CHAPTER 5

ACCUMULATION OF TOTAL PHENOLICS DUE TO SILICON APPLICATION IN ROOTS OF AVOCADO TREES INFECTED WITH *PHYTOPHTHORA CINNAMOMI*

5.1 ABSTRACT

The accumulation of soluble and wall-bound phenolics and phenolic polymers in *Persea americana* Mill. roots from thirteen year-old Hass on Edranol trees exposed to the pathogen *Phytophthora cinnamomi*, and treated with water soluble potassium silicate was investigated. Following elicitation, the conjugated and non-conjugated phenolic metabolites present in the induced root tissue were extracted and quantified. From March 2005 to January 2006, three applications (Si x 3) of soluble potassium silicate per season resulted in significantly higher concentrations of crude phenolic compounds in the roots compared to the untreated control. From March to May 2006, the control treatment (133.66µg.l^{-1}; 109.08µg.l^{-1}) resulted in higher crude phenolic levels compared to Si x 3 (94.61µg.l^{-1}; 67.98µg.l^{-1}). Significantly higher crude phenolic concentrations in avocado roots were obtained in Si x 3 during March and May 2006 (94.61µg.l^{-1}; 67.98µg.l^{-1}) when compared to potassium phosphonate (Avoguard®) (49.07µg.l^{-1}; 59.46µg.l^{-1}). Glucoside bound phenolic acid concentrations in trees treated with Si x 3 differed significantly from the untreated control for the period from January to May 2006. Concentrations of glucoside bound phenolic acids obtained with Si x 3 treatment are comparable to that of potassium phosphonate (Avoguard®) with exceptions during March 2005 and May 2006. Three silicon applications per season resulted in significantly lower cell wall bound phenolic acid concentrations on avocado roots compared to the control treatment during May and Sept 2005, and March and May 2006. This trend was negated during January 2006 when a significantly higher cell wall bound phenolic concentration was obtained in Si x 3 (0.71µg.l^{-1}) than the control (0.36µg.l^{-1}). Three silicon treatments per season resulted in significantly lower cell wall bound phenols during May, Jul and Sept 2005 and May 2006 compared to Si x 1, with higher concentrations obtained in Si x 3 only during Jan 2006 (0.71µg.l^{-1} vs. 0.38µg.l^{-1}). Results indicate that potassium silicate application leads to lower cell wall bound phenolics. Silicon treatment of avocado trees resulted in fewer identifiable phenols in avocado roots compared to the untreated control and potassium phosphonate (Avoguard®) treatments. HPLC separation of hydrolysed

phenolic acids extracted from roots revealed all non-conjugated phenolic acid hydrolysed samples to contain 3,4-hydroxibenzoic acid. The glucoside bound samples of both the potassium phosphonate and the untreated control treatments contained 3,4-hydroxibenzoic acid and vanillic acid, while the control also contained syringic acid in the hydrolysed glucoside bound extract. These results indicate that potassium silicate application to avocado trees under *P. cinnamomi* infectious conditions increase total phenolic content of avocado root tissue.

5.2 INTRODUCTION

Due to the threat of infection, plants have evolved a multitude of chemicals and structures that are incorporated into their tissue for the purpose of protection. These defences can repel, deter, or intoxicate including resin-covered or fibrous foliage, resin-filled ducts and cavities, lignified or phenol-impregnated cell walls, and cells containing phenols or hormone analogues (Berryman, 1988).

Various antimicrobial compounds which are synthesized by plants after infection, have been identified. Most phenolic compounds are phenolic phenyl-propanoids that are products of the shikimic acid pathway. Non-pathogenic fungi induce such high levels of toxic compounds in the host, that their establishment is prevented, while pathogenic fungi either induce only non-toxic compounds or quickly degrade the phytoalexins (Macheix *et al*., 1990; De Ascensao and Dubery, 2003). Rapid and early accumulation of phenolic compounds at infection sites is a characteristic of phenolic-based defence responses. This accumulation of toxic phenols may result in effective isolation of the pathogen at the original site of entrance (De Ascensao and Dubery, 2003).

Wehner *et al*. (1982) reported on the sensitivity of pathogens to antifungal substances in avocado tissue. They concluded that no consistent tendencies exist in the antifungal compound concentration in different avocado cultivars, although marked differences were found between plant parts, with avocado leaves containing the highest levels, followed by fruit mesocarp, root, seed and skin extracts.

In avocado some phenolics may act as antioxidants and induce resistance. These phenolic antioxidants are present in plant lipophylic regions. The soluble phenol flavan-3-ol epicatechin is an antioxidant and acts as a trap for free radicles (Vidhyasekaran, 1997). Diene (1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene) inhibits mycelial growth (Prusky *et al*., 1982; Prusky *et al*., 1983) and spore germination (Prusky *et al.,* 1982), and

is degraded by lipoxygenase extracted from avocado peel. An 80% increase in the specific activity of lipoxygenase in peel extracts occurs coincident with a rapid decrease of diene in fruit peel (Prusky *et al.,* 1983).

