

EFFECT OF POSTHARVEST SILICON APPLICATION
ON 'HASS' AVOCADO (*Persea americana* MILL.)
FRUIT QUALITY

By

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ABSTRACT

The South African avocado industry is export-orientated with forty percent of total production sold overseas. The avocado fruit is a highly perishable product with a relatively high rate of respiration which results in the quick deterioration of fruit quality. Good phytosanitary procedures are a necessity in ensuring good product quality. Due to the threat of pests and diseases becoming resistant to the conventional chemicals currently used to control them, there has been a great need to diversify from their usage.

Silicon (Si), being the second most abundant element (28%) in the earth's crust after oxygen, is a major constituent of many soils and has been associated with disease resistance in plants for a long time. It has been used in a number of crop species to provide resistance against pathogenic agents. In some horticultural crops Si has been found to offer protection against fungal infections by strengthening cell walls, thus making it more difficult for the fungi to penetrate and colonize the plant. The aim of this research was to investigate the effects of post-harvest silicon application on the quality of 'Hass' avocado fruit. The specific objectives included investigating the effect of silicon on the ripening pattern as well as the metabolic physiology of the avocado fruit.

Avocado fruit were obtained from two locations in the KZN Midlands (Everdon Estate in Howick and Cooling Estate in Wartburg). Fruit were treated with different forms of Si (potassium silicate (KSil), calcium silicate (CaSil), sodium silicate (NaSil) and Nontox-silica® (NTS)) at concentrations ranging from 160 ppm to 2940

ppm. After dipping for 30 minutes in the silicon treatments, the fruit were stored at -0.5°C, 1°C, 5°C or at room temperature (25°C). Energy dispersive x-ray (EDAX) analysis was then conducted on the exocarp and mesocarp tissues to determine the extent of silicon infiltration within each treatment. Firmness measurements, ethylene evolution and CO₂ production were recorded as fruit approached ripening. The CO₂ production of fruit that were stored at room temperature was analysed daily until they had fully ripened, while fruit from cold storage were removed weekly to measure respiration. Mesocarp tissue from each fruit was extracted using a cork borer and subsequently freeze-dried and stored for physiological analysis. The freeze-dried mesocarp tissue was then finely ground and later analysed for sugar content, total anti-oxidant capacity (TAOC), total phenolic (TP) content and phenylalanine ammonia lyase (PAL) activity using their respective assays. Statistical analyses were carried out using GenStat® version 11 ANOVA. Treatment and storage temperature means were separated using least significant differences (LSD) at 5% ($P = 0.05$). The experimental design in this study was a split-plot design with the main effect being storage temperature and the sub-effect being treatments. Each replication was represented by a single fruit.

EDAX analysis revealed that Si passed through the exocarp into the mesocarp tissue in fruit treated with high concentrations of silicon, i.e., KSil 2940 ppm. Significant differences ($P < 0.001$) were observed in temperature means with regards to firmness. Fruit treated with KSil and NTS only and stored at 5°C were firmer than fruit stored at other temperatures. Fruits treated with Si in the form of KSil 2940 produced the least amount of CO₂, while non-treated fruits (Air) had the

highest respiration rate. Fruit stored at room temperature (25°C) produced significantly higher amounts of CO₂ and peaked much earlier than fruit stored at other temperatures. Ethylene results showed that there were differences ($P < 0.05$) between temperature means with the highest net ethylene being produced by fruit stored at 25°C. There were also significant differences amongst treatment means ($P < 0.001$), with fruits treated with KSil 2940 ppm producing the least ethylene.

There were significant differences ($P < 0.001$) in temperature means with regards to the total phenolic concentration with fruits stored at 1°C having the highest TP concentration (26.4 mg L⁻¹ gallic acid). Fruit treated with KSil 2940 ppm had the highest total phenolic concentration whilst the control fruit (Air and Water) had the lowest. There were also differences ($P < 0.05$) in storage temperature means with respect to the total antioxidant capacity. Fruit stored at -0.5°C had the highest TAOC (52.53 μmol FeSO₄.7H₂O g⁻¹ DW). There were no significant differences in TAOC ($P > 0.05$) with regards to treatment means although fruit treated with KSil 2940 ppm and stored at -0.5°C showed the highest TAOC of 57.58 μmol FeSO₄.7H₂O g⁻¹ DW. With regards to the concentration of major sugars in avocado, mannoheptulose and perseitol (mg g⁻¹), no significant differences ($P > 0.05$) were observed in temperature means. However, fruit stored at -0.5°C had the highest concentration of these C7 sugars compared with fruit stored at other temperatures. There were significant differences in treatment means ($P < 0.001$) showing that fruit treated with KSil 2940 ppm had the highest concentration of both

mannoheptulose (18.92 mg g^{-1}) and perseitol (15.93 mg g^{-1}) in the mesocarp tissue.

Biochemical analyses showed differences ($P < 0.05$) in storage temperature means with respect to PAL enzymatic activity. Fruit stored at 5°C had the highest PAL activity ($18.61 \text{ mmol cinnamic acid g}^{-1} \text{ DW h}^{-1}$) in the mesocarp tissue compared with fruit stored at other temperatures. There were significant differences in treatment means ($P < 0.001$) with regard to PAL activity. Fruit treated with KSil 2940 ppm had the highest PAL activity ($23.34 \text{ mmol cinnamic acid g}^{-1} \text{ DW h}^{-1}$).

This research has demonstrated the beneficial effects, particularly applications of 2940 ppm Si in the form of KSil. This treatment successfully suppressed the respiration rate of avocado fruit. Biochemical analyses of total antioxidants, total phenolics and PAL activity in the mesocarp tissue have shown the usefulness of Si in improving the fruit's metabolic processes. The C7 sugars (*D*-mannoheptulose and perseitol) also seem to be more prevalent in avocado fruit treated with Si (particularly KSil 2940 ppm) than in non-treated fruit. This suggests that an application of Si to avocado fruit can aid in the retention of vital antioxidants (C7 sugars).

DECLARATION

I hereby declare that the research work reported in this thesis is as a result of my own investigation, except where acknowledged.

Signed: _____

Kamukota Kaluwa

I certify that the above statement is correct.

Signed: _____

Dr. Isa Bertling

Supervisor

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“Consider it pure joy, my brothers, whenever you face trials of many kinds, because you know that the testing of your faith develops perseverance. Perseverance must finish its work so that you may be mature and complete, not lacking anything.” – James 1: 2 – 4.

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CHAPTER 1

GENERAL INTRODUCTION

The avocado (*Persea americana* Mill.) belongs to the Lauraceae family and has been classified into three botanical races, viz. *Persea americana* var. *americana*, (West Indian types), *Persea americana* var. *drymifolia*, (a race of Mexican origin), and *Persea americana* var. *guatemalensis*, (a race of Guatemalan origin) (Bergh and Ellstrand, 1986).

In South Africa, forty percent of the avocado produced is exported. Good phytosanitary measures are a requirement for export and are needed in ensuring good product quality. The avocado fruit is a highly perishable product with a relatively high rate of respiration (Wills *et al.*, 1989), which resulting in the quick deterioration of fruit quality. Due to the threat of pest and disease resistance to the currently used conventional chemicals, there has been a great need to diversify from their usage (Stevens, 2006). The recognition of the importance of silicon (Si) began in the early 1900's (Emanuel, 2005). Silicon has been applied to a number of crop species, mainly members of the Poaceae family, and has been found to confer host-resistance to the crop (Belanger *et al.*, 2003). In some horticultural crops that do not belong to the Poaceae family (tomato, dry beans, green peas and cucumber), Si has been found to offer protection against fungal infections by strengthening cell walls, thus making it more difficult for the fungi to penetrate and colonize the plant (Fawe *et al.*, 2001).

1.1 Silicon and its role in agriculture

Silicon (Si) being the second most abundant element (28%) in the Earth's crust after oxygen, is a major constituent of many soils (Datnoff *et al.*, 2001; Ma and Takahashi, 2002) and has been associated with disease resistance in plants for a long time (Liang *et al.*, 2007). Si dioxide comprises 50 – 70% of the soil mass. As a consequence, all plants rooting in soil contain some Si in their tissues. However, it generally occurs as a relatively insoluble component of primary minerals and secondary minerals (e.g. clays). Si is lost as soils weather, so highly weathered soils, such as in the humid subtropics and tropics, contain less Si than soils in temperate regions (Epstein, 1999).

The concentration of Si in the soil solution ranges from 1 to 40 mg L⁻¹ and seems to be controlled more by chemical kinetics than by thermodynamics (Hallmark *et al.*, 1982). Plants absorb Si from the soil in the form of monosilicic acid (H₄SiO₄), also called orthosilicic acid, which is chemically very active and can react with Al, Fe, and Mn to form slightly soluble silicates. Monosilicic acid combines with heavy metals such as Pb, Cd, Zn, and Hg. The anion of monosilicic acid (Si(OH)₃⁻) can replace phosphate anions (e.g., HPO₄²⁻) from Ca, Mg, Al, and Fe phosphates, thereby making P more readily available to plants (Datnoff *et al.*, 2007).

The adsorption of Si on soil particles is pH-dependant. The higher the pH, the greater this adsorption and, therefore, less Si is available to the plant (McKeague and Cline, 1963). In addition to monosilicic acid, polymerized forms (polysilicic acids) are also an integral component of the soil solution. The mechanism of polysilicic acid formation is not clearly understood. It generally forms when Si in

the soil solution exceeds 65 mg L^{-1} . Unlike monosilicic acid, polysilicic acid is chemically inert. It affects the physical properties of soil, acting as an adsorbent and forming colloidal particles (Savant *et al.*, 1997).

Benefits attributed to Si have not been demonstrated for all crops, but substantial yield increases and pest resistance have been reported when Si fertiliser is applied to rice (*Oryza sativa* L.) and sugarcane (*Saccharum* sp.) grown in soils containing relatively little soluble Si (Liang *et al.*, 2007). The beneficial effects of Si are particularly realised in crops that readily take up Si (accumulators), although some non-accumulator species also accrue benefits with Si nutrition (Ma and Takahashi, 2002).

1.2 Si and its role in the soil

Soil minerals and organic matter control physical and chemical soil properties. Silicon compounds in the soil exist usually in the form of silicon dioxide and various aluminosilicates. Quartz, together with Si-rich minerals (kaolinite, vermiculite, smectite) and amorphous silica form the skeleton of the soil (Orlov, 1985). Numerous physical-chemical soil properties are influenced by minerals. Both mineral components and organic matter determine soil fertility.

According to Hou *et al.* (2006), Si is the only element that does not cause serious injuries in excess amounts. Silicon fertilisation has been reported to result in increased soil exchange capacity, improved water uptake, transformation of P-containing minerals and formation of aluminosilicates and heavy metal silicates (Ma *et al.*, 2001). Silicon substances (amorphous fine silica, calcium silicates, Si-

rich clay minerals) usually exhibit very good adsorption ability. Tokunaga (1991) reported a reduction in the leaching of potassium and other mobile nutrients from the soil surface due to Si fertilisation.

According to world crop production data, 210 to 224 million tons of plant available Si is removed from the soil annually (FAO, 1998). This rapid removal of Si from the soil leads to an acceleration in mineral weathering, de-polymerization of polysilicic acids, increase in Al, heavy metals and Mn toxicity, degradation of soil humic compounds, increase in erosion, decreased microbial population, and decreased plant Si nutrition (Bazilevich, 1993).

Silicon fertilisation is needed on all soils, except for unique soils with an abnormally high level of Si, such as volcanic soils, or soils of an extremely accumulative type of geomorphology, formed in zones with Si fertilisers which generally are Si-rich inorganic substances that increase the content of plant available Si (monosilicic) compounds in the soil (Hou *et al.*, 2006).

Si fertilisers have certain direct effects on soil properties. Phosphate fertiliser efficiency is increased due to transformation of slightly soluble phosphates into plant-available forms (Gladcova, 1982). Similarly, K fertilisation efficiency is also increased. Aluminium toxicity is decreased, and heavy metal mobility in the soil is changed (Jones and Handreck, 1963). Additionally Si can initiate the soil mineral formation process and improve the adsorption properties of the soil (Hou *et al.*, 2006).

1.2.1 Si's interaction with other nutrients in the soil

Si plays an important role in balancing nutrients, often interacting with other elements (Ma *et al.*, 2001). According to Jones and Handreck (1963), toxicities of Al and other metal ions in the soil solution, common in highly leached, acidic, and desilicified soils, are often mitigated by Si. Experiments by Corrales *et al.* (1997) have shown the same effect in solution cultures. Si may also retard or minimise Na uptake by plants, therefore decreasing salinity in the soil (Liang, 1999). Dense plantings and high N applications are usually adopted to optimise production of crops. Under such cultural conditions, leaf erectness is an important factor affecting light interception. Leaf erectness decreases with increasing N fertilization, but Si application increases leaf erectness, reducing mutual shading caused by dense planting and high N application (Ma *et al.*, 2001).

Various Si fertilisers (amorphous dioxide of Si, silica-gel, silicates of Ca, K, or Na) can increase the quantity of mobile phosphates in the soil (Gladcova, 1982). Thermodynamic calculations show that the reaction of displacing phosphate-anion by silicate-anion from slightly soluble phosphates of the corresponding silicates is possible (Matichenkov and Ammosora, 1996).

It is possible to postulate five different mechanisms of Al toxicity reduction by Si-rich compounds. Firstly, monosilicic acid can increase soil pH (Lindsay, 1979); secondly, monosilicic acid can be adsorbed on aluminium hydroxides, impairing their mobility (Panov *et al.*, 1982); thirdly, soluble monosilicic acid can form slightly soluble substances with ions of Al (Horigushi, 1988). Fourthly, Al toxicity reduction by Si-rich compounds can also be achieved by strong adsorption of mobile Al on

silica surfaces (Shulthess and Tokunda, 1996), and finally, mobile Si compounds can increase plant tolerance to Al (Rahman *et al.*, 1998). All of these mechanisms may work simultaneously, with certain ones prevailing under certain soil conditions.

Si compounds are shown to affect heavy metal behaviour in the soil (Treder and Cieśliński, 2005). Monosilicic acids are able to combine with heavy metals (Cd, Pb, Zn, Hg, and others), insoluble complex compounds (Schindler *et al.*, 1976) and poorly soluble heavy silicates (Lindsay, 1979). Energy dispersive X-ray microanalysis revealed that considerable amounts of Cd can be detected in the cytoplasm, vacuole and cellular organelles of plants that are natural Si-accumulators, while very little Si can be found in non Si-accumulator plants (Wang *et al.*, 2001).

1.3 Use of Si on horticultural crops

The role of Si in the nutrition of plant species used in horticulture has not been well investigated in comparison with other agricultural crops, like rice and sugarcane (Iler 1979). Miyake and Takahashi (1978) demonstrated the relevant uptake by species like cucumber, strawberry and tomato. Horst and Marschner (1978) found that an application of Si in bean plants alleviated manganese toxicity. However, the interest in Si application to support growth and development of soil-grown horticultural crops has been very limited (Voogt and Sonneveld, 2001).

1.4 Silicon as a tool for improving crop quality and productivity

Beneficial effects of Si, direct or indirect, on plants under biotic or abiotic stresses have been reported to occur in a wide variety of crops, such as rice, oat, barley, wheat, cucumber, and sugarcane (Datnoff *et al.*, 2001; Ma and Takahashi, 2002). Leaves, stems, and culms of plants grown in the presence of Si grow more erect, thereby increasing the distribution of light within the canopy (Elawad and Green, 1979; Ma *et al.*, 1989; Epstein, 1991). Si increases resistance to lodging and drought in rice and dry matter accumulation in cucumber and rice (Lee *et al.*, 1985; Adataia and Besford, 1986). It also increases the activity of some enzymes involved in photosynthesis in rice and turf grass (Schmidt *et al.*, 1999) and can reduce rice leaf senescence (Kang, 1980 cited by Datnoff *et al.*, 2007). Agarie *et al.* (1998) demonstrated that Si lowers the electrolyte leakage of rice leaves, therefore promoting greater photosynthetic activity in plants grown under water deficit or heat stress. Si decreases injury caused by climate stress, such as typhoons and cool summer damage in rice, alleviates frost damage in sugarcane and other plants and favours supercooling of palm leaves (Savant *et al.*, 1997; Hodson and Sangster, 2002). It reduces the availability of potentially toxic elements, such as manganese (Mn), iron (Fe), and aluminium (Al), to roots of plants such as rice and barley (Horiguchi, 1988; Liang *et al.*, 1996). Moreover, the most significant effect of Si on plants, besides improving their fitness in nature and increasing agricultural productivity, is the restriction of grazing and parasitism (Datnoff *et al.*, 2007).

1.5 The role of Si in controlling plant diseases

The beneficial effects of Si are more evident under stress conditions. This is because Si is able to protect plants from multiple abiotic and biotic stresses

(Miyaki and Takahashi, 1983). Besides the many agronomic benefits gained by maintaining adequate levels of Si in the soil, this element has been found to reduce the intensity of many diseases of important crops, such as rice, cucurbits, muskmelon, wheat, sugarcane, sorghum, and turf grass (Fawe *et al.*, 2001). In cucurbits, the application of Si in the form of potassium or sodium silicates to recirculating nutrient solutions reduced the severity of powdery mildew, caused by *Sphaerotheca fuliginea* (Adata and Besford, 1986). Menzies *et al.* (1991) reported that foliar sprays of potassium silicate (KSil) at concentrations of 17 mM (1000 ppm) and above were effective in controlling powdery mildew in muskmelon (*Cucumis melo* L.) and zucchini (*Cucurbita pepo* L.). Susceptible cucumber cultivars grown in nutrient solution containing KSil at 100, 150, or 200 mg L⁻¹ exhibited a slight, but statistically significant, reduction in powdery mildew (Belanger *et al.*, 2003). Cherif *et al.* (1992) found that 1.7 mM KSil (100 ppm) significantly reduced plant death, root decay, and yield loss caused by *Pythium ultimum* (Trow) in cucumber. The number of cucumber plants attacked by *Pythium aphanidermatum* (Edson Fitzp.) also decreased when Si was added to the nutrient solution (Datnoff *et al.*, 2007). Miyaki and Takahashi (1983) observed a certain degree of control of *Fusarium* wilt in cucumber by applying Si to the soil.

