Pots
p-value=	0.0002		66.22	33.23
SD=	55.52000		SP5.0	AGS
			10.12	41.02
			SP1.7	AGL
			32.22	37.67

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

Table 4.2: Mean Si content (%) in Navel leaves.

Navel	Months from treatment application			
Treatment	0	6	12	AUC (%days)
Water	0.09	0.20	0.16	72.90a
Pots	0.17	0.27	0.42	138.96bc
SP1.7	0.10	0.39	0.60	187.02c
AGL	0.13	0.29	0.39	134.28b
SP5.0	0.13	0.33	0.54	176.40bc
AGS	0.11	0.32	0.67	187.92c
f=	5.3588	Cv%	water	Pots
p-value=	0.0019		57.34	19.67
SD=	51.01000		SP5.0	AGS
			22.22	17.05
			SP1.7	AGL
			32.54	36.17

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

In Table 4.3, all Si treatments provided a positive uptake of SI into the lemon leaves. Lemon trees treated with SP1.7 and AGS had the highest rates of Si taken up into the leaves and were not significantly different. AGS had an AUC value of 255.96%days. There were no significant differences between the rates of Si uptake into the leaves between the variations of application for the slag products (SP5.0 and SP1.7).

Table 4.3: Mean Si content (%) in lemon leaves.

Lemon	Months from treatment application			
Treatment	0	6	12	AUC (%days)
Water	0.18	0.19	0.25	93.96a
Pots	0.17	0.43	0.51	183.60bc
SP1.7	0.15	0.45	0.75	229.86cd
AGL	0.20	0.34	0.47	163.44b
SP5.0	0.14	0.34	0.61	187.92bc
AGS	0.22	0.51	0.80	255.96d
f=	8.9635	Cv%	water	Pots
p-value=	0.0001		27.65	37.43
SD=	50.04000		SP5.0	AGS
			14.27	9.81
			SP1.7	AGL
			11.87	34.22

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

In Table 4.4, all treatments provided a positive uptake of Si into avocado leaves. Avocado trees treated with SP1.7 had the highest rate of Si taken up into the leaves, with an AUC value of 28.29%days. Trees treated with Pots and AGS had similar rates of Si taken up into the leaves, with AUC values at 19.44%days and 19.89%days, respectively. There were no significant differences in the rate Si was taken up into avocado leaves from treatments SP1.7, AGL and SP5.0.

Table 4.4: Mean Si content (%) in avocado.

Avocado	Days from treatment application				
Treatment	0	30	60	90	AUC (%days)
Water	0.07	0.07	0.22	0.07	11.67a
Pots	0.04	0.15	0.20	0.28	19.44b
SP1.7	0.09	0.17	0.27	0.45	28.29c
AGL	0.06	0.19	0.25	0.32	23.43bc
SP5.0	0.08	0.19	0.24	0.32	24.12bc
AGS	0.10	0.16	0.19	0.26	19.89b
f=	4.3005	Cv%	water	Pots	SP1.7
p-value=	0.0062		95.23	24.21	28.12
SD=	7.25000		SP5.0	AGS	AGL
			17.23	24.21	11.61

AUC = Area under the Curve used to estimate the rate which silicon was taken up by the plant leaves as %days.

4.4 Discussion

All treatments provided a positive Si uptake into the plant leaves in citrus, but there was a variation in the rates at which Si was taken up. The rates at which Si was taken up by the citrus trees may have been dependent on the cultivars of the different citrus trees used in this study. This was expected due to the variation in the genetic composition among citrus related to the genes required for Si uptake.

Si was taken up into the citrus species at the highest rate for SP1.7. This was a fine granular form with particle sizes around 1.7mm in diameter. This could have played a role in the rate was Si was taken up into the citrus leaves. Pots and AGL were in liquid form, their rates were lower in comparison to the granular forms of Si treatments. Possible factors for lower rates could have been affected by seepage through the soil, evaporation and run off (Fan *et al*., 2021). This is why liquid forms of SI treatments have to be applied regularly to ensure adequate Si is available for plant up take. This further emphasises why steel slag (SP1.7 and SP5.0) are time efficient and economical. Time and resources taken to maintain the Si content in the soil is far greater than using the steel slag.

The final Si content would have been dependent on the amount of Si that was made available through the treatments. This relates with the report from Kaur and Greger (2019), whereby the rate of Si uptake in plants depends primarily on the phylogenetic position of the plant, compared with the environmental effects that encompass Si concentrations in the soil. Although various amounts of Si uptake are reported for a given plant species and Si accumulation is primarily a phylogenetic feature, the amount of plant-available Si in the soil affects the final amount of Si that is absorbed by the plant (Kaur and Greger, 2019).

Si nutrition enhances host resistance to pests and diseases and also alleviates abiotic stresses, thus protecting the crops. In the context of organic farming, the application of siliceous materials and Si sources, not only to agricultural crops but also to horticultural crops, especially fruit crops, may pave the way for increasing the yield and reducing the use of chemical fertilizers, pesticides and fungicides.