Epicatechin inhibits lipoxygenase *in vitro*, and may act as a regulator of membrane-bound lipoxygenase. Epicatechin concentration in avocado fruit peel is inversely correlated with lipoxygenase activity and decreases significantly when lipoxygenase increases (Marcus *et al*., 1998). It is suggested that epicatechin plays a role in induced resistance by inhibiting lipoxygenase. Diene decrease is regulated by lipoxygenase activity, which in turn is regulated by a decrease in the antioxidant, epicatechin, concentration (Karni *et al.,* 1989; Prusky *et al.,* 1991). Exposure of avocado fruit to CO_2 for 24h increased diene as well as epicatechin concentrations, while lipoxygenase activity was inhibited (Prusky *et al.,* 1991). Diene has also been isolated from avocado leaves (Carman and Handley, 1999), and appears to accumulate in order of magnitude in Hass ($4.5\mu g.g^{-1}$), Pinkerton, Fuerte, Duke 7 and Edranol ($0.4\mu g.g^{-1}$) avocado leaves.

In addition to diene, numerous other compounds with fungitoxic characteristics are produced in avocado plants. Domergue *et al*. (2000) isolated (E,Z,Z,)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-5,12,15-triene, which inhibited spore germination of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Brune and van Lelyveld (1982) conducted studies on the biochemical composition of avocado leaves and its correlation to susceptibility to root rot caused by *Phytophthora cinnamomi*. They concluded the majority of phenols detected in avocado plant material to be either phenolic acid (C_6-C_1) or cinnamic acid derivatives (C_6-C_3). The possibility exists that avocado plants may convert specific phenolics into coumarins, from which coumarin phytoalexins may be derived.

The current study was initiated to determine if the application of potassium silicate to avocado trees increases the phenolic concentration in avocado tissue. If possible, specific phenol increases are to be determined, thus confirming the hypothesis that silicon increases the phenolic concentration of host tissues, resulting in the inhibition of Phytophthora root rot severity in avocados.

5.3 MATERIALS AND METHODS

5.3.1 Chemicals

Potassium silicate was obtained from Ineos Silicas (Pty) Ltd, and potassium phosphonate (Avoguard®) from Ocean Agriculture (Johannesburg, South Africa). Analytical grade solvents used in the extractions and HPLC were obtained from Merck Chemicals (Merck, Halfway House, South Africa).

5.3.2 Experimental Layout

An avocado orchard (latitude 23° 43' 60S; longitude 30°10'0E) at an altitude of 847m was selected in the Tzaneen area, South Africa. Trees consisted of thirteen year old "Hass" on "Duke7" seedling rootstocks planted at a density of 204trees.ha^{-1} (7 x 7m spacing). Trees were on a southern facing slope. The trial layout consisted of 50 trees (n) with 10 trees randomly assigned per treatment, and organised in a randomised block design (Appendix B).

5.3.3 Treatments

Treatments consisted of a soil drench with a 20 litre solution of 20ml.l^{-1} soluble potassium silicate (20.7% silicon dioxide) (Bekker *et al*, 2006) per tree either once, twice or three times in a growing season. Trees injected with potassium phosphonate (Avoguard®) were incorporated as a standard fungicide treatment. Untreated trees served as controls. Data was collected from January 2005 to July 2006. Root samples were taken every second month on the northern side of the tree.

5.3.4 Extraction and Quantification of Total Phenolic Compounds

Root samples were freeze dried for 120h. The dried material was ground with an IKA® A11 basic grinder (IKA Werke, GMBH & Co., KG, D-79219 Staufen) to a fine powder. Three extractions were done per sample. One millilitre of a cold mixture of methanol: acetone: water (7:7:1, v:v:v) solution was added to 0.05g powdered plant sample, ultrasonicated for 5min by means of a VWR ultrasonic bath, and centrifuged at 24000g for 1min. No antioxidant (ascorbic acid or $Na_2S_2O_5$) was used, as it would have interfered with the folin-ciocalteau reagent used for total phenol determination (Regnier, 1994). This extraction procedure was repeated twice, and the supernatant fractions pooled. The

solid material left in the eppendorf tube after extraction was saved for cell wall-bound phenolic acid determination. Chlorophyll was removed from the leaf sample solutions by adding 0.5ml chloroform to the supernatant, shaking it for 30s and thereafter centrifuging it for 30s. The organic solvent mixture was evaporated in a laminar flow cabinet at room temperature, whereafter the residue was dissolved in 1ml distilled water. Crude samples were stored at 4°C until extraction.

5.3.5 Non-Conjugated Phenolic Acids

An aliquot of 0.25ml from the crude sample for total soluble phenolic determination was acidified by addition of 25µl 1M HCl before extraction with 1ml anhydrous diethyl ether. The ether extract was reduced to dryness at 4°C and the resulting precipitate was resuspended in 0.25ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

5.3.6 Glycoside-Bound Phenolic Acids

An aliquot of 0.25ml from the crude sample for total soluble phenolic determination was hydrolysed in 40µl concentrated HCl for 1h at 96°C, and extracted with 1ml anhydrous diethyl ether. The ether extract was reduced to dryness at 4°C and the resulting precipitate was resuspended in 0.25ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