The mechanism or mechanisms underlying the effect of Si in increasing the resistance of crops to diseases remain poorly understood and inconclusive. In the rice – *Magnaporthe grisea* pathosystem, an increase in resistance through Si treatment has been associated with the density of silicified bulliform, long and short cells in the leaf epidermis, which act as a physical barrier to impede penetration by *M. grisea* (Ito and Hayashi, 1931; Suzuki, 1935). This physical

barrier hypothesis is strengthened by the findings of Yoshida *et al.* (1962), who reported the existence of a layer of Si of approximately 2.5 µm thick beneath the cuticle of rice leaves and sheaths. This cuticle Si double layer can impede penetration by *M. grisea* and, consequently, decrease the number of blast lesions on leaf blades. According to Volk *et al.* (1958), Si can form complexes with organic compounds in the cell walls of epidermal cells, therefore increasing their resistance to degradation by enzymes released by *M. grisea*. Indeed, Si can be associated with lignin-carbohydrate complexes in the cell wall of rice epidermal cells (Inanga *et al.*, 1995).

Phenol-like compounds such as phytoalexins also play a crucial role in the defence response in rice, as reported by Maekawa *et al.* (2002), against infection by *M. grisea*. Rodrigues *et al.* (2004) found that leaf extracts from plants inoculated with *M. grisea* and amended with Si contained higher levels of phytoalexins than leaf extracts obtained from inoculated plants, but not amended with Si or un-inoculated plants amended or not amended with Si. The more efficient stimulation of the terpenoid pathway in plants amended with Si and, consequently, the increase in the levels of phytoalexins, appear to be factors contributing to enhanced resistance to blast in rice. Maekawa *et al.* (2002) observed a dramatic increase in superoxide (O_2^-) generation in leaves of rice plants treated with Si, in as little as 15 min after inoculation with *M. grisea* (Datnoff *et al.*, 2007).

In oat plants deprived of Si, the activity of phenylalanine ammonia lyase (PAL) decreased, and consequently the accumulation of phenolic compounds in epidermal cells colonized by *Blumeria graminis* f. sp. *avenae* (Em. Marchal.) also

decreased resulting in a spread of the disease (Carver *et al.*, 1998). Menzies *et al.* (1991) described a rapid accumulation of phenolic-like compounds in the leaf cells of cucumber plants amended with Si and inoculated with *Sphaerotheca fuliginea*, indicating the presence of flavonoids and phenolic acids. Belanger *et al.* (2003) found that the greatest cytochemical difference between wheat plants amended with Si and plants not amended with Si was the extensive deposition of glycosylated phenolics in the cell wall of infected epidermal cells of Si-treated plants and in the extrahaustorial membrane of *B. graminis* f. sp. *tritici*.

Leaf samples from cucumber plants amended with Si and inoculated with *Pythium ultimum* showed an increase in the activity of chitinases, peroxidases, and polyphenoloxidase in comparison with samples from infected plants not amended with Si (Cherif *et al.*, 1994). Dann and Muir (2002) also reported an increase in the activity of chitinase as well as of β -1,3-glucanase in pea seedlings amended with potassium silicate, before the plants were challenged with *Mycosphaerella pinodes*.

1.6 Si as a suitable alternative to conventional agrochemicals

Horticultural production in the 21st century focuses on safe and functional food production including environmentally safe practises. The use of fertilisers and agriculture chemicals must be at an optimum level for sufficient and safe food production as well as minimal environmental hazards (Park, 2001). With an increased global environmental awareness, the role of Si has become increasingly important as a means to achieve a more sustainable production of horticultural crops.

1.7 Aims and objectives

The aim of this research was to investigate the effect of post-harvest Si applications on the quality of 'Hass' avocado fruit. The specific objectives included investigating the effect of Si on the ripening pattern as well as the metabolic physiology of the avocado fruit, particularly important parameters of post-harvest fruit quality. Furthermore, the metabolic physiology of the fruit after treatment with Si is investigated. Specific parameters that were measured include: total antioxidant capacity, total phenolic concentration, the C7 sugars (*D*-mannoheptulose and Perseitol), and the activity of PAL.

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CHAPTER 2

SILICON UPTAKE AND ACCUMULATION IN 'HASS' AVOCADO FRUIT

2.1 Introduction

The avocado fruit is a berry of one carpel containing a single seed. When mature, the fruit is noticeably asymmetrical at the apex as a result of differential growth on opposite sides. The pericarp consists of three layers: the exocarp, which comprises the skin or rind; the fleshy mesocarp, which is the edible portion of the fruit; and the endocarp, which is the thin inner layer next to the outer seed coat (Cummings and Schroeder, 1942). The exocarp is made up of epidermal, parenchyma and sclerenchyma tissues and is the layer which is removed when the fruit is peeled. A relatively thin cuticle forms a wax-like film over the surface of the fruit. Beneath this film is an epidermis of one layer and a hypodermis of one to three layers of brick-shaped cells (Schroeder, 1953).

2.1.1 Uptake and accumulation of Si by plants

Si is taken up by roots as monosilicic acid (H_4SiO_4 or $\text{Si}(\text{OH})_4$) (Ma and Yamaji, 2006). Plants differ greatly in their ability to accumulate Si, ranging from 0.1% to 10.0% Si (dry weight). Most plants, particularly dicots, are unable to accumulate high levels of Si in their shoots (Ma and Takahashi, 2002). Differences in Si accumulation between species have been attributed to differences in the Si uptake ability of the roots. Ma and Yamaji (2006) studied the uptake mechanisms of Si

using three crops: rice, cucumber and tomato, species that accumulate high, medium and low levels of Si, respectively. The authors found that transportation of Si from the external solution to the cortical cells was mediated by the same transporter with a K_m value of 0.15mM, independent of species (Mitani *et al.*, 2005). However, the V_{max} differs with plant species (i.e., rice > cucumber > tomato), suggesting that the density of the transporter differs among plant species. It appears that this process of transport is energy dependent, because metabolic inhibitors and low temperature inhibit transport (Mitani *et al.*, 2005 cited by Ma and Yamaji, 2006).

2.1.2 Modes of uptake

The physiology of Si uptake is still under discussion, but it is likely that both passive and active uptake mechanisms are involved (Raven, 2001). Plants take up Si in the form of monosilicic acid, which is transported to the shoot. Once in the xylem, uncharged H_4SiO_4 readily moves with the transpiration stream and is eventually deposited as solid amorphous silica ($SiO_2 \cdot nH_2O$) in the cell wall matrix, cell lumen, and extracellular spaces of shoot, leaf, culm, and root tissues and in the inflorescences of grasses (Sangster *et al.*, 2001).

The large variation in Si concentration among plant species is the result of differentiated abilities of Si uptake (Takahashi and Miyake, 1976). Based on the Si concentration, three groups of plants are distinguished. In group A, the Si concentration is lower than 0.5% DW Si, suggesting that these plants take up Si more slowly than water. In contrast to group A, the Si concentration in group C is more than 0.5% DW, suggesting that these plants take up Si faster than water

using an active uptake mechanism. Group B has similar Si concentration as the criterion value (0.5% DW). These different modes of Si uptake are also defined as rejective, passive, and active uptake, and differ with plant species (Ma *et al.*, 2001).

2.1.3 Silicon accumulators

Many plants are able to accumulate Si. Depending on the species, the Si percentage of the biomass can range from 1% to more than 10% DW (Elawad and Green, 1979). Plant species are considered accumulators when the concentration of Si is greater than 1% DW (Epstein, 1991). Dicotyledons, such as tomato and soybean, with less than 0.1% DW Si in their biomass, are relatively poor accumulators of Si, compared with monocots. Dry-land grass species, such as wheat, oat, rye, barley, sorghum, maize, and sugarcane, contain about 1% DW Si in their biomass, while aquatic grasses contain up to 5% DW Si (Jones and Handreck, 1967; Epstein, 1999; Datnoff *et al.*, 2001).

Silicon is taken up at levels equal to or greater than essential nutrients such as nitrogen and potassium in plant species belonging to the families Poaceae, Equisetaceae, and Cyperaceae (Savant *et al.*, 1997). The Si-Ca ratio is another criterion used to determine if a plant species is a Si accumulator (Takahashi *et al.*, 1990). Although Si has not been considered an essential element for crop plants - for lack of supportive data - species such as *Equisetum* spp. and some of the Diatomaceae cannot survive without an adequate level of Si in their environment (Epstein, 1991; 1999).

2.1.4 Aims and Objectives

The aim of this experiment was to determine the extent of Si infiltration through the exocarp and mesocarp tissues of 'Hass' avocado fruit after treatment with various Si dips at different concentration. This would allow us to determine whether the effects observed after treatment are a result of Si incorporation into the tissue or are independent of the tissue Si concentration. It would also indicate how well the avocado fruit can take up Si through dipping applications. The specific technique used for this determination was EDAX (Energy Dispersive X-ray) analysis.

2.2 Materials and Method

2.2.1 Origin of Fruit

Avocado (*Persea americana* Mill. cv. 'Hass') fruit were obtained from Everdon Estate in Howick (29°27'S, 30°16'E, KwaZulu-Natal) and from Cooling Estate in Wartburg (29°43'S, 30°58'E, KwaZulu-Natal).

2.2.2 Treatments

Fruit were treated with different forms of Si (potassium silicate – KSil, calcium silicate – CaSil, sodium silicate – NaSil and Nontox-silica® – NTS) at concentrations ranging from 160 ppm to 1470 ppm. Fruit were submerged in the Si solutions for 30 minutes and stored at -0.5°C, 1°C, 5°C or room temperature (25°C). This procedure was replicated three times.

Statistical analyses were carried out using GenStat® version 11 (VSN International, Hemel Hempstead, UK) ANOVA. Treatment means were separated using least significant differences (LSD) at 5% ($P = 0.05$). The experiment was laid out as a complete block design (CBD). Three fruits per treatment were used. Each fruit represented a replicate. There were three replicates used for each treatment block. EDAX analysis was then conducted on the exocarp and mesocarp tissues to determine the extent of Si infiltration within each treatment.

2.2.3 EDAX analysis

EDAX analysis is a technique used for identifying the elemental composition of a specimen. The EDAX analysis system works as an integrated feature of a scanning electron microscope (SEM), and cannot operate on its own without the latter.

In this experiment, Si was analysed by slicing off a piece of fresh avocado tissue (exocarp or mesocarp) and placing it on a specimen stub which was then put in the SEM for EDAX analysis. During the analysis, the specimen was bombarded with an electron beam inside the SEM. The bombarding electrons collide with the specimen atoms' own electrons, knocking some of them off in the process. A position vacated by an ejected inner shell electron is eventually occupied by a higher-energy electron from an outer shell. To be able to do so, however, the transferring outer electron must give up some of its energy by emitting an X-ray.

The amount of energy released by the transferring electron depends on which shell it is transferring from, as well as which shell it is transferring to. Furthermore,

the atom of every element releases X-rays with unique amounts of energy during the transferring process. Thus, by measuring the amounts of energy present in the X-rays released by a specimen during electron beam bombardment, the identity of the atom, from which the X-ray was emitted, can be established (Goldstein *et al.*, 2003).

The only disadvantage with this technique is that it is destructive to the fruit. Once a fruit is analysed, it has to be discarded.

2.3 Results

In all treatments, increased concentrations of Si were observed in the exocarp. EDAX analysis revealed that Si passed through the exocarp into the mesocarp tissue in fruit treated with high concentrations of Si, i.e., KSil 1470 ppm (Table 2.1). Fruit dipped into very dilute Si solutions (80 ppm and 160 ppm) showed very little (KSil, NaSil) to no (CaSil, NTS) infiltration of Si into the mesocarp. NTS was only tested at 80 and 160 ppm and not at 160 and 1470 ppm like the other treatments due to its low solubility in water; it did not dissolve at concentrations higher than 160 ppm. The Si uptake could have occurred directly through the exocarp or it could have taken place via the pedicel cut surfaces.

Table 2.1: Percentage of Si in mesocarp and exocarp tissue after treating with various Si compounds at different concentrations. $LSD_{(P = 0.05)} = 3.928$.

Treatment	Concentration (ppm)	Area of fruit scanned	Si in tissue (%)
KSil	1470	Exocarp	14.59 (a)

		Mesocarp	4.68 (cd)
KSil	160	Exocarp	4.46 (cd)
		Mesocarp	1.11 (e)
CaSil	1470	Exocarp	9.23 (b)
		Mesocarp	2.41 (d)
CaSil	160	Exocarp	5.05 (cd)
		Mesocarp	0.41 (ef)
NaSil	1470	Exocarp	8.54 (b)
		Mesocarp	2.14 (d)
NaSil	160	Exocarp	6.51 (c)
		Mesocarp	1.2 (e)
NTS	160	Exocarp	5.62 (c)
		Mesocarp	1.44 (e)
NTS	80	Exocarp	4.49 (cd)
		Mesocarp	0.2 (f)
Water	0	Exocarp	0.11 (f)
		Mesocarp	0.1 (f)
Air	0	Exocarp	0.08 (g)
		Mesocarp	0.24 (f)

2.4 Discussion

Uptake and accumulation of Si is more common in grasses, like rice and sugarcane, than in tree crops (Ma and Yamaji, 2006). The avocado plant can be classified as relatively poor accumulator of Si (Sangster *et al.*, 2001). It is therefore not surprising that only a small percentage of Si was observed in the mesocarp tissue for all treatments. A possibly more successful method insuring Si uptake by the fruit could be application of Si as a foliar spray during flowering or before fruit set. This would allow uptake of Si over an extended time period.

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CHAPTER 3

EFFECTS OF SILICON APPLICATION ON THE RIPENING PATTERN OF 'HASS' AVOCADO FRUIT

3.1 Introduction

Fruit ripening has been described as the “processes resulting in changes in colour, taste, and texture, which make the fruit acceptable for consumption” (Eaks, 1978). Once started, these ripening processes cannot be reversed. Large amounts of energy are required to trigger the multitude of necessary catabolic and anabolic changes that aid in prolonging the integrity of membranes (Bruinsma, 1981). Tissue senescence finally occurs, leading to the over-ripe state. The avocado (*Persea americana* Mill.) differs from most other fruits in that ripening does not take place on the tree, but only after picking (Schroeder, 1953). Softening is almost always associated with fruit ripening, which leads to increased susceptibility to physical damage and pathological attack (Brady, 1987), thereby reducing the shelf life of the product (Donkin, 1995). Normal avocado softening with acceptable taste occurs only when a certain level of maturity has been reached. Before this state of maturity is reached, only slight softening may occur due to shrivelling from water loss, resulting in poor flavour (Bower and Cutting, 1988). Once horticultural maturity has been reached, the rate of post-harvest softening becomes progressively faster with increasing maturity (Forero, 2007).

The avocado fruit is highly perishable. The relatively high rate of respiration results in a relatively quick deterioration of fruit quality (Wills *et al.*, 1989). Many external and internal factors of biotic and abiotic nature participate in this process. Keeping the quality of avocado is, however, particularly important for the export industry, where fruit quality has to be maintained for long periods of time.

It is necessary to understand the physiology of avocado ripening in the search for possible causes of common avocado disorders, and also to develop pre- and post-harvest practices effective in producing high quality fruit with extended shelf life. The avocado fruit is climacteric, which implies a marked rise in respiration rate on the onset of ripening, followed by a decline (Bower and Cutting, 1988).

3.1.1 Factors affecting fruit ripening

Factors influencing the ripening rate can affect fruit quality. Pre-harvest factors, like water stress and nutrient imbalances, have a major influence on avocado fruit ripening, affecting the variability of ripening in fruit subjected to these stress factors so that not all fruit ripen at the same time. An increase in water stress reduces the normal time of ripening which then leads to an increase in the incidence of postharvest disorders, such as mesocarp discoloration and anthracnose (Bower and Cutting, 1988).

The rate of avocado fruit ripening is also related to fruit maturity, as fruit picked at the beginning of the picking season takes longer to ripen than fruit picked at the end of the season (Zauberman and Schiffman-Nadel, 1972). Hopkirk *et al.* (1994) noted that fruit that ripened more rapidly had a lower incidence of stem-end and

body rots, although the incidence of internal browning was not affected. Susceptibility to disorders is influenced by many cultural factors including cultivar, maturity, production location, rootstock, crop load, tree vigour and mineral nutrition (Ferguson *et al.*, 1999). 'Fuerte' is more susceptible to these disorders than 'Hass' (Bower and Cutting, 1988). Internal disorders increase with maturity, but skin discolouration due to chilling injury (CI) decreases with maturity (Cutting and Wolstenholme, 1992).

3.1.2 Colour change as an indication of ripening

The skin colour of 'Hass' avocado (*Persea americana* Mill.) fruit changes from green to purple/black during ripening. This colour change is important as an indicator of ripeness for both industry and consumers. However, little is known about the anthocyanins responsible for these changes. In addition, late-season skin darkening can be observed on fruit before harvest. Although ripening time is reduced for late-season fruit, the fruit may be dark, but un-ripe. This colouration can confuse customers as fruit appears to be ripe, but is not soft (Cox *et al.*, 2004).

3.1.3 Aims and objectives

The objective of this study was to investigate whether the application of silicon (Si) as a post-harvest dip has an effect on the ripening pattern (softening, carbon dioxide production, ethylene evolution and skin colour) of 'Hass' avocado fruit. Fruit ripening is directly correlated to stress. Therefore when fruit is subjected to stressful conditions, it is likely to ripen quicker than when subjected to favourable

conditions. It is expected that Si will have an effect on the ripening pattern due to its stress-reducing ability (Ma *et al.*, 2001).

3.2 Materials and Methods

Avocado (*Persea americana* Mill. cv. Hass) fruit were obtained from Everdon Estate in Howick (29°27'S, 30°16'E, KwaZulu-Natal) and Cooling Estate in Wartburg (29°43'S, 30°58'E, KwaZulu-Natal). Fruit were treated with Si sources at concentrations ranging from 80ppm to 2940 ppm and subsequently stored at either -0.5°C, 1°C, 5°C or room temperature (25°C). Fruit from Everdon were treated with four different sources of Si namely, potassium silicate (KSil), Nontox-Silica® (NTS), calcium silicate (CaSil) and sodium metasilicate pentahydrate (NaSil) as dips. Three separate trials were done throughout the year representing early (July), middle (September) and late (December) season fruit. Fruit from Wartburg were treated with three sources of Si (potassium silicate - KSil, Nontox-Silica® - NTS, and Biosilicate) applied as soil drenches throughout the season. Only one concentration (manufacturer's recommendation) of each treatment was used.