Slag products SP1.7 and SP5.0 were efficient sources of Si for the uptake of Si into citrus trees. The applications of these products are convenient, time efficient and economical. They are applied once-off during the growing season, which results in reduction in labour and time compared to the traditional liquid fertilizers which have to be applied regularly.

In conclusion, steel slag is an efficient and effective source of Si for the uptake into citrus and avocado leaves.

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Dissertation overview

Introduction

Silicon (Si) is the second most abundant element on the planet, after oxygen, contributing about 25% of the Earth's crust. Since it exists in the Earth's crust, many plants can accumulate it in large concentrations, in amounts similar to macronutrients. Si enhances the growth and yield of all annual and vegetable crops, promotes upright growth (stronger and thicker stems, shorter internodes), prevents lodging, promotes favorable exposure of leaves to light, and provides resistance to bacterial and fungal diseases. It also decreases the effect of abiotic stresses.

The objectives of this study were to:

1. Investigate the efficiency of steel slag as a source of Si for uptake into plants.
2. Investigate the effect steel slag has on plant growth under disease and drought stress.
3. Investigate the effectiveness of steel slag as a pre-harvest Si treatment to control post-harvest anthracnose disease on green pepper fruit.
4. Investigate the efficiency of steel slag as a source of Si for uptake in citrus and avocado.

Chapter Two: Determining the effectiveness of steel slag as a source of silicon using Energy Dispersive X-ray (EDX) microanalysis

Using an EDX was an efficient and simple method to obtain the amount of Si content that was in the leaf samples. However, despite its efficiency, it is not the ideal method to get accurate and precise results. Data analysed using an EDX had a large coefficient of variance (CV%), indicating it is not a precise method.

For this study, a Microwave Plasma-Atomic Emission Spectrometer (MP-AES) would have provided precise measurements for the amount of Si content that was taken up into the plant leaves. A Tiron extraction method for leaf sample preparations was tested, using methods from various published papers, on the MP-AES at the Institute for Commercial Forestry Research

(ICFR). However, the Tiron-based Si analysis by ICP-OES, ICP-MS or MP-AES was problematic. Prepared leaf samples were diluted in nitric acid, which initially worked on the MP-AES, but over a short period of time, the samples blocked and fused with the glass nebulizer. This could have been due to the Tiron not being fully deactivated by the hydrogen peroxide or due to the colloidal Si formation causing a blockage/fusion with the glass nebulizer. An alternative method would have been to use a plastic nebulizer, but the aperture was much narrower, and a blockage from colloidal Si would have resulted in the same problem. Due to the financial risks involved in damaging the MP-AES, this assay was aborted.

Major findings:

- Steel slag was an efficient source of Si for uptake into plants.
- Steel slag enhanced plant growth under disease and drought stress.

Si content accumulated in the plant leaves over time when treated with the slag products, indicating that slag can be used as fertilizer for providing Si for uptake into plants. A positive uptake of Si into the plant leaves treated with the slag products had a positive effect on the growth of the plants under biotic and abiotic stresses.

Chapter Three: Effect of pre-harvest silicon application to inhibit *Colletotrichum capsici* on post- harvest pepper fruit (*Capsicum annuum* L.)

Major finding:

- Pre-harvest steel slag application reduced the disease progress of post-harvest anthracnose disease in green pepper.

Steel slag as a pre-harvest Si application reduced disease progress of post-harvest anthracnose disease in green pepper. This could reduce the use of chemicals used on fruit which may have toxic effects on consumers. The reduction in post-harvest diseases could lead to longer shelf-life in green pepper fruit.

Chapter Four: Determining the effectiveness of steel slag as a source of silicon for citrus and avocado uptake using Energy Dispersive X-ray (EDX) microanalysis

Using an EDX was an efficient and simple method to determine the amount of Si content that was in the leaf samples. Despite its efficiency, it is not the ideal method to get accurate and precise results. Data analysed using an EDX had a large coefficient of variance (CV%), indicating it is not a precise method.

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Major finding:

- Steel slag was an efficient source of Si for uptake into citrus and avocado leaves.

Steel slag is an economical and time-saving treatment which can be used as an alternative to the traditional Si-liquid formula treatments. Citrus treated with the slag products had similar Si content taken up into their leaves compared to the other Si treatments.

Future research and way forward

- The long-term effects of steel slag application on soil and following growing seasons should be researched further. Steel slag contains other compounds and elements, which

may have toxic effects on the soil and plants if applied too often or at very high dosages. This will provide a better understanding of the use of steel slag from an economical and practical point of view.

- Testing the effect of steel slag as a pre-harvest treatment on post-harvest diseases for various crops and diseases needs to be done to a greater extent, in order to find economical alternative methods to control post-harvest diseases, and to reduce the use of chemicals.