5.3.7 Ester-Bound Phenolic Acids

Extraction of soluble ester-bound phenolics took place after hydrolysis under mild conditions. To an aliquot of 0.25ml for total soluble phenolic determination, 0.1ml 2M NaOH was added and the solutions were allowed to stand in the Eppendorf tubes for 3h at room temperature. After hydrolysis 40µl 1M HCl was added and the phenolics extracted with anhydrous diethyl ether. The ether extract was reduced to dryness at 4°C and the resulting precipitate was resuspended in 0.25ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

5.3.8 Cell Wall-Bound Phenolic Acids

The solid material left in the Eppendorf tube after extraction was dried, weighed and resuspended in 0.5M NaOH for 1h at 96°C. Cell wall esterified hydroxycinnamic acid

derivatives were selectively released under these mild saponification conditions. The supernatant was acidified to pH 2 with HCl, centrifuged at 12000g for 5min and then extracted with anhydrous diethyl ether. The extract was reduced to dryness and the precipitate was resuspended in 0.25ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

5.3.9 Quantification of Phenolics by the Folin-Ciocalteau Method

The concentration of phenolic compounds in the various extracts was determined using the folin-ciocalteau reagent (Merck) (Regnier, 1994). The reaction mixture used was reduced proportionally to enable the use of 96-well ELISA plates for the quantification of phenolics. For the quantification of phenolic content, a dilution series (10 – 1000µg.ml^{-1} methanol) was used to prepare standard curves for furellic and gallic acid, which is a modification to the folin-ciocalteau method as described by Regnier and Macheix (1996). The reagent mixture comprised: 170µl distilled water, 5µl standard or plant extract sample, 50µl 20% (v/v) Na_2CO_3 and 25µl folin-ciocalteau reagent. After incubation at 40°C for 30min the absorbance was read at 720nm using an ELISA reader (Multiskan Ascent VI.24354 – 50973 (version 1.3.1)). Spectrometric measurements of the phenolic concentrations in the various extracts was calculated from a standard curve ($y = 0.0013x + 0.0177$, $r^2 = 0.9982$) and expressed as mg gallic acid equivalent per gram of dry weight.

5.3.10 Reverse Phase – High Performance Liquid Chromatography

Extracted phenolic fractions were analysed by means of reverse phase - high performance liquid chromatography (RP-HPLC) (Hewlett Packard Agilent 1100 series) with DAD detection (diode array detector, 280, 325, 340nm). A Luna 3u C-18 (Phenomenex®) reverse phase column (250mm length, 5µm particle size, 4.6mm inner diameter) was used. An excess injection volume of 50µl of each sample was used in a 20µl loop**.** A gradient elution was performed with water (pH 2.6 adjusted with H_3PO_4) and acetonitrile (ACN) as follows: 0min, 7% ACN; 0 – 20min, 20% ACN; 20 – 28min, 23% ACN; 28 – 40min, 27%, ACN; 40 – 45min, 29%, ACN; 45 – 47min, 33%, ACN; 47 – 50min, 80%. The flow rate was 0.7ml.min^{-1}. The identification of the phenolic compounds was carried out by comparing their retention times and UV apex spectrum to those of standards (purchased from Sigma Chemical Company, USA) which included syringic, gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, ferulic, caffeic, and chlorogenic acids. After

each run, the column was re-equilibrated with the initial conditions for 10min. The detector was programmed for peak detection at 280nm, which, although not optimum for ferulic acid and its derivatives, allowed simultaneous detection of hydroxybenzoic and hydroxycinnamic acids and their derivatives (Zhou *et al*., 2004).

5.3.11 Statistical Analysis

Data were subjected to analysis of variance (ANOVA). Mean differences were separated according to Duncan's multiple range test ($P < 0.05$).

5.4 RESULTS AND DISCUSSION

Extraction of phenolics in the present study yielded concentrated samples. Apart from crude extract phenolic content determination, four targeted extractions were done to obtain glycoside bound phenolic acids, free phenolic acids, ester bound phenolic acid and cell wall bound phenolic acids. A gallic acid equivalent calibration curve ($y = 0.013x + 0.0177$, $R^2 = 0.9982$) was used to determine the amount of each fraction contained in the sample material. The targeted extract values are representative of the relative amount of each fraction in the crude extract. This is in agreement with phenolic acid functionality as discussed by several authors (Dixon and Paiva, 1995; Beckman, 2000; Zhou *et al*., 2004). Although high concentrations were obtained in the crude extracts, crude extract values do not reflect the combined values of the four other phenolic acid fractions extracted with more specific hydrolysis reactions. This is because phenols are bound to large molecules in the cell cytoplasm, and by hydrolysis, these molecules are split, resulting in the relevant concentrations being measured.