The potassium silicate was supplied by Plant Health Products, South Africa (SA), NTS by Plant Bio Regulators (Pty) Ltd (Wierda Park, SA), the calcium silicate was supplied by National Plant Food (Camperdown, SA) and the Biosilicate was supplied by Biotechnica (product manufactured by Hexachem, SA). Firmness measurements, ethylene evolution and CO₂ production were recorded as fruit approached ripening. The CO₂ production of fruit stored at room temperature was

analysed daily until fruit had fully ripened, while fruit from cold storage were removed weekly to measure respiration and were allowed to ripen.

3.2.1 Measurement of fruit firmness

Fruit firmness of each fruit was measured daily as soon as softening was initiated using a hand-held firmness tester (5 mm anvil; Densimeter, Bareiss, Oberdischingen, Germany) (Figure A3). The same fruit was measured each day, taking two measurements along the equatorial region of the fruit.

3.2.2 Measurement of respiration rate

Fruit respiration rate, as determined by CO₂ production, was measured using an infrared gas analyser (EGM-1, PP Systems, Hitchin, Hertfordshire, UK) (Figure A4). Fruit were incubated in 1 litre plastic jars for 12 minutes. Net CO₂ production per kilogram fruit was calculated by adjusting for head space, ambient CO₂ in the jar, fruit volume and fruit mass. Carbon dioxide concentration ($\mu\text{l L}^{-1}$) was determined daily and results were calculated as rate of CO₂ production per hour ($\text{ml kg}^{-1} \text{FW h}^{-1}$).

3.2.3 Measurement of ethylene evolution

Ethylene evolution was measured every second day until the fruit were “ripe”; fruit were considered to be “ripe” when softness measurements reached less than 60 (45.61 N) on the hand-held firmness tester (5 mm anvil; Densimeter, Bareiss, Oberdischingen, Germany). Ethylene production was measured using a gas chromatograph (DANI 1000, DANI Instruments S.p.A., Monzese, Italy) in

conjunction with an auto-sampler (HT250D, HTA S.r.L., Brescia, Italy) (Figure A5). The gas chromatograph (GC) was equipped with a flame ionization detector (FID) and a stainless steel packed column with an alumina-F1 stationary phase. Fruit ethylene production was measured by placing a single fruit in a 1L plastic jar containing a 20 ml glass vial, sealing the jar and incubating for 30 minutes. The ethylene produced by the fruit during that time was retained in the glass vial which was transferred to the GC autosampler where the ethylene ($\mu\text{l L}^{-1}$) concentration was determined. Taking into account head space, fruit volume, fruit mass and period of incubation, net ethylene values were calculated as ($\mu\text{L C}_2\text{H}_2 \text{ kg}^{-1} \text{ FW h}^{-1}$).

3.2.4 Assessment of skin colour change

Skin colour was rated by one trained assessor using the following scale: 1 – emerald green; 2 – forest green; 3 – green to brown; 4 – brown to purple; and 5 – black (Table A1 Appendix). Colour variability around the fruit was averaged.

3.2.5 Experimental design

Statistical analyses were carried out using GenStat® version 11 (VSN International, Hemel Hempstead, UK) ANOVA. Treatment and storage temperature were separated using least significant differences (LSD) at 5% ($P = 0.05$). The experiment was laid out as a split-plot design with the main effect being storage temperature and the sub-effect being the four Si treatments at their various concentrations. Each fruit represented a replicate. There were three fruit used for each treatment.

3.3 Results

3.3.1 Firmness

As expected fruit stored at 25°C were the first to become “eat-ripe” (5 days after treatment) followed by fruit stored at 5°C. There were significant differences in temperature means with regards to firmness. The firmness of all fruit decreased with time (Figure 3.1). Fruit treated with KSil and NTS, which were stored at 5°C were firmer than fruit stored at other temperatures (Figure 3.2) three days after removal from storage.

3.3.2 CO₂ production

After removal from cold storage, CO₂ production increased with most fruit reaching the respiratory peak five days after removal from storage. Thereafter respiration decreased gradually (Figure 3.3). The late-season (Harvest 7) fruit treated with Si in the form of KSil at 2940 ppm produced the least amount of CO₂ and reached their respiratory peak later than non-treated fruit (Air) which had the highest respiration rate. There was no significant difference in the respiration rate of early (Harvest 5) and mid-season (Harvest 6) fruit (data not shown). Fruit stored at room temperature (25°C) produced significantly higher amounts of CO₂ and peaked much earlier than fruit stored at other temperatures (Figure 3.4).

3.3.3 Ethylene production

There were differences ($P < 0.05$) between temperature means with the highest net ethylene being produced by fruit stored at 25°C (Figure 3.5). There were also significant differences amongst treatment means ($P < 0.001$), with fruits treated with KSil 2940ppm producing the least ethylene (Figure 3.6).

With regards to colour change, there were no significant differences ($P > 0.05$) observed in both storage temperature means and treatment means (data not shown). The application of Si had no effect on the change in colour of 'Hass' avocado fruit during ripening.

3.4 Discussion

Climacteric fruit are characterised by a rise in respiration during ripening followed by a quick decline (Millerd *et al.*, 1962). The storage life of agricultural produce, like avocado fruit, is inversely related to the rate of respiration because respiration supplies compounds that determine the rate of metabolic processes directly related to quality parameters, e.g., firmness, sugar content, aroma and flavor (Zauberman and Schiffman-Nadel, 1972). Agricultural commodities and cultivars with high rates of respiration tend to have a shorter storage-life than those with low rates of respiration (Saltveit, 1996).

Carbon dioxide is a by-product of respiration and was measured in this study to determine the respiration rate of each fruit. A high CO₂ evolution means that the sample/fruit was undergoing a high level of respiration at the time and that it will deteriorate quickly resulting in reduced quality.

The higher concentrations of Si applied to the fruit only slowed down the respiration rate in the late season fruit. Late season fruit take a shorter time to ripen than early season fruit because their development is more advanced as they have stayed on the tree for a longer period of time (Cutting and Wolstenholme, 1992). This rapid ripening of "older" fruit is a result of metabolic processes of respiration resulting in a faster ripening rate. Therefore, the presence of Si could

slow down the rate of these processes in the fruit and reduce the rate of respiration.

Stress is one of the post-harvest factors that affects the respiration rate in agricultural products. The higher the stress levels, the higher the respiration rate (Saltveit, 1996). A possible explanation as to why Si-treated fruit displayed the lowest respiration rate is that Si reduces stress in plants (Ma *et al.*, 2001).

Post-harvest applications of 2940 ppm Si in the form of KSil seem to be most beneficial as respiration was most suppressed in this treatment. The Si concentration had no effect on the deposition of Si in the mesocarp tissue, but the amount of Si found on the exocarp was high in fruits treated with high Si concentrations, i.e., KSil 2940 ppm (Chapter 2). It is therefore useful to consider the use of Si compounds in improving the quality of avocado fruit as this has proven to be beneficial in enhancing the ripening processes by reducing the respiration rate.

Figures

Firmness

Early season (Harvest 5)

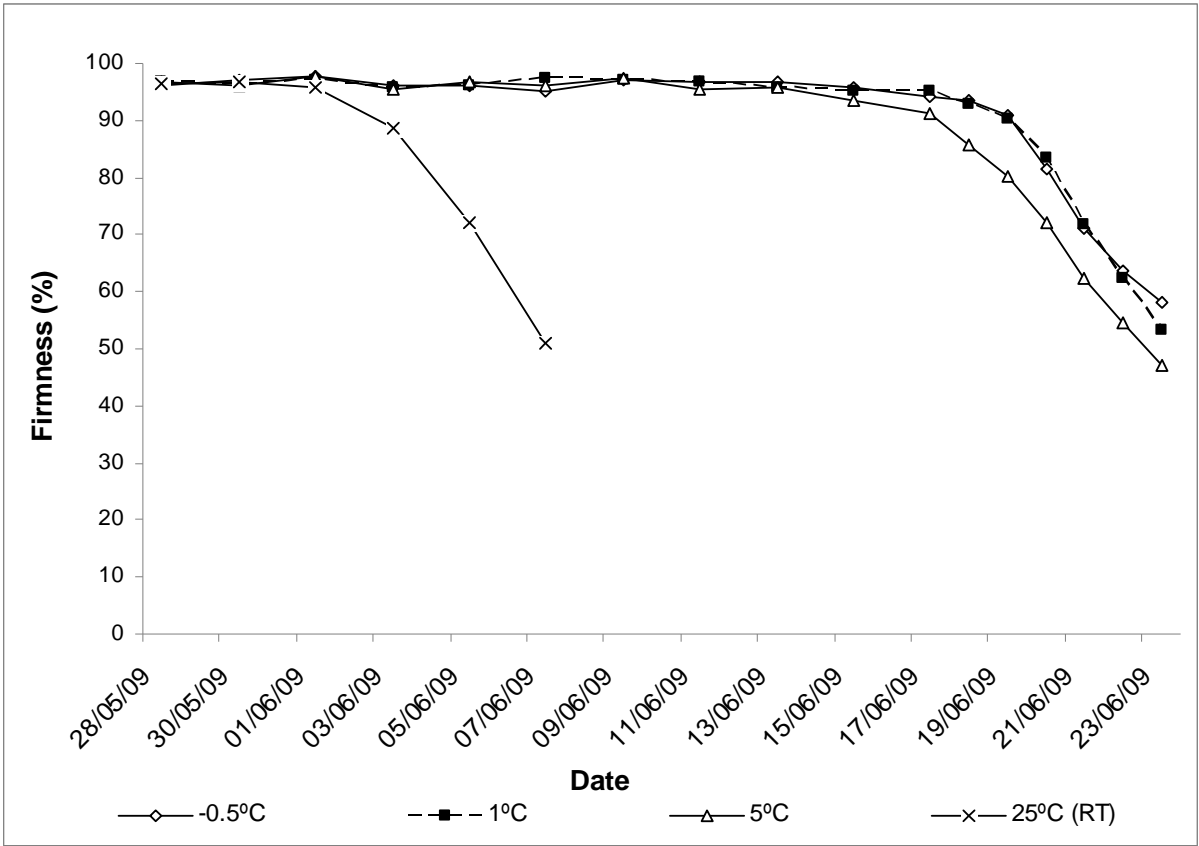


Figure 3.1: Firmness of early season (May 2009) 'Hass' avocado fruit stored at different temperatures. Fruit were removed from storage after 21 days and kept at room temperature until eat-ripe softness was reached. LSD ($P = 0.05$) = 0.914.

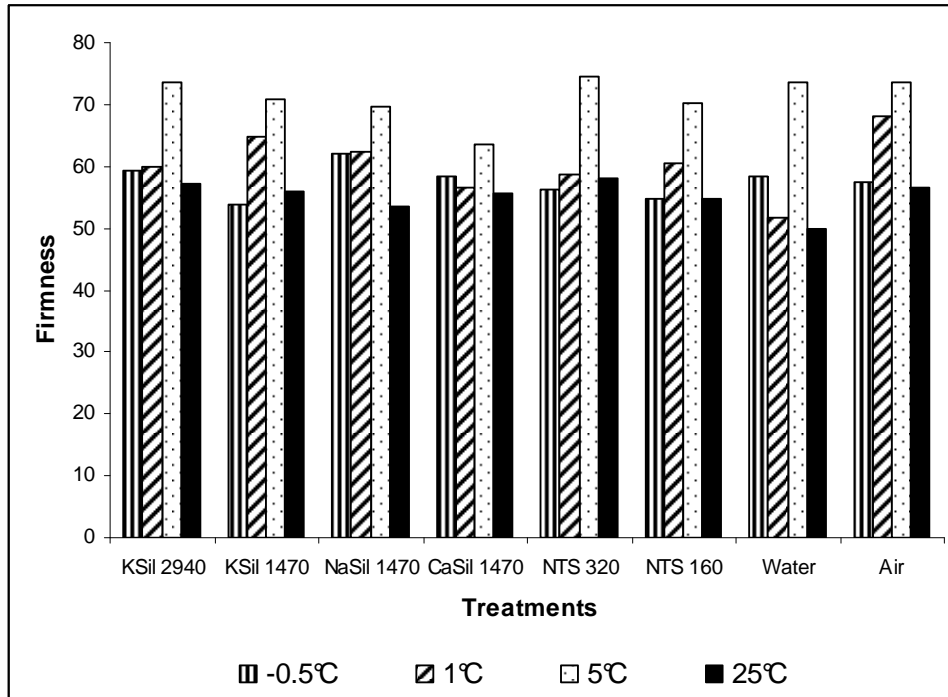


Figure 3.2: Firmness of 'Hass' avocado fruit treated with different Si compounds three days after removal from cold storage. LSD ($P = 0.05$) = 4.791.

Respiration rate

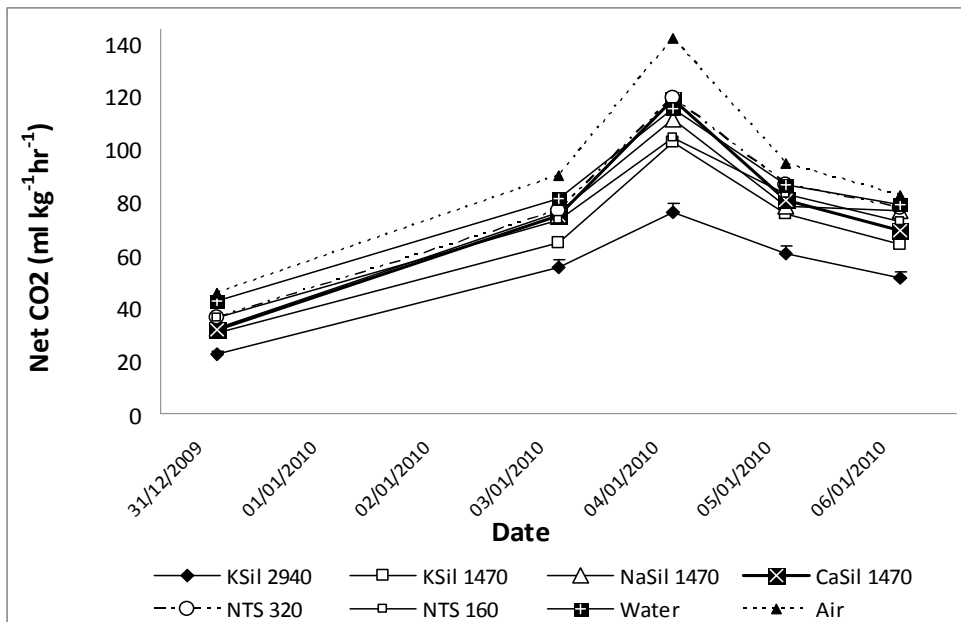


Figure 3.3: Net CO₂ production of late-season (December 2009) 'Hass' avocado fruit treated with different Si compounds. LSD ($P = 0.05$) = 5.623.

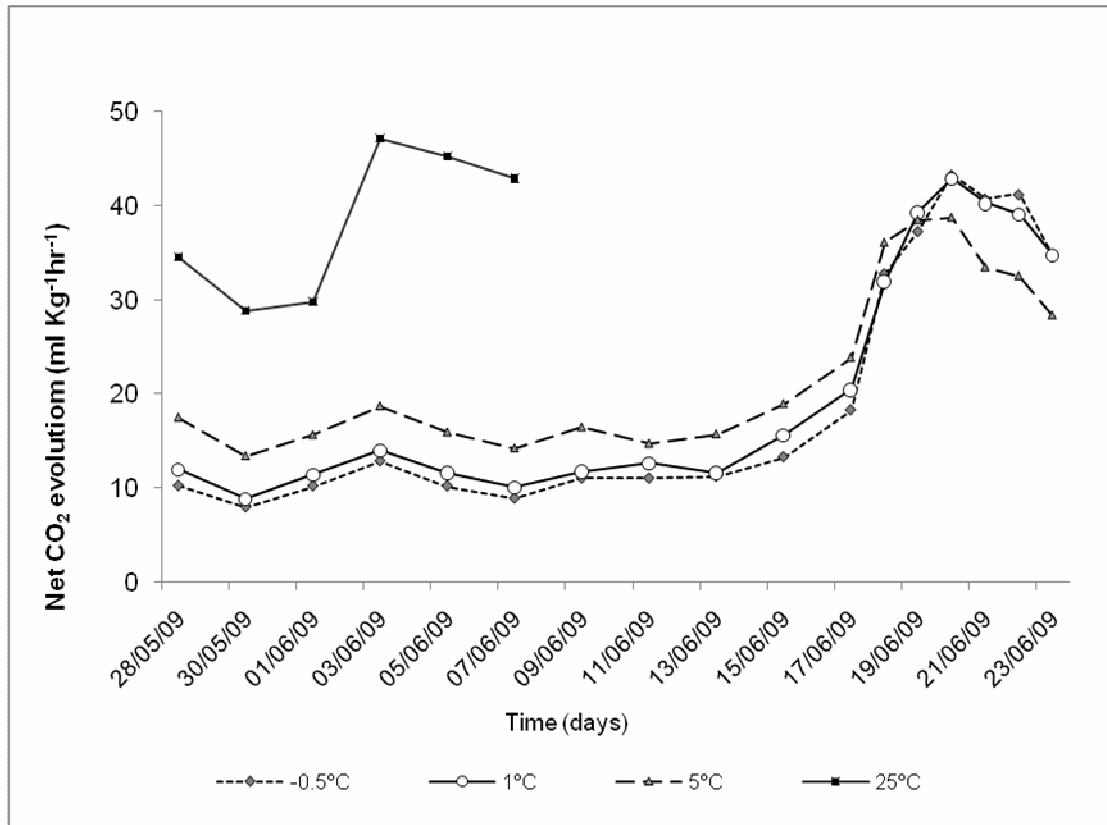


Figure 3.4: Net CO₂ (ml kg⁻¹ h⁻¹) production of early-season (Harvest 5) 'Hass' avocado fruit stored at -0.5°C, 1°C, 5°C or room temperature (25°C). LSD ($P = 0.05$) = 0.624.

Ethylene evolution

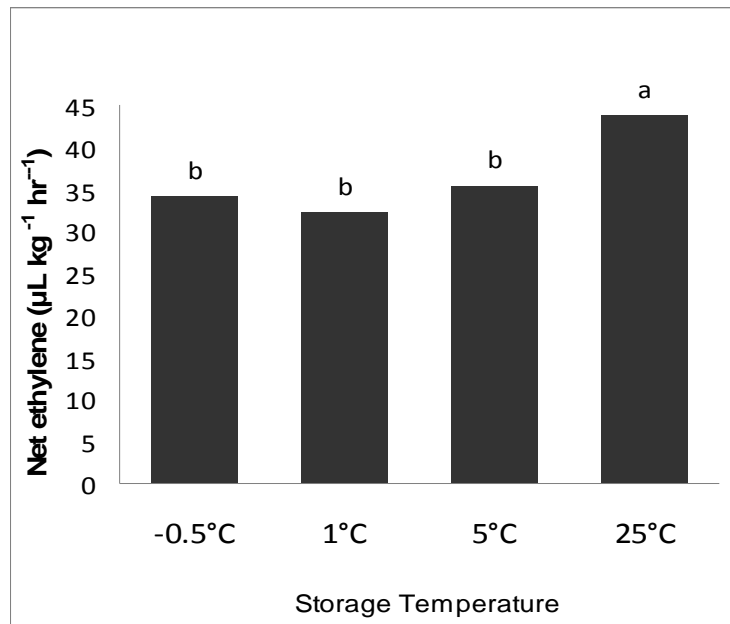


Figure 3.5: Net ethylene ($\mu\text{L kg}^{-1} \text{h}^{-1}$) production of ripe 'Hass' avocado fruit (firmness readings less than 60%) stored at -0.5°C, 1°C, 5°C or at room temperature (25°C). Fruit were harvested late in the season (Harvest 7). LSD ($P = 0.05$) = 4.762

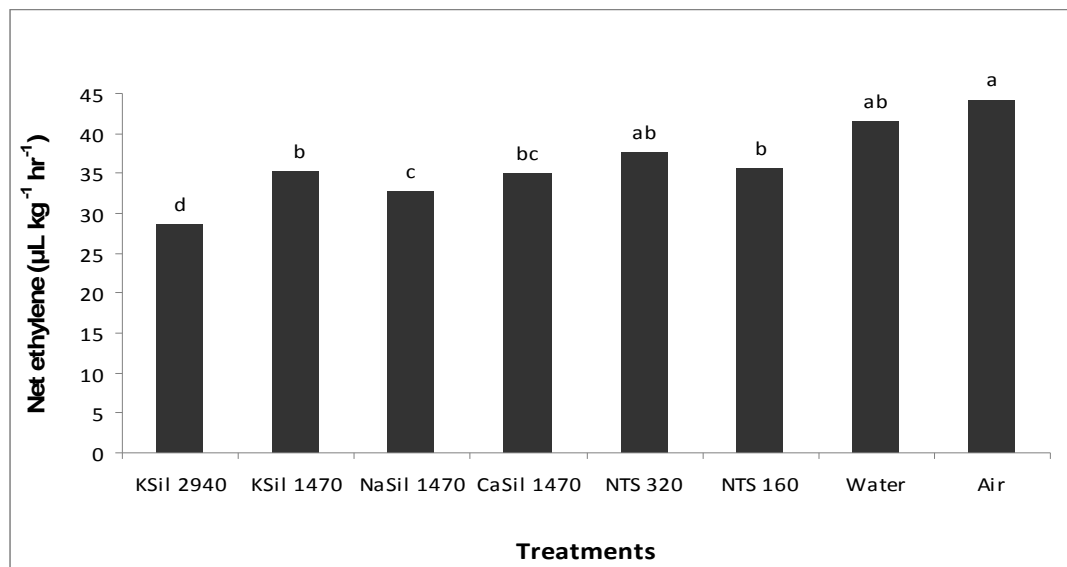


Figure 3.6: Net ethylene ($\mu\text{L kg}^{-1} \text{h}^{-1}$) production of ripe 'Hass' avocado fruit (firmness readings less than 60%) treated with different concentrations of Si treatments. LSD ($P = 0.05$) = 6.734.

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CHAPTER 4

EFFECT OF SILICON APPLICATION ON 'HASS' AVOCADO FRUIT PHYSIOLOGY

4.1 Introduction

Avocado (*Persea americana* Mill.) fruit are relatively rich in antioxidants (Pellegrini *et al.*, 2003). Antioxidants are found inside cells and their purpose is to guard cellular structures against naturally occurring, extremely reactive compounds, the reactive oxygen species (ROS) such as the hydroxyl radical, the superoxide ion, hydrogen peroxide and other free radicals (Benzie and Strain, 1996). Due to their high chemical reactivity, these compounds have a high potential of damaging proteins, DNA and lipids – a damage which will eventually lead to cell death (Apel and Hirt, 2004). The removal of such compounds from cells is made possible either by ROS scavenging enzymes (e.g. superoxide dismutase, ascorbate peroxidase and catalase) that inactivate the ROS or by small scavenging molecules (e.g. ascorbic acid, glutathione and polyphenols) that combine with these ROS to form non-toxic compounds (Apel and Hirt, 2004).

When growing conditions are favourable, the production of antioxidants in plant cells is low and ROS occurring in cells are scavenged by the antioxidants present (Mittler, 2002). During regular cell metabolism, ROS and antioxidants exist in equilibrium. According to Mittler (2002) stress (drought, chilling, heat shock, UV, pathogens and light) can increase the production of ROS dramatically, which would then be counteracted by an increase in antioxidants. However, when exposed to stressful conditions, the pool of antioxidants present cannot scavenge all ROS produced, which results in cell damage in the fruit (Wang *et al.*, 1996).

Tesfay *et al.* (2010) revealed that in avocado fruit, the mesocarp tissue has a significantly lower total antioxidant capacity compared with seed and exocarp tissues. The authors argue that this might be why particularly the mesocarp is sensitive to various disorders, like anthracnose and vascular browning, as the capacity of flesh tissue to counteract oxidative stress is significantly lower than that of other tissues (Prusky, 1988). Although the mesocarp tissue has the lowest concentration of antioxidants, like polyphenolics and ascorbic acid, Tesfay *et al.* (2010) found that this tissue had the highest concentration of the C7 sugars, *D*-mannoheptulose and perseitol, which act as the major antioxidants in this tissue.

4.1.1 Sugars as a source of energy for avocado fruit

Sugars are important components in the fruit with the major purpose of providing energy. Little is known on either sugar metabolism or sugar content and composition in young developing avocado fruit (Bertling *et al.*, 2007). Five major soluble sugars have been identified in 'Hass' fruit, viz. the disaccharide sucrose, the hexoses glucose and fructose, the C7 reducing sugar *D*-mannoheptulose and its poly-hydroxyl derivative, the sugar alcohol perseitol. These five sugars together constitute 98% of the total soluble solids (TSS) in 'Hass' fruit (Liu *et al.*, 1999a).

Liu *et al.* (1999a; 1999b) found C7 sugars to be the major non-structural carbohydrates present in avocado fruit. They assumed that the C7 sugars played an essential role in fruit growth and proposed that avocado fruit growth follows two physiological phases, carbon accumulation and carbon utilization. During the first phase, soluble sugars provide carbon for the increase in fruit biomass and storage

of photo-assimilates, while the second phase is accompanied by the slowing of fruit growth and coincides with the cessation of TSS accumulation, a decrease in soluble sugars, and the accumulation of oil (Liu *et al.*, 1999a).

4.1.2 Phenolic compounds

Fruit phenolics play an important role in avoiding damage due to free radicals and reactive oxygen species (ROS) that may impede fruit growth and development. ROS have also been implicated in negative effects on humans, such as initiating events resulting in cancer, arteriosclerosis and cataracts (Morello *et al.*, 2005). Phenolic compounds are a diverse range of secondary metabolites derived from the shikimate pathway. They originate from the general phenylpropanoid metabolism, which is characterised by three early steps in the conversion of L-phenylalanine to various hydroxycinnamic acids. The enzymes catalysing the individual steps in this sequence are, from first to last, phenylalanine ammonia lyase (PAL, EC 4.3.1.5), cinnamate-4-hydrolase (EC 1.14.13.11) and 4-coumarate: CoA ligase (EC 6.2.1.12), respectively (Haslam, 1998).

4.1.3 Phenylalanine Ammonia-Lyase (PAL) activity

Phenylalanine ammonia-lyase (PAL) is considered to be the key enzyme in the biosynthesis of phenolics, since it catalyses the reductive deamination of L-phenylalanine to form *trans*-cinnamic acid, the first step in the biosynthesis of plant phenylpropanoid compounds, which results in the formation of compounds like lignin, flavanoids and hydroxycinnamic acids (Tovar *et al.*, 2002). PAL activity varies with the development stage of the plant, cell and tissue differentiation, as well as various stresses such as irradiation, wounding, nutrient deficiencies,

herbicide treatment, and viral, fungal and insect attacks, incidents that increase PAL synthesis or PAL activity in various plants (Hussain *et al.*, 2010).

4.1.4 Aims and Objectives

The aim of this study was to investigate the effect of post-harvest Si applications on the metabolic physiology of 'Hass' avocado fruit. The specific objectives included measuring the following physiological parameters in mesocarp tissue: total antioxidant capacity (TAOC), carbon-7 (C7) sugars – *D*-mannoheptulose and perseitol, total phenolic concentration and activity of PAL. These parameters are good indicators of the level of stress incurred by the fruit. High stress levels result in an accumulation of ROS which then trigger the build-up of antioxidants to counteract them (Mittler, 2002). If Si reduces the stress levels in plants (e.g chilling, pathogen damage), as Ma *et al.* (2001) postulate, then Si-treated fruit should have a higher accumulation of total antioxidants than non-treated fruit.

4.2 Materials and Method

4.2.1 Fruit origin

Avocado (*Persea americana* Mill. cv. Hass) fruit were obtained from Everdon Estate in Howick (29°27'S, 30°16'E, KwaZulu-Natal) on 9 December 2009 (late season).

4.2.2 Treatments

Fruit were treated with Si sources at concentrations ranging from 80 ppm to 2940 ppm and subsequently stored at -0.5, 1, 5°C or at room temperature (25°C) for 28 days. Fruit were treated with four different sources of Si, namely, potassium

silicate (KSil), Nontox-Silica® (NTS), calcium silicate (CaSil) and sodium metasilicate pentahydrate (NaSil) as dips. Two concentrations were used for each Si treatment. Potassium silicate was applied at 2940 ppm (KSil 2940) and 1470 ppm (KSil 1470), due its high solubility in water, whilst CaSil and NaSil were applied at 1470 ppm and 160 ppm. NTS was applied at 160 ppm and 80 ppm, because these are the highest concentrations at which the product can be dissolved in cold water. The fruit were then dipped into the various treatments for 30 minutes.

Chemicals were supplied by Plant Health Products, Nottingham Rd, SA (KSil), Plant Bio Regulators (Pty) Ltd, Wierda Park, SA (NTS) and National Plant Food, Camperdown, SA (CaSil). All fruit were treated in the Horticultural labs at the University of KwaZulu-Natal, Pietermaritzburg.

Mesocarp tissue was extracted from each fruit weekly using a cork borer and subsequently freeze-dried and stored for physiological analysis. The extraction was initiated one week after the fruits were treated. The freeze-dried mesocarp tissue was then finely ground and later analysed for sugars, total anti-oxidant capacity (TAOC), total phenolics (TP) and PAL activity using the procedures described below. All statistical analyses were carried out using GenStat® version 11 ANOVA. Treatment and storage temperature means were separated using least significant differences (LSD) at 5% ($P = 0.05$). The experiment was laid out as a split-plot design with the main effect being storage temperature and the sub-effect being the different concentrations of Si products. Each fruit represented a replicate.

4.2.3 Determination of Total Phenolic (TP) concentration

The extraction of both, bound and soluble, free phenolic compounds was carried out according to Böhm (2006). An amount of 100 to 500 mg sample was weighed into centrifuge tubes. After mixing the samples with 1 mL hydrochloric acid (1.0 M) and incubating at 37°C for 30 min, 1 mL NaOH solution (2.0 M in 75% methanol) was used for alkaline hydrolysis. A second incubation (37 °C, 30 min) was followed by mixing the samples with 1 mL meta-phosphoric acid (0.75 M), and centrifuging at 5,000 rpm (4193g). One milliliter of acetone/water (1:1) was added at the end of the extraction. The mixture was again centrifuged at 5,000 rpm for 5 min. After transferring the supernatant into a flask, the extraction with acetone/water was repeated twice. The combined extracts were filled up to 10 mL with acetone/water (Böhm, 2006). The content of polyphenols was analysed spectrophotometrically at 765 nm using Folin-Ciocalteu reagent. Gallic acid monohydrate was used as the standard and the TP concentration expressed as gallic acid equivalents (GAE) (Singleton and Rossi, 1965).

4.2.4 Determination of Total Antioxidant Capacity (TAOC)

The total anti-oxidant capacity (TAOC) was measured using the FRAP (ferric-reducing ability of plasma) assay (Benzie and Strain, 1996). The assay depends upon the reduction of the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. An amount of 0.1 g sample was weighed out, and 5 mL of 1 N perchloric acid was added. The mixture was vortexed and centrifuged at 12,402 g for 10 min at 4°C. The supernatant was transferred to a test tube. Fresh FRAP reagent solution was prepared for every assay prior to measurement by mixing 300 mM sodium acetate

buffer (pH 3.6), 10 mM of TPTZ dissolved in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (10:1:1). An aliquot of the samples (30 μl) was mixed with 900 μl of FRAP reagent. After mixing, the change in absorbance was measured at 593 nm. Standards were prepared using 100-1000 $\mu\text{mol/L}$ of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The total antioxidant capacity was expressed as $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O g}^{-1}$ DW.

4.2.5 Analysis of sugar profiles in mesocarp tissue

Finely ground lyophilised mesocarp tissue sample was weighed (0.05 g) and mixed with 10 ml 80% (v/v) ethanol and homogenized using an Ultraturrax® for one minute. After homogenizing, the mixture was incubated in a water bath (80°C) for 60 min to extract the soluble sugars. The mixture was then stored at 4°C overnight. The following day, the mixture was centrifuged at 10,000 rpm (11953g) for 10 min at 4°C. The supernatant was then filtered through glass wool and transferred into scintillation vials and taken to dryness in a vacuum concentrator. Dried samples were re-suspended in 2 ml ultra pure water and vortexed. The suspension was later filtered through 0.45 μm nylon filters and analysed using an isocratic high-performance liquid chromatograph (LC-20AT; Shimadzu Corp., Kyoto, Japan) equipped with a refractive index detector (RID-10A; Shimadzu Corp., Kyoto, Japan) using a 300 mm X 7.8 mm Rezex RCM-Monosaccharide column (8 μm pore size; Phenomenex®, Torrance, CA, USA).

4.2.6 Phenylalanine ammonia-lyase (PAL) activity

The activity of PAL in the mesocarp tissue of 'Hass' avocado fruit was determined by weighing out 0.1 g ground mesocarp tissue. Extraction of PAL enzyme was carried out using a 0.05 M sodium borate buffer (pH 8.8) containing 5 mM

mercaptoethanol and 1 g of 4% (w:v) polyvinylpyrrolidone (PVP). This mixture was then shaken and homogenized using an Ultraturrax® and later centrifuged at 10,000 rpm (11953g) (4°C) for 15 min. The supernatant was collected for determination of PAL activity. PAL activity was assayed using a reaction mixture of 1.9 ml 0.05 M sodium borate buffer (pH 8.8) containing 1 ml of 20 mM L-phenylalanine and 0.1 ml of enzyme extract (Jiang and Joyce, 2003). The cinnamic acid concentration was estimated by measuring the absorbance at 290 nm (A₂₉₀) of the supernatant. The reaction mixture was then incubated at 37°C for 1 h. Following this, the reaction was stopped by adding 0.2 ml of 6 M trichloroacetic acid (TCA) and measured at 290 nm. The enzyme activity was measured by recording the difference in absorbance at 290 nm within the 1 h period. PAL activity was expressed as mmol cinnamic acid liberated per gram of dry weight mesocarp tissue per hour (mmol cinnamic acid g⁻¹ DW h⁻¹).

4.3 Results

4.3.1 TP concentration

There were significant differences ($P < 0.001$) in temperature means with regards to the TP concentration (Figure 4.1), with fruit stored at -0.5°C having the highest TP concentration. The TP concentration of fruit treated with KSil 2940 ppm was greatest whilst the control fruit (Air and Water) had the lowest concentration of phenolics (Figure 4.2).

4.3.2 TAOC

There were differences ($P < 0.05$) in storage temperature means with respect to TAOC of mesocarp tissue in 'Hass' avocado fruit (Figure 4.3). Fruit stored at -0.5°C had the highest TAOC (52.53 $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O g}^{-1}$ DW). With regards to

treatment means, there were no significant differences in TAOC ($P > 0.05$), although fruit treated with KSil 2940 ppm and stored at -0.5°C had a tendency towards highest total antioxidant capacity (Figure 4.4).

4.3.3 Sugar Profile

There were significant differences ($P > 0.001$) in temperature means with regards to the concentration of mannoheptulose and perseitol (mg g^{-1}) in mesocarp tissue of 'Hass' avocado fruit (Figure 4.5). Fruit stored at -0.5°C had the highest concentration of both C7 sugars and the concentration of mannoheptulose was generally higher than that of perseitol in all storage temperatures. There were also significant differences in treatment means ($P < 0.001$) showing that fruit treated with KSil 2940 ppm had the highest concentration of both mannoheptulose (18.92 mg g^{-1}) and perseitol (15.93 mg g^{-1}) in the mesocarp tissue (Figures 4.6 and 4.7).

4.3.4 PAL Activity

Further analysis showed differences ($P < 0.05$) in storage temperature means with respect to PAL activity. Fruit stored at 5°C had the highest mesocarp PAL activity ($18.61 \text{ mmol cinnamic acid g}^{-1} \text{ DW h}^{-1}$) with fruit stored at other temperatures (Figure 4.8). There were significant differences in treatment means ($P = 0.001$) with regard to PAL activity. Fruit treated with KSil 2940 ppm had the highest PAL activity ($23.34 \text{ mmol cinnamic acid g}^{-1} \text{ DW h}^{-1}$), whereas non-treated fruit (water and air) had the lowest (Figure 4.9).