During the harvesting period (July 2005 & 2006), no significant differences were seen between any treatments with regards to crude phenolic concentrations. For the period of March 2005 to January 2006, three silicon applications (Si x 3) per season resulted in significantly higher total phenolic concentrations in root tissue compared to the control (Figure 5.1). From March to May 2006, the control treatment (133.66µg.l^{-1}; 109.08µg.l^{-1}) resulted in higher crude phenolic levels compared to Si x 3 (94.61µg.l^{-1}; 67.98µg.l^{-1}). Although this data does not correlate with any of the parameters of the phenological model proposed by Kaiser (1993), it is proposed that the lower metabolic plant levels are as a result of lowered physiological activity in the plant, due to lower temperatures, leading to sub-optimal photosynthesis. Although Si x 3 resulted in significantly higher

phenolic concentrations in avocado roots only during March and May 2006 (94.61µg.l^{-1}; 67.98µg.l^{-1}) compared to potassium phosphonate (Avoguard®) (49.07µg.l^{-1}; 59.46µg.l^{-1}), Si x 3 is statistically comparable to the current control method implemented to suppress *Phytophthora* infection. Statistically similar total phenol concentrations in avocado roots were obtained by two silicon applications per season (Si x 2) throughout the duration of the experiment except for March 2005 compared to Si x 3. Two (Si x 2) silicon applications per season mostly resulted in significantly higher phenol concentrations in avocado tissue compared to the control, except for July 2005, Sept 2005, March 2006 and Jul 2006.

Glucoside bound phenolic acid concentrations (Figure 5.2) for Si x 3 differed significantly from the control for the period January to May 2006. Significant differences between these two treatments prior to Jan 2006 were only detected during May 2005. This could possibly be related to the dry period experienced during that time (Appendix B). It is expected that a significant difference will be seen between Si x 3 and the control treatment under conditions where the trees are subjected to environmental stress. Concentrations of glucose bound phenolic acids obtained with Si x 3 treatment were comparable to that of the potassium phosphonate (Avoguard®) treatment with exceptions during March 2005 and May 2006.

Three silicon applications per season resulted in significantly lower cell wall bound phenolic acid concentrations in avocado roots (Figure 5.3) compared to the control treatment during May and Sept 2005, and March and May 2006. This trend was negated during January 2006 when a significantly higher cell wall bound phenolic concentration was obtained in Si x 3 (0.71µg.l^{-1}) than the control (0.36µg.l^{-1}). Although the trend was not consistent compared to Si x 3, the potassium phosphonate (Avoguard®) treatment did not differ from the control throughout the tested period. Three silicon treatments per season resulted in significantly lower cell wall bound phenols during May, Jul and Sept 2005 and May 2006 compared to Si x 1, with higher concentrations obtained in Si x 3 only during Jan 2006 (0.71µg.l^{-1} vs. 0.38µg.l^{-1}). No significant difference was obtained between Si x 1 and Si x 3, except during Jan 2006, when Si x 3 (0.71µg.l^{-1}) resulted in higher cell wall bound phenols compared to Si x 2 (0.35µg.l^{-1}). Results indicate that potassium silicate application leads to lower cell wall bound phenolics. If the hypothesis of silicon being build into cell walls as part of a physical barrier is correct, it is possibly that silicon replaces phenol-binding molecules, or is bound in the place of phenolics, resulting in lower cell wall bound phenols. Epstein (2001) reported an accumulation of

phenolic compounds in the epidermis of silicon-deprived plants inoculated with a phytopathogenic fungus. It was accounted by Carver *et al*. (1998) that silicon-deprived leaves have been shown to exhibit higher phenylalanine ammonia lyase (PAL) activity compared to silicon-replete leaves, concluding that silicon deprivation may have been compensated for by the rise in PAL activity, in turn contributing to plant fungal resistance. Menzies *et al*. (1991) reported an extreme change in defence response expression of infected silicon-fertilized epidermal plant cells. Their results indicated that silicon accumulation was subsequent to phenol appearance in infected tissue, challenging the physical barrier-hypothesis that silicon accumulation in plant cell walls in close contact with the pathogen confers resistance to fungal penetration by physical means.

No significant differences were seen between treatments for ester bound phenolic concentrations throughout the duration of the trial (Figure 5.4).

Non-conjugated phenolic concentrations did not differ significantly between treatments during March, Jul and Sept 2005, and Jan and March 2006. Three silicon applications per season (1.62µg.l^{-1}) and potassium phosphonate (Avoguard®) (2.44µg.l^{-1}) resulted in significantly lower non-conjugated phenol concentrations compared to that of the control (2.80µg.l^{-1}) only during Nov 2005 (Figure 5.5), while the concentrations between Si x 3 and potassium phosphonate (Avoguard®) were statistically similar.

After silicon is taken up by a plant, it goes through a silicification process, and is either deposited in the cell wall, cell lumen, or intercellular spaces (Epstein, 1999; Sangster *et al.,* 2001). Silicon possesses a strong affinity for organic poly-hydroxyl compounds which participate in lignin synthesis. This partly explains its tendency to accumulate in cell walls during plant maturation or pathogen attack, which both corresponds to a radical change in cell wall constitution, with the apposition of lignin (Jones and Handreck, 1967; Inanaga and Okasaka, 1995). Electron microscopy and dispersive x-ray analysis led Samuels *et al.* (1991) and Chérif *et al*. (1992a) to conclude that enhanced defence reactions in the cucumber plant to *Pythium ultimum* Trow. and *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci appear to be the result of silicon present in the plants' transpiration stream, and not because it becomes bound to the plant cell wall. Although Menzies *et al*. (1992) and Chérif *et al*. (1992b) deemed the possibility of silicification of cell walls as not to be completely discarded, silicon is more likely to affect signalling between the host and pathogen, resulting in more rapid activation of a hosts' defence mechanisms. Heath (1976, 1979, 1981) and Chong and Harder (1980, 1982) investigated the effect of silicon on haustoria formation, and concluded that heavy silicon deposition