4.4 Discussion

Silicon has been used in agriculture for many years and its beneficial effects are evident in many plant species (Datnoff *et al.*, 2001). It is therefore not surprising that these positive effects of Si may also be applicable to 'Hass' avocado fruit.

The antioxidant defense system in fruits is composed of a mixture of antioxidants. Avocado fruits are a good source of antioxidants and may be more effective and economical than supplements in protecting the body against oxidative damage under different conditions (Leong and Shui, 2002). The pool of antioxidants occurring naturally in a system usually increase as ROS increase (Wang *et al.*, 1996). ROS will normally increase if there is a certain level of stress that has been introduced (Mittler, 2002). The results showed that fruit treated with the highest concentration of Si had the highest TAOC (Figure 4.4), possibly preparing fruit for better resistance against stress due to an initiation of a high antioxidant pool.

The prevailing antioxidant system in the mesocarp tissue is the pool of C7 sugars – *D*-mannoheptulose and perseitol (Tesfay *et al.*, 2010). The C7 sugars found in avocado fruit play a vital role in preventing oxidative damage caused by ROS due to their antioxidant properties.

Fruit treated with high concentrations of Si had a greater TP concentration than control fruit. This is in support of Bekker *et al.* (2007) who showed that potassium silicate application significantly increases the amount of phenolic compounds available to the plant via the roots.

Biochemical analyses revealed that total antioxidants, total phenolics and PAL activity increase in the mesocarp tissue of avocado fruit as a result of applying Si. Further investigations should address how this “immune system” of antioxidant compounds can be enhanced pre-harvest as such treatments could improve fruit quality.

Figures

Total Phenolic (TP) concentration

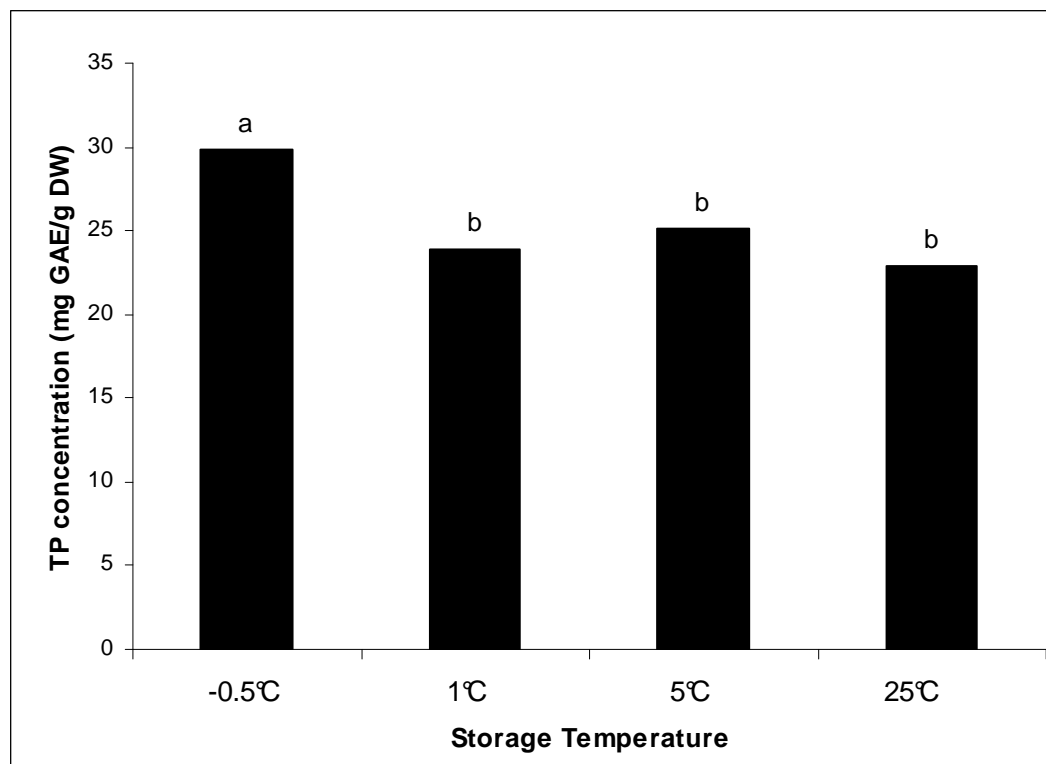


Figure 4.1: Total phenolic concentration expressed as GAE (Gallic Acid Equivalents) for mesocarp tissue of ‘Hass’ avocado fruit stored at different temperatures for 28 days. LSD ($P = 0.05$) = 4.182

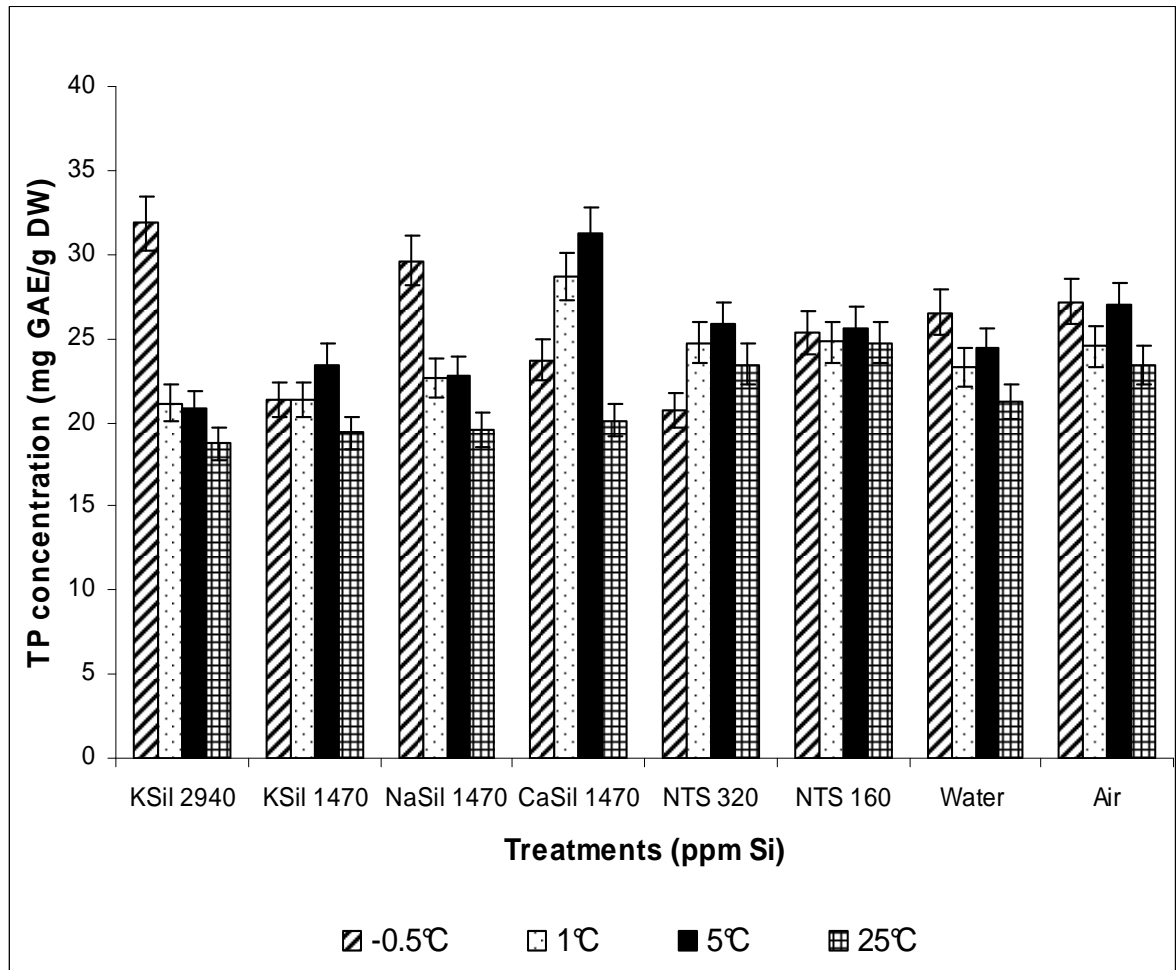


Figure 4.2: Total phenolic concentration (GAE) for mesocarp tissue of 'Hass' avocado fruit treated with different silicon compounds and stored at -0.5°C, 1°C, 5°C or at room temperature (25°C) for 28 days. LSD ($P = 0.05$) = 7.287.

Total Antioxidant Capacity (TAOC)

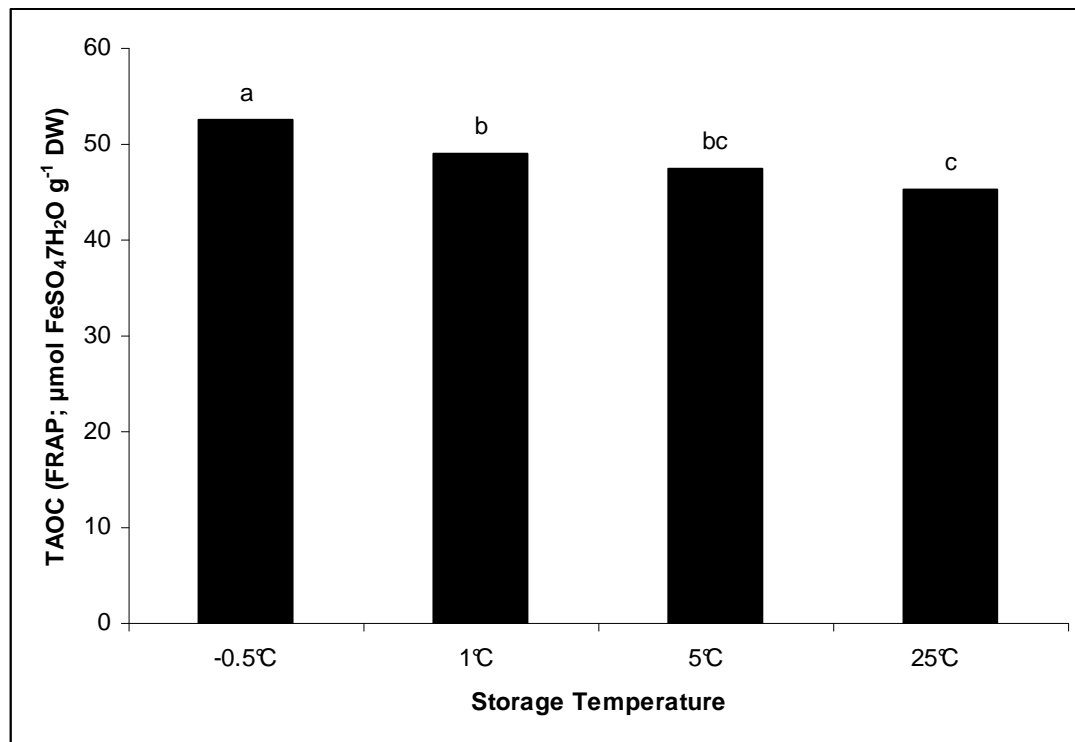


Figure 4.3: Total antioxidant capacity (TAOC) in the mesocarp of 'Hass' avocado fruit stored at -0.5°C, 1°C, 5°C or at room temperature (25°C) for 28 days. LSD ($P = 0.05$) = 2.806

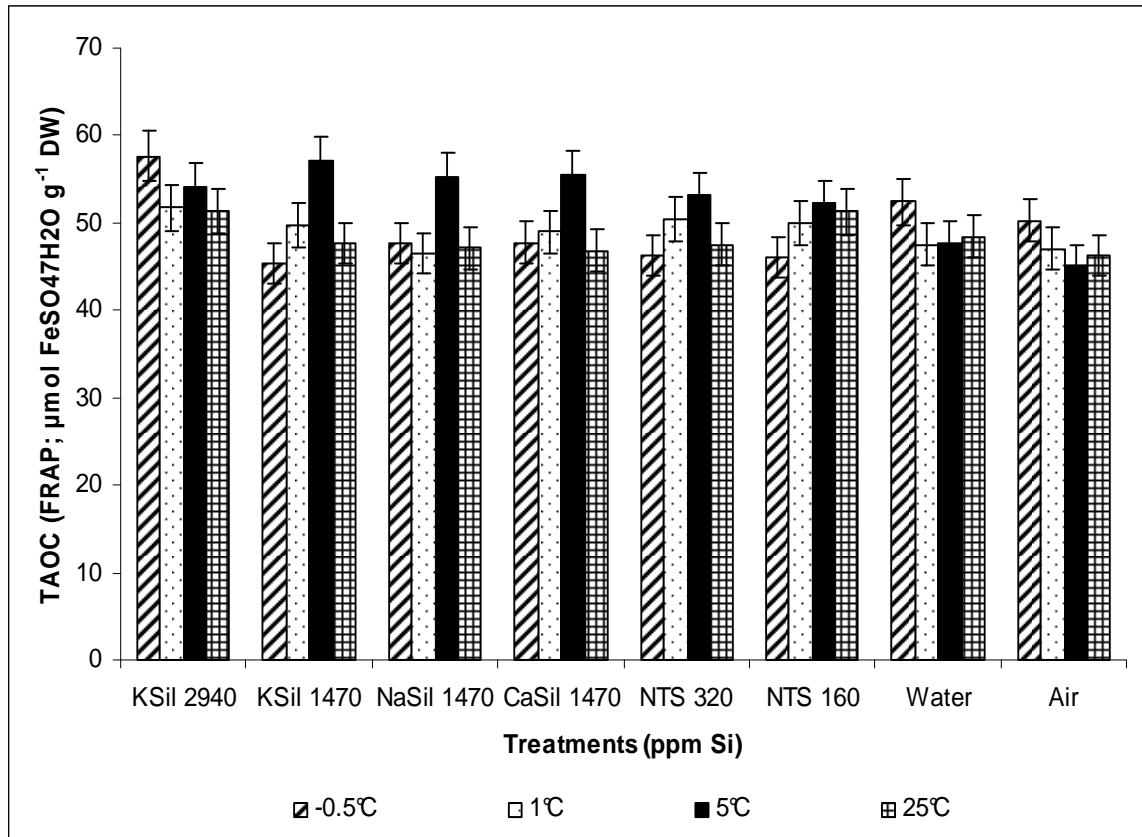


Figure 4.4: Total antioxidant capacity (TAOC) in the mesocarp of 'Hass' avocado fruit treated with different silicon compounds and stored at -0.5°C, 1°C, 5°C or at room temperature (25°C) for 28 days. LSD ($P = 0.05$) = 7.936; $P = 0.115$.

Sugar Profile

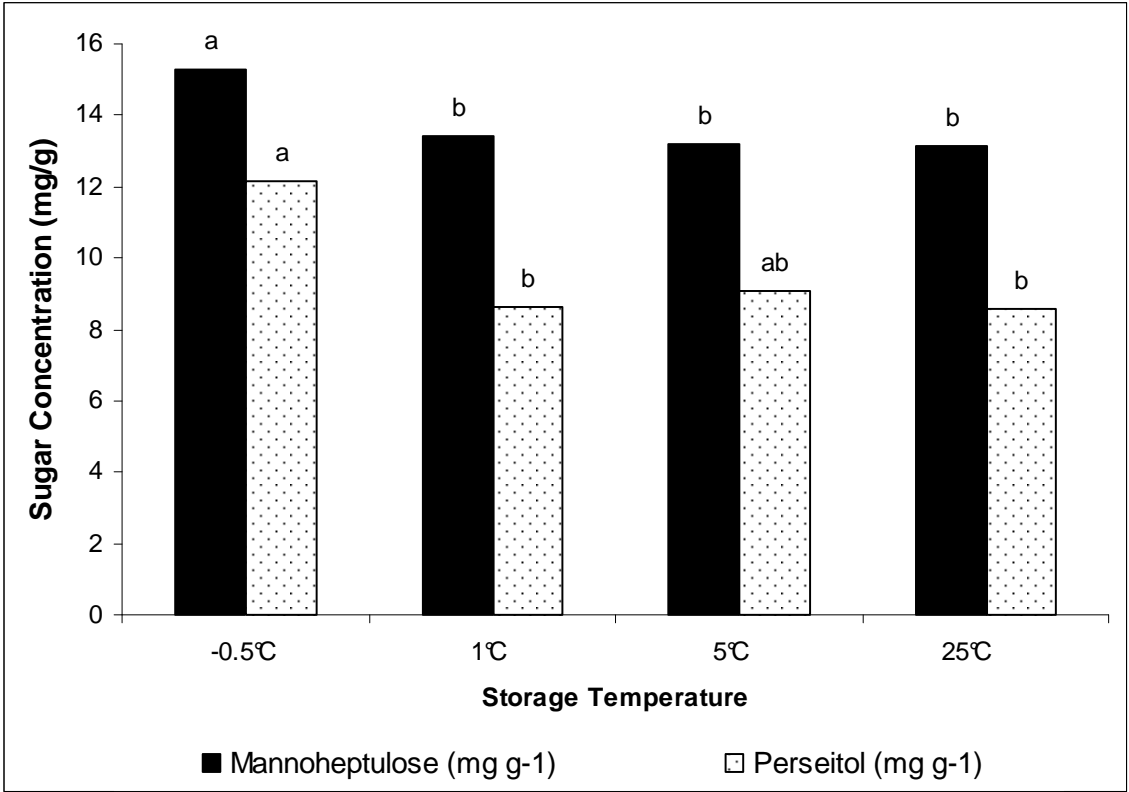


Figure 4.5: Concentration of mannoheptulose and perseitol (mg g⁻¹) in the mesocarp tissue of 'Hass' avocado fruit stored at -0.5°C, 1°C, 5°C or at room temperature (25°C) for 28 days. LSD (P = 0.05) = 7.936.