in the haustorial mother cells located at or near the centres of infection colonies was a protective mechanism of the plant to pathogen penetration. This mechanism acted as a permeability barrier to minimize passage of deleterious cell breakdown products to the rest of the pathogen mycelia. Heath (1979) reported that silicon accumulation as a response to infection is not limited to silicon accumulating plants (Epstein, 1999). Heath (1981) reported silicon accumulation not to be related to haustoria formation and, although uncertain on the significance of silicon in the cell walls and necrotic cytoplasm, suggested silicon accumulation to reflect a passive secondary association of silicon with phenolic compounds present in the disorganized host cell. Heath and Stumpf (1986) suggested the high levels of wall-associated phenolics in silicon-depleted tissue to result in faster inhibition of fungal enzymes involved in fungal-penetrating peg formation. In untreated tissue, the presence of silicon in the cell walls acted to 1) restrict substance flow to the haustorial mother cell, 2) reduce the interchange between the fungus and plant, so lesser amounts of phenolics are produced by the host, and 3) acted as a physical barrier to the penetration peg if it reached the cell wall (Heath, 1981). Results from the current study indicate that potassium silicate application to avocado trees leads to higher crude extract phenolic concentrations but lower cell wall bound phenolics compared to the control. Silicon accumulation was subsequent to phenol appearance in infected tissue, challenging the physical barrier-hypothesis that silicon accumulation in plant cell walls in close contact with the pathogen confers resistance to fungal penetration by physical means.

The accumulation of crude phenols in avocado roots treated with potassium silicate corresponds to higher root densities and lower canopy ratings (Chapter 4). The accumulation of phenols in avocado roots due to potassium silicate treatment could therefore be responsible for the increased resistance to *Phytophthora* observed in nursery trees and avocado orchards.

Phenols derived from cinnamic and ferulic acid would be polar (hydrophilic), while polymeric phenolics would be less polar (hydrophobic), and co-extracted biopolymers possibly present (e.g. terpenes) would be very hydrophobic (Regnier and Macheix, 1996). In the current study effective separation by gradient extraction was achieved by tapping the differences in the hydrophobic/hydrophilic nature of mobile phase components, as well as extracted molecule polarity. Phenolics were adsorbed onto the stationary phase at low solvent strength through Van der Waals forces and, according to their decreasing ability to participate in hydrogen bonding at distinct solvent concentrations, selectively

released (Cunico *et al*., 1998). Results from the RP-HPLC investigation in the current study could not be quantified to satisfaction and are therefore presented qualitatively only (Figure 5.6). Representative chromatograms for potassium phosphonate (Avoguard®), Si x 3 and control treatments are included.

Crude extracts of avocado roots used for determination of total phenol concentration were separated using HPLC, but, although separated peaks were obtained, the compounds were unidentifiable as phenols were present as glycosides. Nuutila *et al*. (2002) reported that, for quantitative determination of individual flavonoid glycosides to occur, glycosides need to be hydrolyzed and the resulting aglycones are then identified and quantified. Chérif *et al*. (1994) reported the fungitoxicity of these compounds to be apparent only after acid hydrolysis of the plant extracts. Hydrolysis of samples was therefore imperative to determine specific compounds within the phenol constitution of avocado root extracts. After hydrolysis, peak sizes reduced dramatically. Phenolic compounds were however identified on the basis of peak shape and retention time.

Silicon application to avocado trees resulted in fewer identifiable phenols in avocado roots compared to the control and potassium phosphonate treatments. HPLC separation of hydrolysed phenolic acids extracted from roots revealed that all non-conjugated phenolic acid samples contain 3,4-hydroxibenzoic acid [retention times of potassium phosphonate, Si x 3 and control treatments being R_t = 14.693, R_t =14.878 and R_t = 14.984, respectively]. The hydrolysed glucoside bound samples of both the potassium phosphonate and control treatments also contained 3,4-hydroxibenzoic acid (R_t = 14.693; R_t = 14.881, respectively) and vanillic acid (R_t = 22.326; R_t = 22.621, respectively). The control treatment contained syringic acid (R_t = 23.154) in the hydrolysed glucoside bound extract.

Nuutila *et al*. (2002) reported that phenol based defence responses are characterised by an accumulation of phenolic compounds within host cell walls, as well as the synthesis and deposition of the phenolic polymer, lignin. Esterification of phenolic cell wall materials is a common occurrence in expression of resistance (Cunico *et al*., 1998). Phenols in the cell wall has been suggested to act as a template for further lignin deposition, indicating esterification and lignification to be contigious rather than separate processes. Lignin formation takes place as a result of cell damage due to mechanical puncturing or infectional penetration (De Ascensao and Dubery, 2003).

5.5 CONCLUSION

The accumulation of phenols and phenolic polymers in *Persea americana* roots exposed to cell wall derived elicitors from the pathogen *Phytophthora cinnamomi*, and treated with water soluble potassium silicate, was investigated. These findings support the hypothesis that silicon application results in heightened resistance against *P. cinnamomi* infection via an elevation of phenolic levels in the roots. Although crude phenolic concentrations differed between treatments and no clear deduction may be made concerning the effect of potassium silicate on the phenolic content of avocado roots in the presence of *P. cinnamomi*, it is clear that similar or higher crude phenolic concentrations are obtained in avocado roots with three silicate applications per season compared to potassium phosphonate treated trees. This was also true for glucoside bound phenolic concentrations in roots from trees treated three time per season with potassium silicon (Si x 3) compared to potassium phosphonate treated trees.

In this study the potassium silicate application lead to lower cell wall bound phenolics. The possibility that silicon replaces phenol-binding molecules is not fully understood. However, this study indicates that the accumulation of silicon was subsequent to phenol appearance in infected tissue; challenging the physical barrier and conferring to the cell wall in close contact with the pathogen some resistance to fungal penetration by physical means. The future search on the use of silicon therefore can be upheld with this strategy to control plant disease in general and avocado root diseases in particular.

5.6 LITERATURE CITED

BECKMAN, C.H., 2000. Phenol-storing cells: Keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiol. Mol. Plant Pathol.* 57, 101-110.

BEKKER, T.F., KAISER, C., VAN DER MERWE, R., & LABUSCHAGNE, N., 2006. *In-vitro* inhibition of mycelial growth of several phytopathogenic fungi by soluble silicon. *S.A. J. Plant Soil* 26(3), 169-172.

BERRYMAN, A.A., 1988. Towards a unified theory of plant defence. In: Mechanisms of Woody Plant Defences against Insects - Search for Pattern, W.J. Mattson, J. Levieux, C. Bernard-Dagan (Eds.), Springer-Verlag, New York, pp 1.

BRUNE, W. & LELYVELD, L.J., 1982. Biochemical comparison of leaves of five avocado (*Persea americana* Mill.) cultivars and its possible association with susceptibility to *Phytophthora cinnamomi* root rot. *Phytopath. Z.* 104, 243-254.

CARMAN, R.M. & HANDLEY, P.N., 1999. Antifungal diene in leaves of various avocado cultivars. *Phytochem.* 50, 1329-1331.

CARVER, T.L.W., ROBBINS, M.P., THOMAS, B.J., TROTH, K., RAISTRICK, M. & ZEYEN, R.J., 1998. Silicon deprivation enhances autofluorescence responses and phenylalanine ammonialyase activity in oat attacked by *Blumeria graminis. Physiol. Mol. Plant Pathol.* 52, 245-257.

CHÉRIF, M., ASSELIN, A. & BELANGER, R.R., 1994. Defence responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathol.* 84, 236-242.

CHÉRIF, M., BENHAMOU, N., MENZIES, J.G. & BÉLANGER, R.R., 1992a. Silicon induced resistance in cucumber plants against *Pythium ultimum. Physiol. Mol. Plant Pathol*. 41, 411-415.

CHÉRIF, M., MENZIES, J.G., BENHAMOU, N. & BÉLANGER, R.R., 1992b. Studies of silicon distribution in wounded and *Pythium ultimum* infected cucumber plants. *Physiol. Mol. Plant Pathol*. 41, 371-385.

CHONG, J. & HARDER, D.E., 1980. Ultrasturcture of haustorium development in *Puccinia coronata avenae*. I. Cytochemistry and electron probe X-ray analysis of the haustorial neck ring. *Can. J. Bot*. 58, 2496-2505.

CHONG, J. & HARDER, D.E., 1982. Ultrasturcture of haustorium development in *Puccinia coronata* f.sp. *avenae*. I. Cytochemistry and energy dispersive X-ray analysis of the haustorial mother cell. *Phytopath*. 72, 1518-1526.

CUNICO, R. L., GOODING K. M. & WEHR, T., 1998. Basic HPLC and CE of Biomolecules. Bay Bioanalytical Laboratory, Richmond, California, pp. 205 – 233.

DE ASCENSAO, A.R.F.D.C. & DUBERY, I.A., 2003. Soluble and wall-bound phenolics and phenolic polymers in *Musa acuminata* roots exposed to elicitors from *Fusarium oxysporum* f.sp. *cubense*. *Phytochem.* 63, 679-686.

DIXON, R.A. & PAIVA, N.L., 1995. Stress-induced phenylpropanoid metabolism. *The Plant Cell* 7, 1085-1097.

DOMERGUE, F., HELMS, G.L., PRUSKY, D. & BROWSE, J., 2000. Antifungal compounds from idioblast cells isolated from avocado fruits. *Phytochem.* 54, 183-189.

EPSTEIN, E., 1999. Silicon. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 50, 641-664.

EPSTEIN, E., 2001. Silicon in plant: Facts vs. concepts. In: Silicon in Agriculture, L.E. Datnoff, G.H. Snyder and G.H. Korndorfer (Eds.), Elsevier Science B.V., Amsterdam, pp 1-15.

HEATH, M.C., 1976. Ultrastructure and functional similarity of the haustorial neck band of rust fungi and the Casparian strip of vascular plants. *Can. J. Bot*. 54, 1484-1489.

HEATH, M.C., 1979. Partial characterization of electron-opaque deposits formed in the non-host plant, French bean, after cowpea rust infection. *Physiol. Plant Pathol*. 15, 141-148.