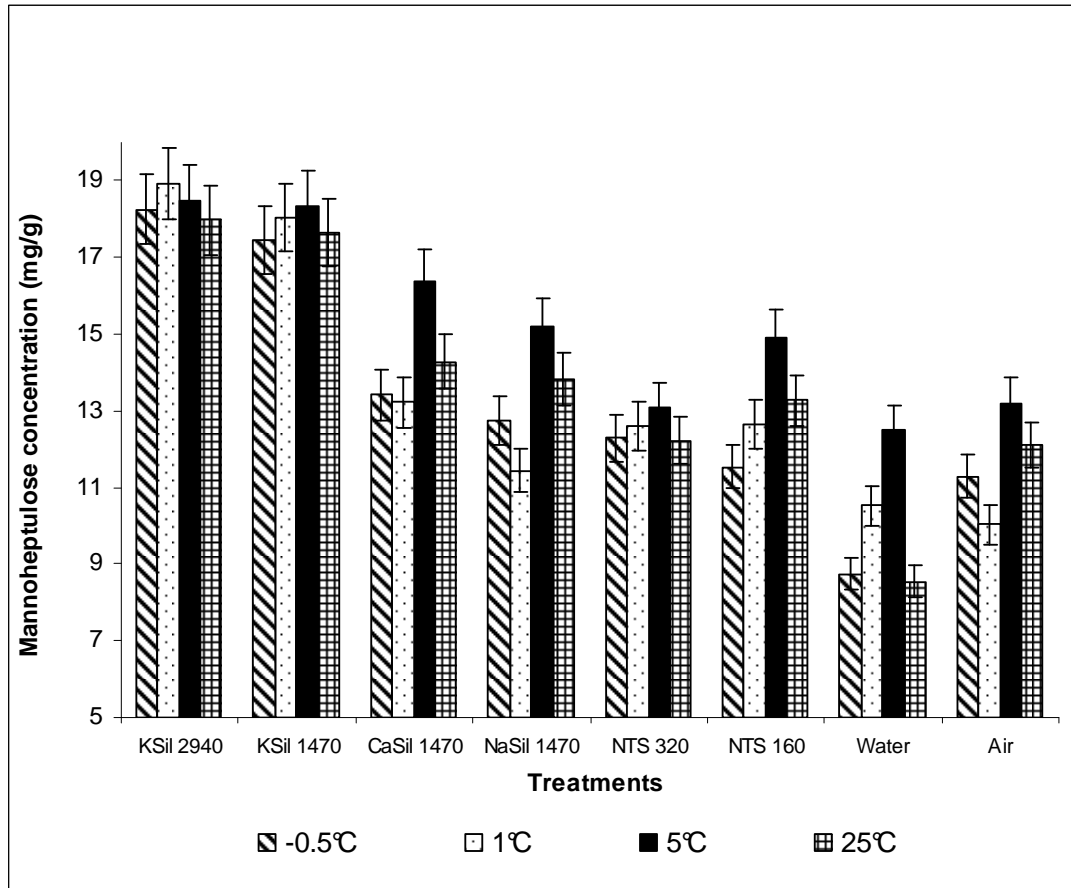


Figure 4.6: Concentration of mannoheptulose (mg/g) in mesocarp tissue of 'Hass' avocado fruit treated with silicon compounds of different concentrations and stored at -0.5°C, 1°C, 5°C or at room temperature (25°C) for 28 days. LSD ($P = 0.05$) = 3.086.

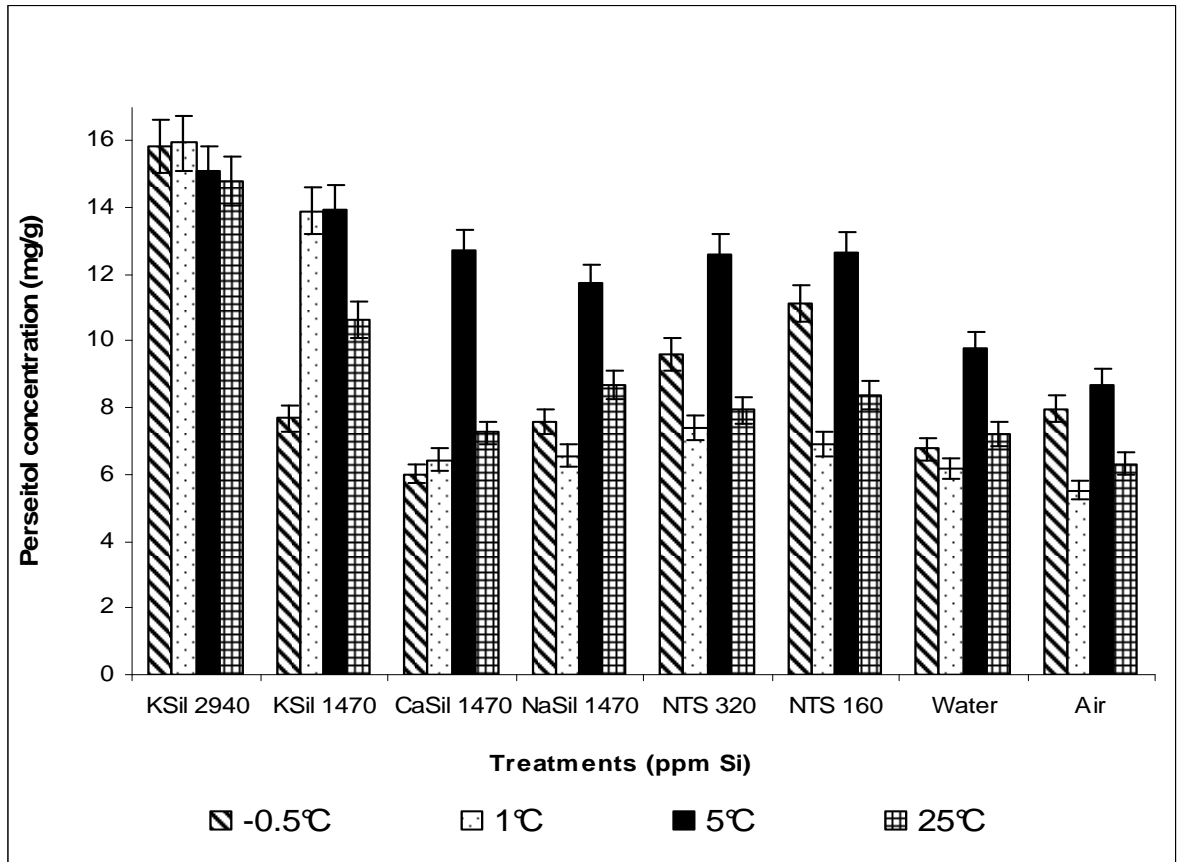


Figure 4.7: Concentration of perseitol (mg/g) in the mesocarp tissue of 'Hass' avocado fruit treated with silicon compounds of different concentrations and stored at -0.5°C, 1°C, 5°C or at room temperature (25°C) for 28 days. LSD ($P = 0.05$) = 3.086.

PAL Activity

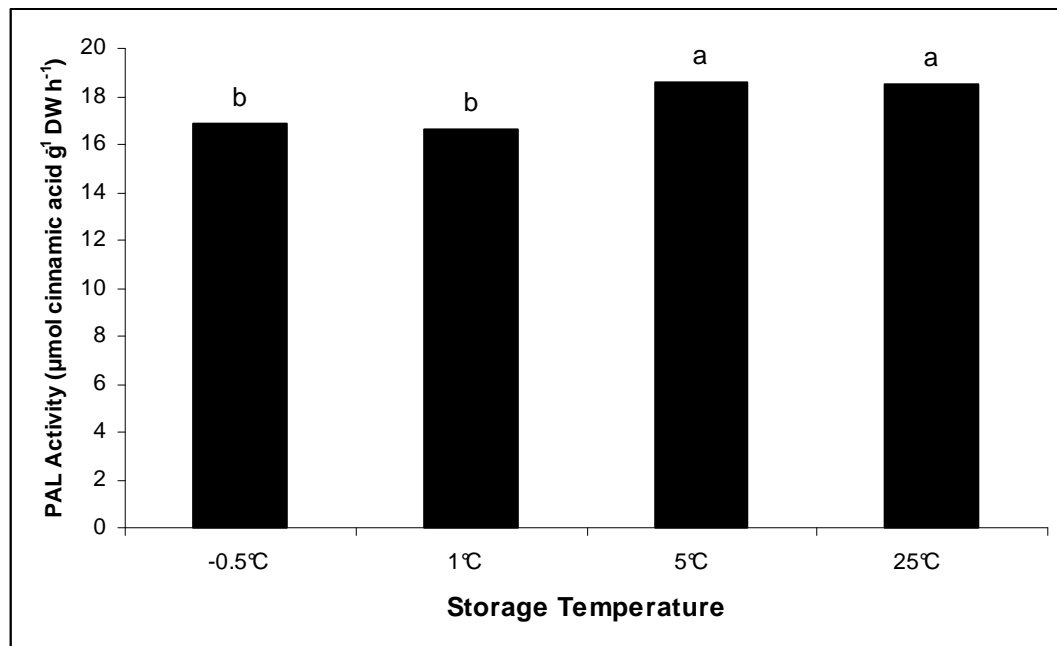


Figure 4.8: PAL activity (expressed as $\mu\text{mol cinnamic acid per gram mesocarp tissue in an hour}$) for 'Hass' avocado fruit stored at different temperatures for 28 days. $\text{LSD}_{(P=0.05)} = 1.159$

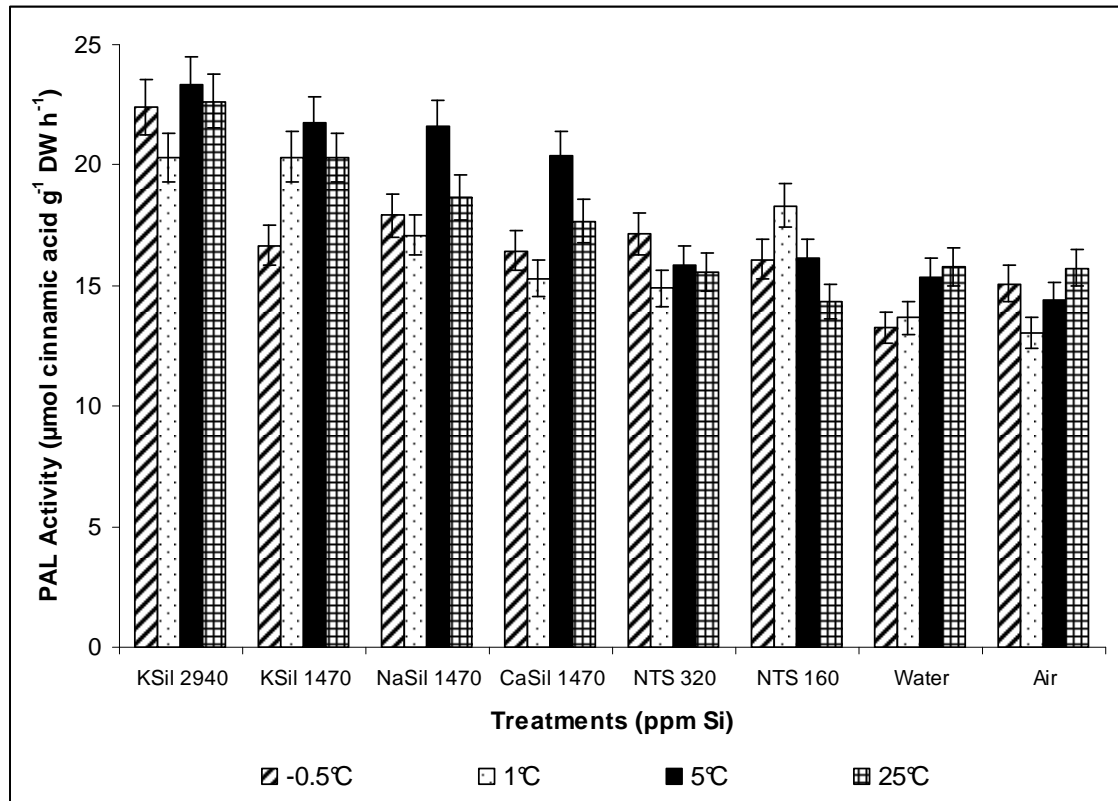


Figure 4.9: PAL activity (expressed as $\mu\text{mol cinnamic acid g}^{-1} \text{DW h}^{-1}$) for 'Hass' avocado fruit treated with silicon compounds of different concentrations and stored at -0.5°C , 1°C , 5°C or room temperature (25°C) for 28 days. $\text{LSD } (P = 0.05) = 3.279$; $P = 0.044$.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

This study was initiated to address the growing urge to find more sustainable and environmentally friendly methods for controlling post-harvest quality problems in avocado fruit. Due to the long shipment period to European destinations, there is a need to maintain fruit quality over a period of 28 days plus a 7 day shelf life period. This has been the focal point for many growers and researchers in this field. In order to manipulate the post-harvest quality of the fruit, it is necessary to understand some of the key factors involved in its physiology. Post-harvest disorders, which originate from pre-harvest practices, are commonly revealed during fruit ripening.

The purpose of this study was to investigate the beneficial effects of silicon (Si) on 'Hass' avocado fruit. Silicon has beneficial effects on growth and development of a wide variety of horticultural crops (Takahashi *et al.*, 1990); however, the role of Si in physiological processes has not been well-investigated in horticultural crops, which are mainly dicots, in comparison with agronomical crops, which are mainly monocots, like rice (*Oryza sativa* L.) (Iler, 1979).

It was demonstrated that post-harvest applications of 2940ppm Si in the form of potassium silicate were the most effective treatment, improving the quality of avocado fruit, by suppressing respiration. Although it has been shown that Si suppresses respiration in ripening avocado fruit, commercial applications may not

be feasible, as there was no significant effect of Si on CO₂ production in early- and mid-season fruit. Applications of Si only successfully suppressed fruit respiration in late-season fruit.

Biochemical analyses revealed that total antioxidants, total phenolics and PAL activity increased in mesocarp tissue of avocado fruit as a result of applying Si as a post-harvest dip. The C7 sugars were also more prevalent in avocado fruit treated with Si, suggesting that an application of Si to avocado fruit can aid in the retention of these vital antioxidants. These findings are supported by the fact that fruit treated with high Si concentrations (KSil 2940 ppm) have the highest total anti-oxidant capacity and also a higher total phenolic concentration compared with untreated or those that were treated with lower Si concentrations.

There is no doubt that Si plays an important role in the mineral nutrition of many plant species, as well as, efficiently controlling several plant diseases (Datnoff *et al.*, 2007). As the need for environmentally friendly strategies for management of plant diseases increases, Si could provide a valuable tool for use in a variety of crops. The use of Si for controlling plant diseases would be well-suited for inclusion in an integrated pest management program and would permit reductions in fungicide use. In this research Si-treated fruit were also found to have the lowest incidence of anthracnose infection (data not presented).

As researchers and growers become aware of Si and its potential in agriculture, it is likely that this often-overlooked element will not only be recognised as a viable

means of sustainably managing important plant diseases worldwide (Hou *et al.*, 2006), but also as a postharvest quality management tool.

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Appendix

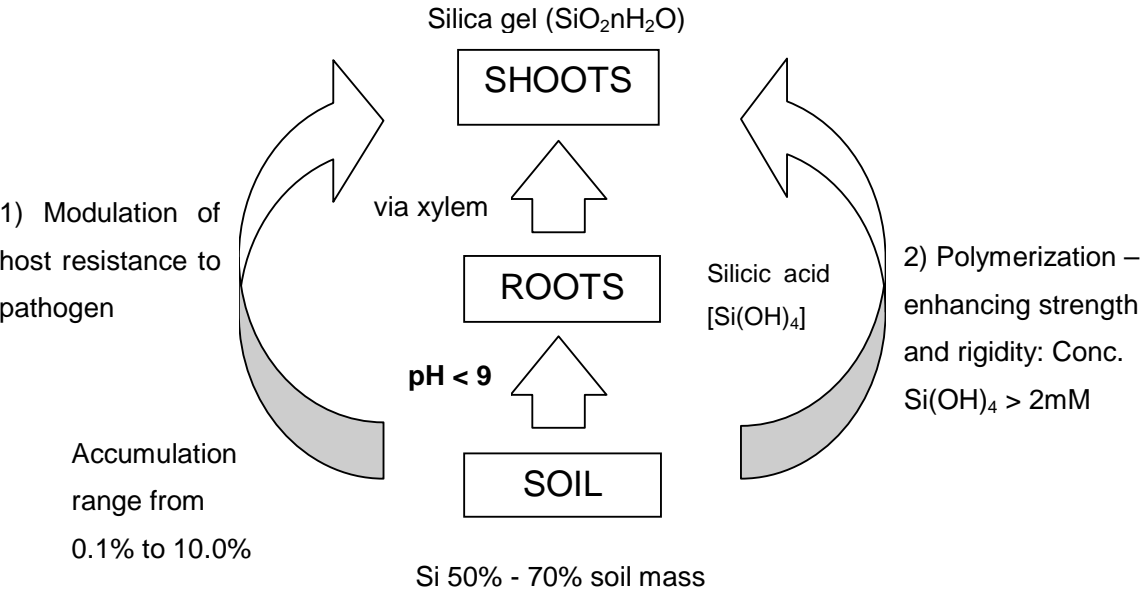


Figure A1: Proposed mechanisms and uptake of silicon.

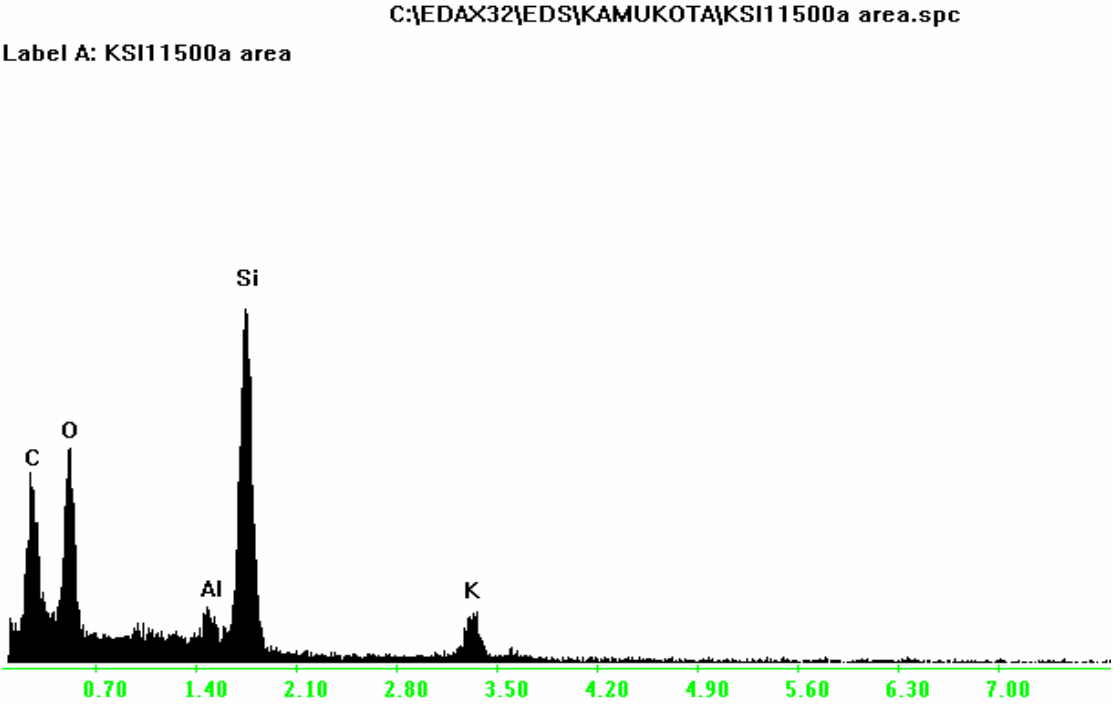


Figure A2: EDAX graph of avocado mesocarp tissue treated with KSil 1470ppm



Figure A3: Hand-held firmness tester (Densimeter) being used to measure firmness of 'Hass' avocado fruit.