HEATH, M.C., 1981. Insoluble silicon in necrotic cowpea cells following infection with an incompatible isolate of the cowpea rust fungus. *Physiol. Plant Pathol*. 19, 273-276.

HEATH, M.C. & STUMPF, M.A., 1986. Ultrastructural observations of penetration sites of the cowpea rust fungus in untreated and silicon depleted French bean cell. *Physiol Mol. Plant Pathol*. 29, 27-39.

INANAGA, S. & OKASAKA, A., 1995. Induced resistance in cucurbits. In: Induced Resistance to Disease in Plants, R. Hammerschmidt & J. Kuc (Eds.), Kluwer Academic Publishers, Dordrecht, pp 63-85.

JONES, L.H.P. & HANDRECK, K.A., 1967. Silica in soils, plants and animals. *Adv. Agron*. 19, 107-149.

KAISER, C., 1993. Some physiological aspects of delayed harvest of 'Hass' avocado (*Persea americana* Mill.) in the Natal midlands. MSc thesis, Department of Horticulture, Pietermaritzburg, South Africa, pp 97.

KARNI, L., PRUSKY, D., KOBILER, I., BAR-SHIRA, E. & KOBILER, D., 1989. Involvement of epicatechin in the regulation of lipoxygenase activity during activation of quiescent *Colletotrichum gleosporioides* infections of ripening avocado fruits. *Physiol. Mol. Plant Pathol.* 35, 367-374.

MACHEIX, J-J., FLEURIET, A. & BILLOT, J., 1990. Fruit Phenolics. CRC Press, Paris, pp 1.

MARCUS, L., PRUSKY, D. & JACOBY, B., 1998. Purification and characterization of avocado lipoxygenase. *Phytochem*. 27, 323-327.

MENZIES, J., BOWEN, P., EHRET, D. & GLASS, A.D.M., 1991. Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber, muskmelon and zucchini squash. *J. Amer. Soc. Hort. Sci*. 117, 902-905.

MENZIES, J., EHRET, D., GLASS, A.D.M. & SAMEULS, A.L., 1992. The influence of silicon on cytological interactions between *Spaerotheca fuliginea* and *Cucumis sativus. Physiol. Mol. Plant Pathol*. 39, 403-414.

NUUTILA, A.A., KAMMIOVIRTA, K. & OKSMAN-CANDENTEY, K.-M., 2002. Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. *Food Chem*. 76, 519-525.

PRUSKY, D., KEEN, N.T. & EAKS, I., 1983. Further evidence for the involvement of a preformed antifungal compound in the latency of *Colletotrichum gleosporioides* in unripe avocado fruits. *Physiol. Plant Pathol* 22, 189-198.

PRUSKY, D., KEEN, N.T., SIMS, J.J. & MIDLAND, S.L., 1982. Possible involvement of an antifungal compound in latency of *Colletotrichum gloeosporioides* on unripe avocado fruits. *Phytopathol*. 72(12), 1578-1582.

PRUSKY, D., PLUMBLEY, R.A. & KOBILER, I., 1991. Modulation of natural resistance of avocado fruits to *Colletotrichum gleosporioides* by CO_2 treatment *Physiol. Mol. Plant Pathol*. 39, 325-334.

REGNIER, T. 1994. *Les composés phénoliques du blé dur (Triticum turgidum L. var. durum): Variations au cours du developpement et de la maturation du grain, relations avec l'apparition de la moucheture*. PhD Thesis, Montpellier University. France, pp. 43 - 45.

REGNIER, T. & MACHEIX, J. J. 1996. Changes in wall-bound phenolic acids, phenylalanine and tyrosine ammonia-lyases, and peroxidases in developing durum wheat grains (*Triticum turgidum* L var. Durum), *J. Agri. Food Chem.* 44, 1727 - 1730.

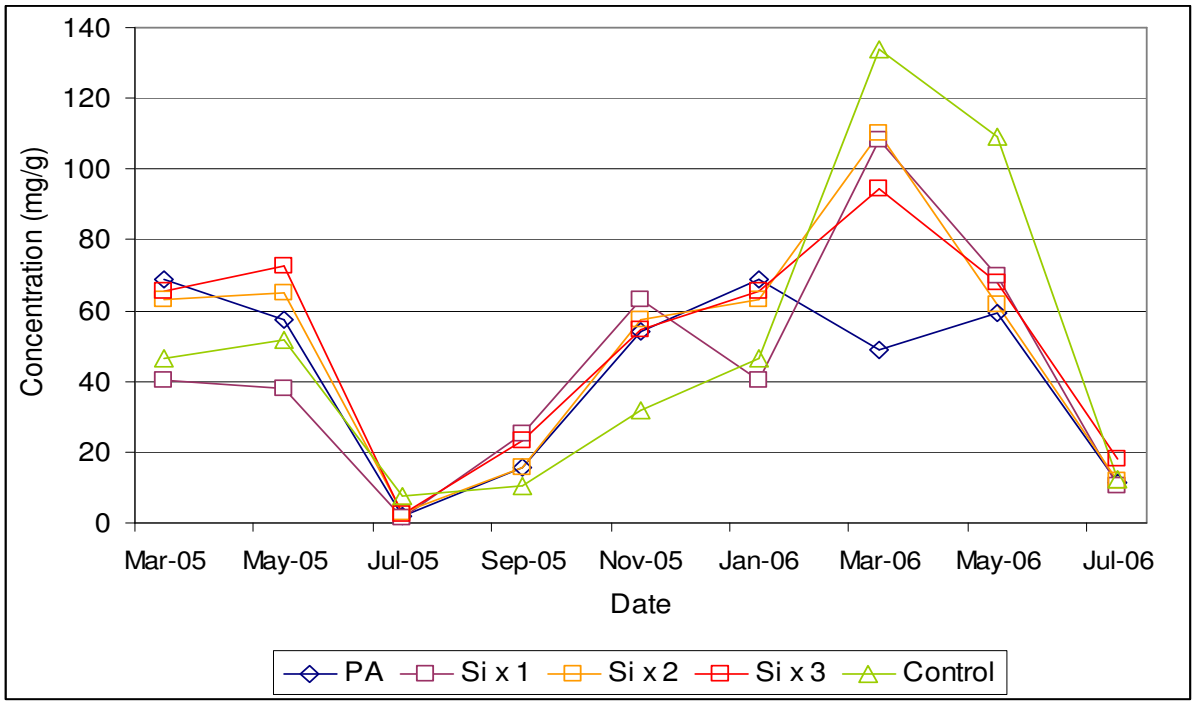
SAMUELS, A.L., GLASS, A.D.M., EHRET, D.L. & MENZIES, J.G., 1991. Mobility and deposition of silicon in cucumber plants. *Plant Cell Environ*. 14, 485-492.