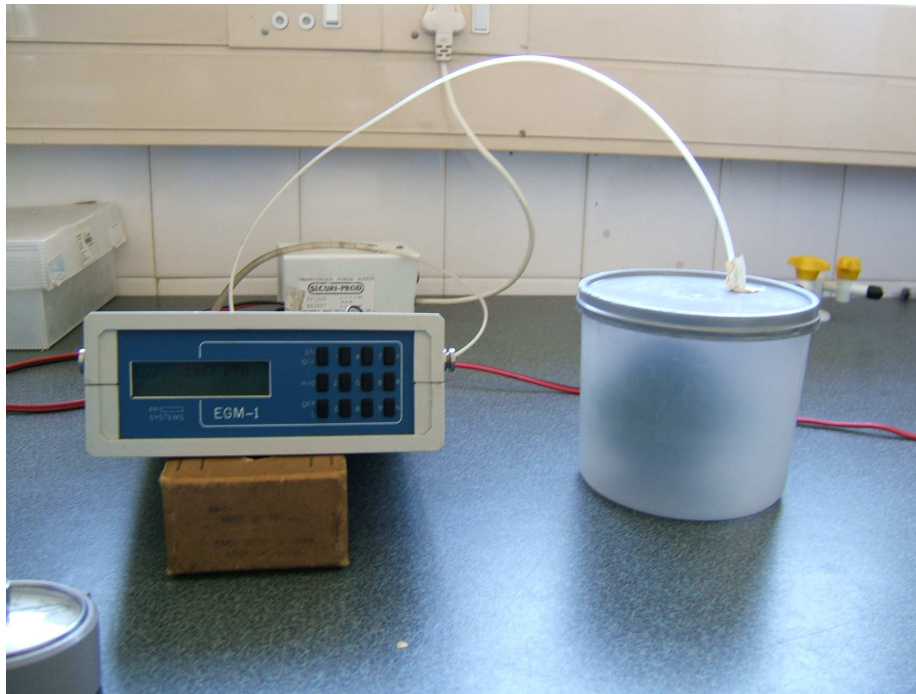


Figure A4: Infra-red gas analyser measuring the respiration rate (CO_2 production) of 'Hass' avocado fruit.



Figure A5: Gas chromatograph (GC) in conjunction with auto-sampler used in the measurement of ethylene gas evolution of Hass avocado fruit.

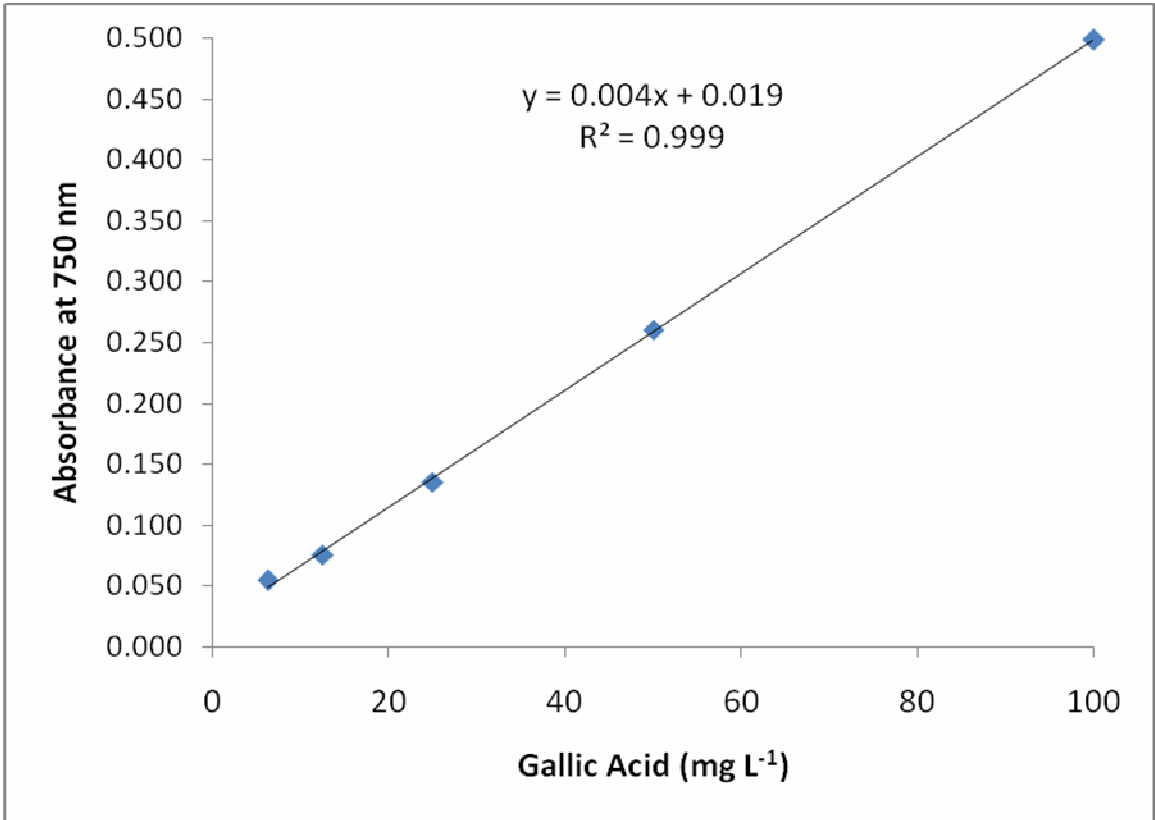


Figure A6: Gallic acid linearity curve (TP determination).

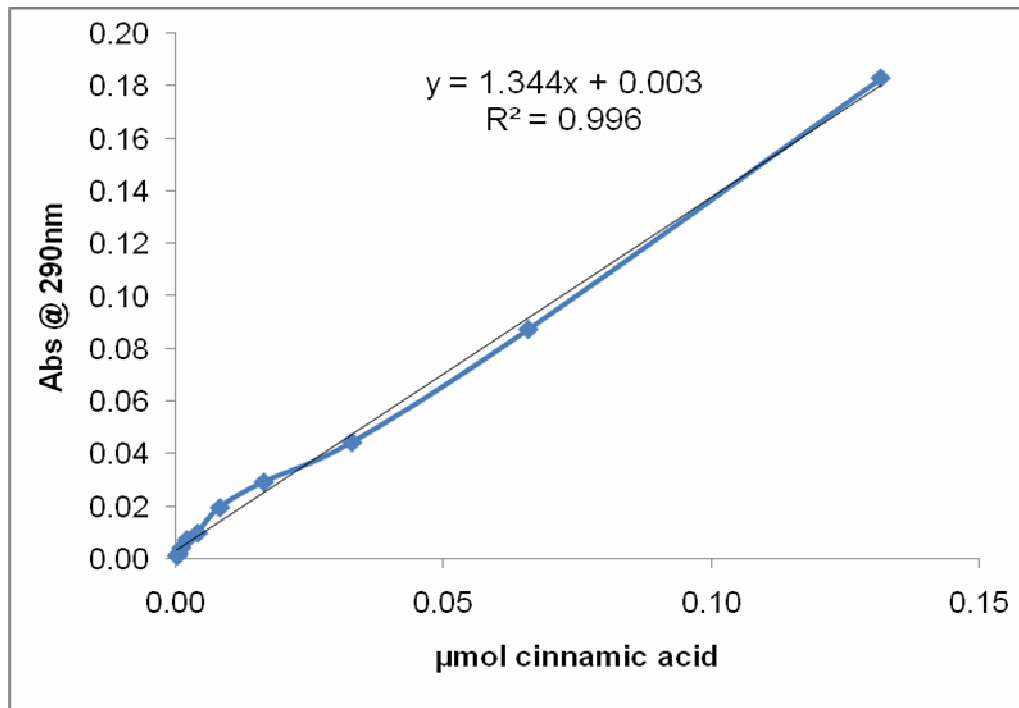







Figure A7: Cinnamic acid linearity curve (PAL activity expressed as $\mu\text{mol g}^{-1} \text{DW h}^{-1}$)

Table A1: Fruit colour rating chart of 'Hass' avocado (*Persea americana* Mill.) fruit.

Sample Photo	Colour	Colour Rating	Ripeness Stage	Average Firmness on densimeter scale
	Emerald Green	1	Very Raw (Unripe)	> 95
	Forest green	2	Raw	> 75
	Green to brown	3	Slightly ripe but not edible	60
	Brown to purple	4	Ripe	50
	Black	5	Fully Ripe	< 40

**EFFECT OF POST-HARVEST SILICON APPLICATION ON 'HASS'
AVOCADO (*Persea americana* Mill.) FRUIT PHYSIOLOGY**

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Abstract

Silicon (Si) has been used to minimize the adverse effects of biotic and abiotic stresses on the quality of various fruit crops. Therefore, the post-harvest effect of Si on fruit firmness, as well as CO₂ and ethylene evolution of 'Hass' avocado fruit was investigated. Four different sources of silicon (potassium silicate (KSil), Nontox-Silica® (NTS), calcium silicate (Ca₂SiO₄) and sodium metasilicate pentahydrate (SiO₃Na₂.5H₂O)) were used as post-harvest applications. Fruit were dipped into the Si sources at 80 ppm to 2940 ppm Si and subsequently stored at either -0.5, 1, 5°C or 25°C (room temperature). Firmness, CO₂ and ethylene measurements were taken every two days as the fruits ripened. Fruit stored at 5°C were firmer than fruit stored at other temperatures. With respect to net CO₂ production, there were significant differences ($P < 0.001$) in temperature means. Fruit stored at -0.5°C produced the least amount of CO₂ whereas fruit stored at 25°C (room temperature) produced the highest. Overall, fruits treated with Si in form of KSil 2940 ppm had the lowest respiration rate and ethylene evolution while non-treated fruits (Air) had the highest respiration rate and the highest ethylene evolution. Using ultra-structural analysis (EDAX), it was found that Si passes through the exocarp into the mesocarp tissue treated with high Si concentrations (KSil 1470) while fruit dipped into very dilute Si solutions (80 ppm and 160 ppm) have very little to no Si infiltration into the mesocarp. Therefore post-harvest application of 2940 ppm Si in the form of KSil seems to be most beneficial to maintain avocado fruit quality, probably due to suppression of respiration and a reduction in ethylene evolution.

Keywords: Avocado (*Persea americana* Mill.), silicon, firmness, carbon dioxide, ethylene

INTRODUCTION

The avocado (*Persea americana* Mill.) fruit is a highly perishable product. The relatively high rate of respiration results in the relatively quick deterioration of fruit quality (Wills *et al.* 1989). Many external and internal factors of a biotic and abiotic nature play a part in this process. Keeping the quality of avocado is, however, particularly important for the export industry where fruit quality has to be maintained for long periods of time.

Silicon (Si), being the second most abundant element (28%) in the Earth's crust and in soils, has long been associated with disease resistance in plants. Silicon is known to effectively mitigate various abiotic stresses (Liang *et al.*, 2007) and also plays an active role in enhancing host resistance to plant diseases by stimulating defence reaction mechanisms (Hou *et al.*, 2006). Silicon also provides protection against fungal diseases by strengthening cell walls, thus making it more difficult for the fungi to penetrate and colonize the plant (Fawe *et al.*, 2001). According to Ma and Takahashi (2002), the beneficial effects of silicon are more evident under stress conditions.

Fauteux *et al.* (2005) proposed two mechanisms for Si-enhanced resistance to diseases. One is that Si acts as a physical barrier as it is deposited beneath the cuticle to form a cuticle-Si double layer. The other mechanism proposed is that soluble Si acts as a modulator of host resistance to pathogens.

Anderson *et al.* (2005) found that injecting soluble silicon into avocado trees prior to harvest significantly decreased the severity and incidence of anthracnose. In contrast to this, these authors found that mixing phosphoric acid and soluble silicon did not provide any control to anthracnose. They also proposed that foliar applications are likely to be ineffective.

As plants vary considerably in their ability to absorb silicon from the soil solution (Ma and Yamaji, 2006), avocado might not take up sufficient Si from the soil to affect resistance to pathogens. The avocado differs from most other fruits in that ripening does not take place on the tree, but only after picking (Schroeder, 1953). Softening is almost always associated with fruit ripening, which leads to increased susceptibility to physical damage and pathological attack (Brady, 1987), which ultimately reduces shelf life of the product (Donkin, 1995).

It is necessary to understand the physiology of avocado ripening in order to search for possible causes of the common disorders, and also to develop pre- and post-harvest practices effective in producing high quality fruit with extended shelf life.

Therefore, the objective of this study was to investigate whether the application of silicon as a post-harvest dip has an effect on the ripening pattern (firmness, carbon dioxide production and ethylene evolution) of 'Hass' avocado fruit.

MATERIAL AND METHODS

Avocado (*Persea americana* Mill. cv. 'Hass') fruit were obtained from Everdon Estate in Howick (29°27'S, 30°16'E, KwaZulu-Natal). Fruit were treated with Si sources at concentrations ranging from 80 ppm to 2940 ppm and subsequently stored at either -0.5, 1, 5°C or room temperature (25°C). Four different sources of silicon namely, potassium silicate (K_2SiO_4), Nontox-Silica® (NTS), calcium silicate (Ca_2SiO_4) and sodium metasilicate pentahydrate ($SiO_3Na_2 \cdot 5H_2O$) were used as dips to treat fruit. Firmness measurements, ethylene evolution and CO_2 production were recorded as fruit approached ripening. The CO_2 production of fruit that were stored at room temperature was analysed daily until they had fully ripened, while fruit from cold storage were removed weekly to measure respiration.

Fruit firmness of each fruit was measured daily as soon as softening was initiated using a hand-held firmness tester (5 mm densimeter; Bareiss, Oberdischingen, Germany). Two measurements were taken along the equatorial region of the fruit at two opposing points.

Fruit respiration rate, as determined by CO₂ production, was measured using an infrared gas analyser (EGM-1, PP Systems, Hitchin, Hertfordshire, UK). Fruit were incubated in 1L jars for 12 minutes with readings taken daily until the respiration peak was reached and CO₂ production decreased. Net CO₂ production per kilogram fruit was calculated by adjusting for ambient CO₂ in the jar, fruit volume and fruit mass. Carbon dioxide concentration ($\mu\text{l L}^{-1}$) was determined and the results were calculated as a rate of CO₂ production ($\text{ml kg}^{-1} \text{FW h}^{-1}$).

Ethylene evolution was measured every second day until the fruits were ripe. Fruit were considered to be “ripe” when softness measurements reached less than 60% on the hand-held densimeter. Ethylene production was measured using a gas chromatograph (GC) (DANI 1000, DANI Instruments S.p.A., Monzese, Italy) in conjunction with an auto-sampler (HT250D, HTA S.r.L., Brescia, Italy). The GC was equipped with a flame ionization detector (FID), stainless steel packed column with an alumina-F1 stationary phase. Fruit ethylene production was measured by placing a fruit in a 1L jar with a 20 ml glass vial, sealing the jar and incubating for 30 minutes. The ethylene produced by the fruit during that time was retained in the glass vial and transferred to the GC auto sampler where the ethylene ($\mu\text{l L}^{-1}$) concentration was determined. Taking into account fruit volume (head space), fruit mass and time of incubation, net ethylene values were expressed as ($\mu\text{L kg}^{-1} \text{FW h}^{-1}$).

Energy dispersive x-ray analysis (EDAX) was conducted to determine the extent of Si infiltration within each treatment on the exocarp and mesocarp tissues of two fruits per treatment.

Statistical analysis was carried out using GenStat® version 11 (VSN International, Hemel Hempstead, UK) ANOVA. Treatment and storage temperature means were separated using least significant differences (LSD) at 5% ($P = 0.05$). The experimental design in this study was a split-plot design with the main effect being storage temperature and the sub-effect being treatments. Each block represents a replicate.

RESULTS AND DISCUSSIONS

There were significant differences in temperature means with regards to firmness. As shown in figure 1, the firmness of all fruit decreased with time (Wills *et al.* 1989). Fruit treated with potassium silicate (KSil) and Nontox-silica® (NTS) and stored at 5°C were firmer than fruit stored at other temperatures (Figure 2) three days after removal from storage.

CO₂ production increased with time with most fruit reaching the respiratory peak four to five days after removal from cold storage, thereafter decreasing gradually (Figure 3). Fruits treated with silicon in the form of KSil 2940 produced the least amount of CO₂, while non-treated fruits (Air) had the highest respiration rate. Fruit stored at room temperature (25°C) produced significantly higher amounts of CO₂ and peaked much earlier than fruit stored at other temperatures (Figure 4).

Results of ethylene evolution showed that there were differences ($P < 0.05$) between temperature means with the highest net ethylene being produced by fruit stored at 25°C (Figure 5). There were also significant differences amongst

treatment means ($P < 0.001$), with fruits treated with KSil 2940 ppm producing the least ethylene (Figure 6).

EDAX analysis revealed that silicon passed through the exocarp into the mesocarp tissue in fruit treated with high concentrations of silicon, i.e., KSil 2940 and 1470 ppm. Fruit dipped into very dilute Si solutions (80 ppm and 160 ppm) showed very little to no infiltration of Si into the mesocarp and treatments with NTS showed very little Si infiltration (Figure 7).

CONCLUSION

Soil-drench applications of silicon on 'Hass' avocado trees in an attempt to control *Phytophthora cinnamomi* have proven to be unsuccessful (results not shown). Other results show that the main effect of silicon is on the suppression of respiration and ethylene production (Figure 3 and Figure 6). In contrast to this, Bekker *et al.* (2007) suggested that the effect of silicon on plant growth and performance is only evident when plants are under some form of stress.

Post-harvest applications of 2940 ppm Si in the form of KSil seem to be most beneficial as respiration was most suppressed in this treatment. The Si concentration had no effect on the deposition of Si in the mesocarp tissue but the amount of Si found the exocarp was high in fruits treated with high Si concentrations, i.e., KSil 1470 ppm.

Therefore we would recommend applying Si to healthier orchards, as the positive effects will be more evident. It would also be beneficial to investigate if Si improves antioxidant accumulation and total phenolic concentration in the fruit, thereby increasing the stress-relieving ability of the fruit.

ACKNOWLEDGEMENTS

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Figures

Firmness

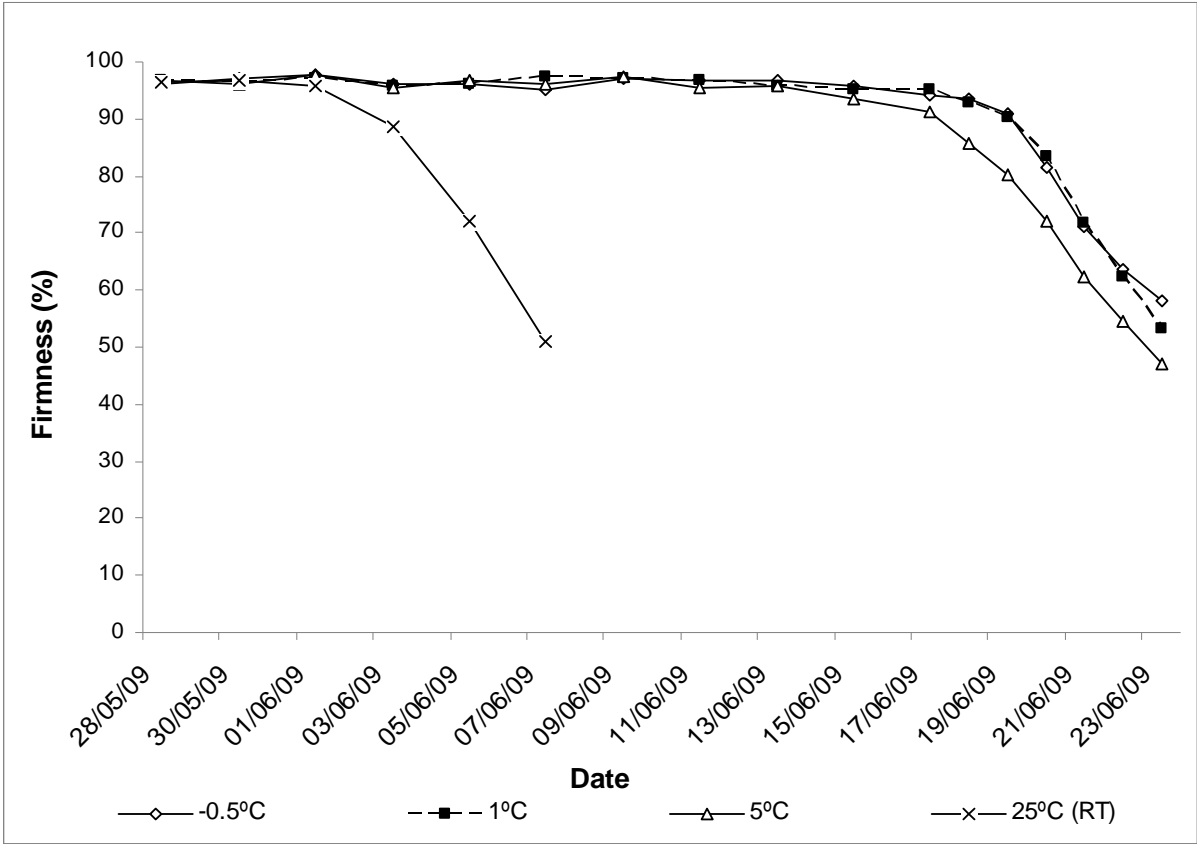


Figure 1: Firmness of early season (May 2009) 'Hass' avocado fruit stored at different temperatures. Fruit were removed from storage after 21 days and kept at room temperature until eat-ripe softness was reached. LSD (P = 0.05) = 0.914.

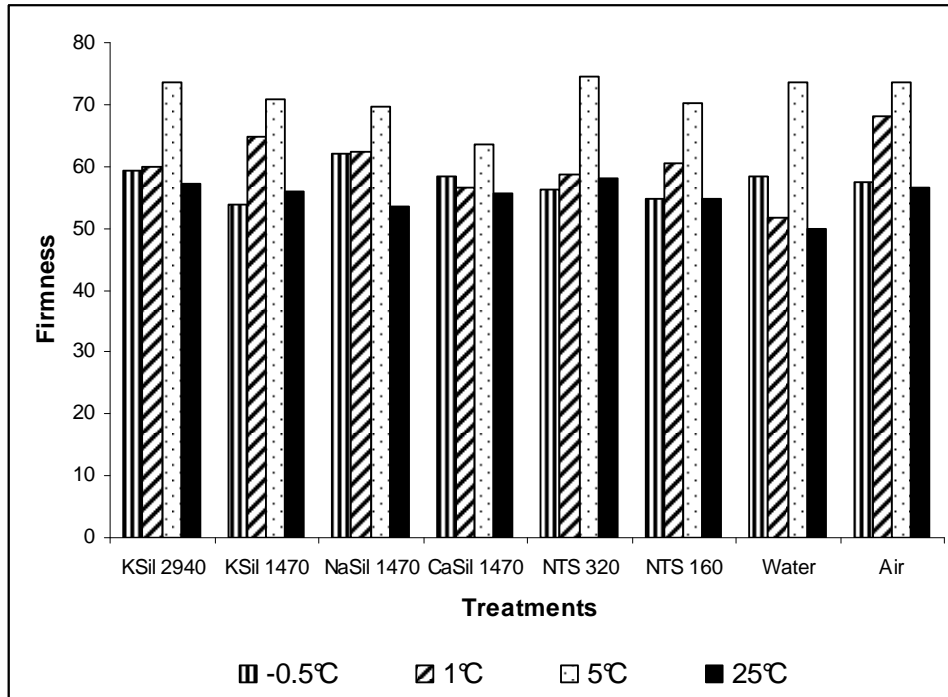


Figure 2: Firmness of 'Hass' avocado fruit treated with different Si compounds three days after removal from cold storage. LSD ($P = 0.05$) = 4.791.

Respiration rate

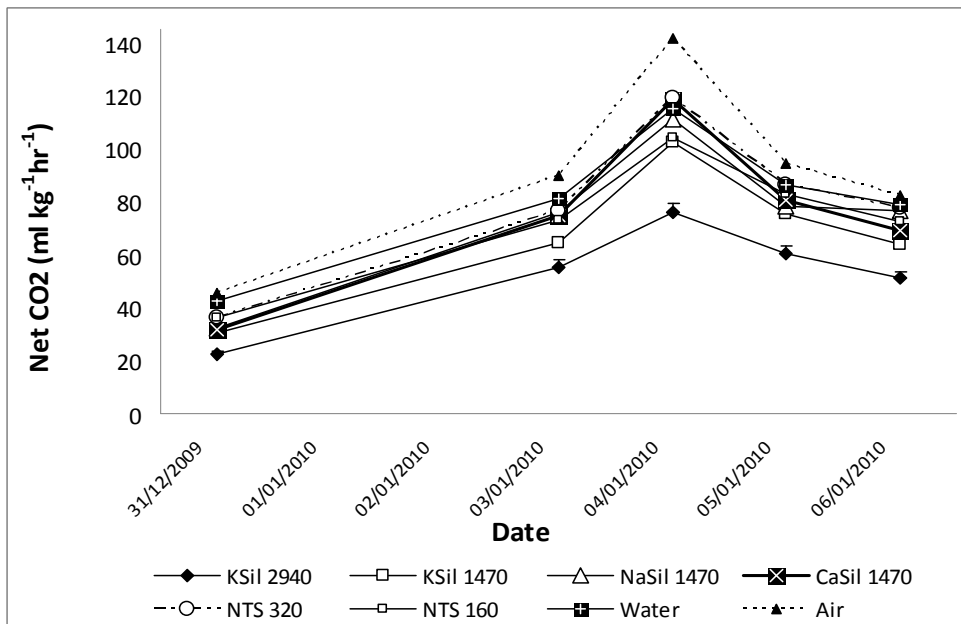


Figure 3: Net CO₂ production of late-season (December 2009) 'Hass' avocado fruit treated with different silicon compounds. LSD ($P = 0.05$) = 5.623.

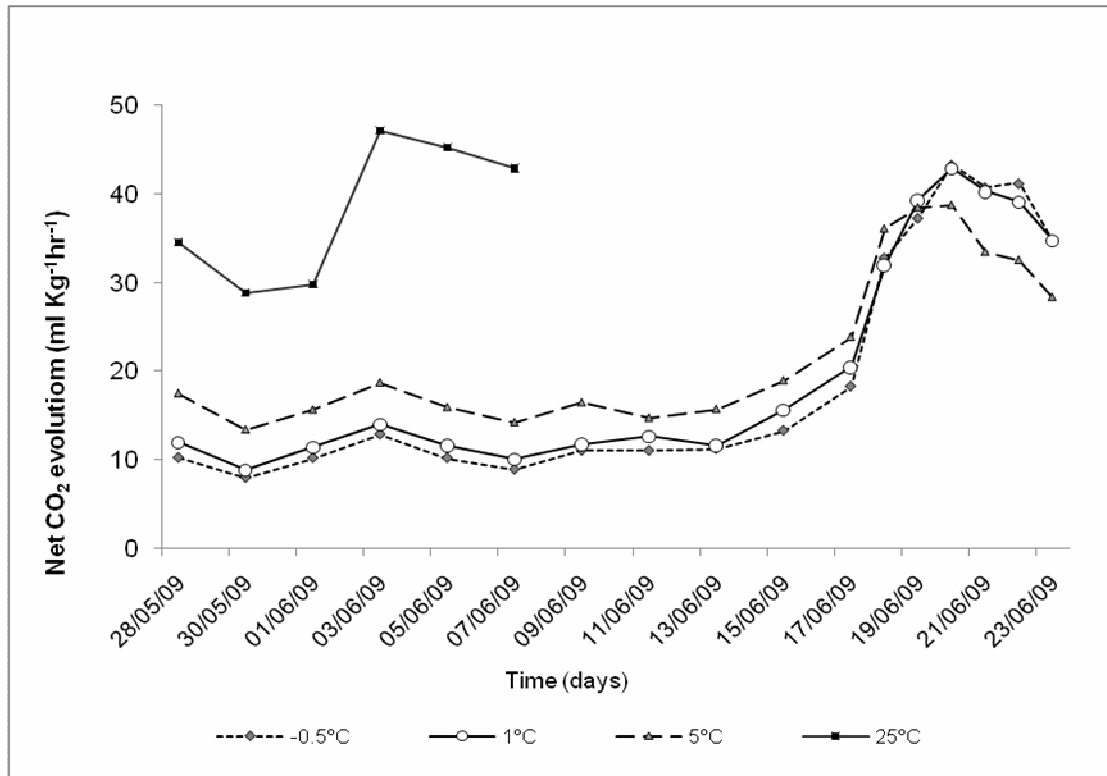


Figure 4: Net CO₂ (ml kg⁻¹ h⁻¹) production of early-season (Harvest 5) 'Hass' avocado fruit stored at -0.5°C, 1°C, 5°C or room temperature (25°C). LSD (P = 0.05) = 0.624.

Ethylene evolution

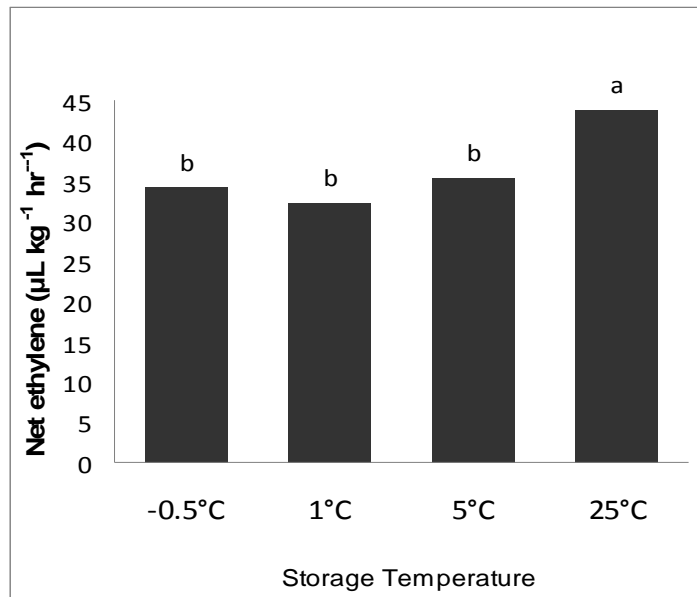


Figure 5: Net ethylene ($\mu\text{L kg}^{-1} \text{h}^{-1}$) production of ripe 'Hass' avocado fruit (firmness readings less than 60%) stored at -0.5°C, 1°C, 5°C or at room temperature (25°C). Fruit were harvested late in the season (Harvest 7). LSD ($P = 0.05$) = 4.762.

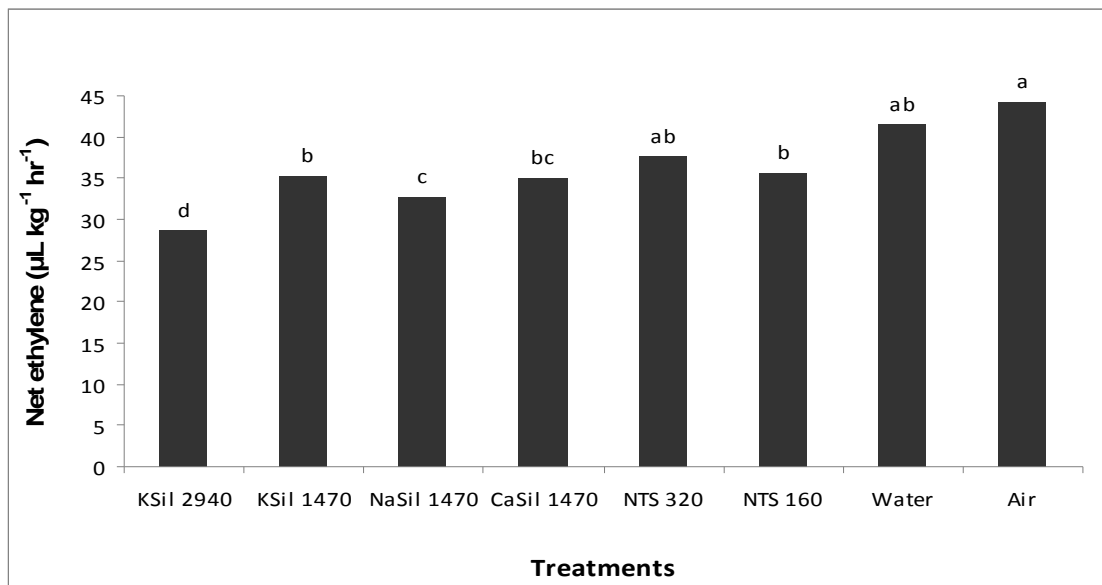


Figure 6: Net ethylene ($\mu\text{L kg}^{-1} \text{h}^{-1}$) production of ripe 'Hass' avocado fruit (firmness readings less than 60%) treated with different concentrations of Si treatments. LSD ($P = 0.05$) = 6.734.

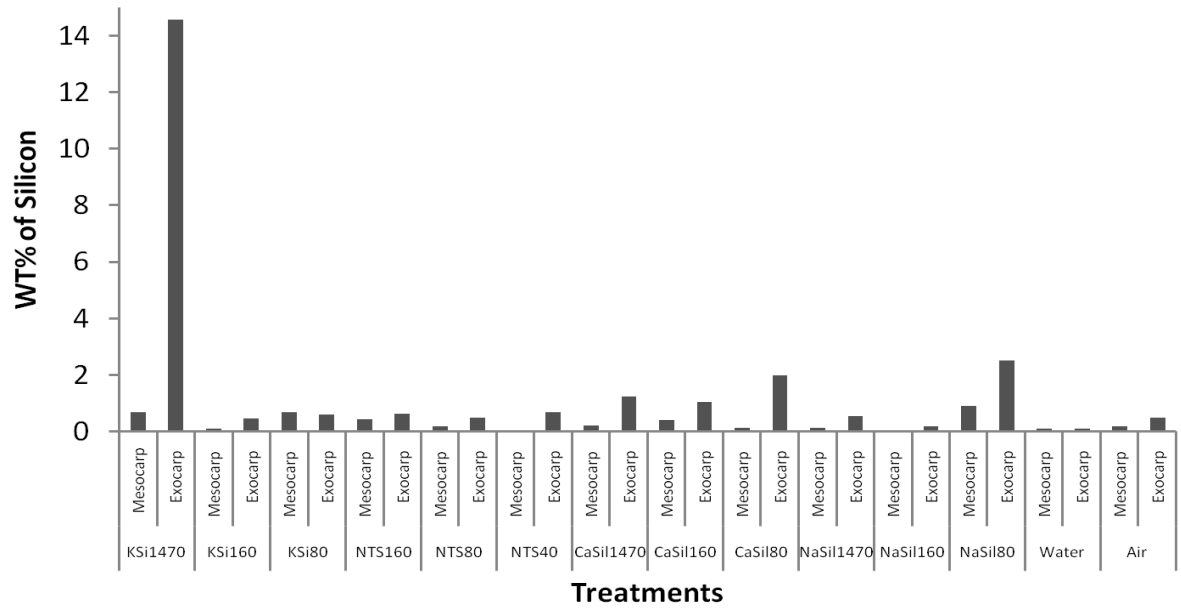


Figure 7: Percentage by weight of silicon in the exocarp and mesocarp tissue after treating with silicon compounds.