SANGSTER, A.G., HODSON, M.J. & TUBB, H.J., 2001. Silicon deposition in higher plants. In: Silicon in Agriculture, L.E. Datnoff, G.H. Snyder and G.H. Korndorfer (Eds.), Elsevier Science B.V., Amsterdam, pp 85-113.

VIDHYASEKARAN, P., 1997. Fungal Pathogenesis in Plants and Crops, Molecular Biology and Host Defence Mechanisms. Marcel Dekker, Inc., New York, pp 223.

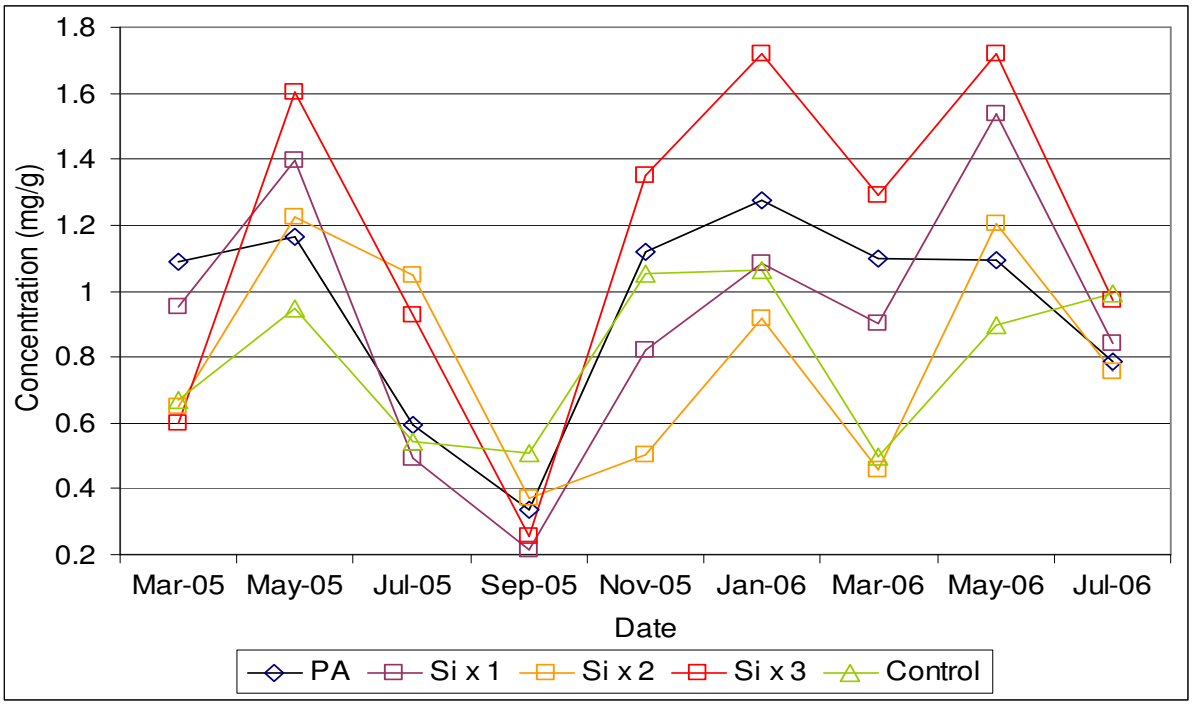
WEHNER, F.C., BESTER, S. & KOTZE, J.M., 1982. Sensitivity of fungal pathogens to chemical substances in avocado trees. *South African Avocado Growers' Association Yearbook* 5, 32-34.

ZHOU, Z., ROBARDS, K., HELLIWEL, S. & BLANCHARD, C., 2004. The distribution of phenolics in rice. *Food Chem.* 87, 401 - 406.



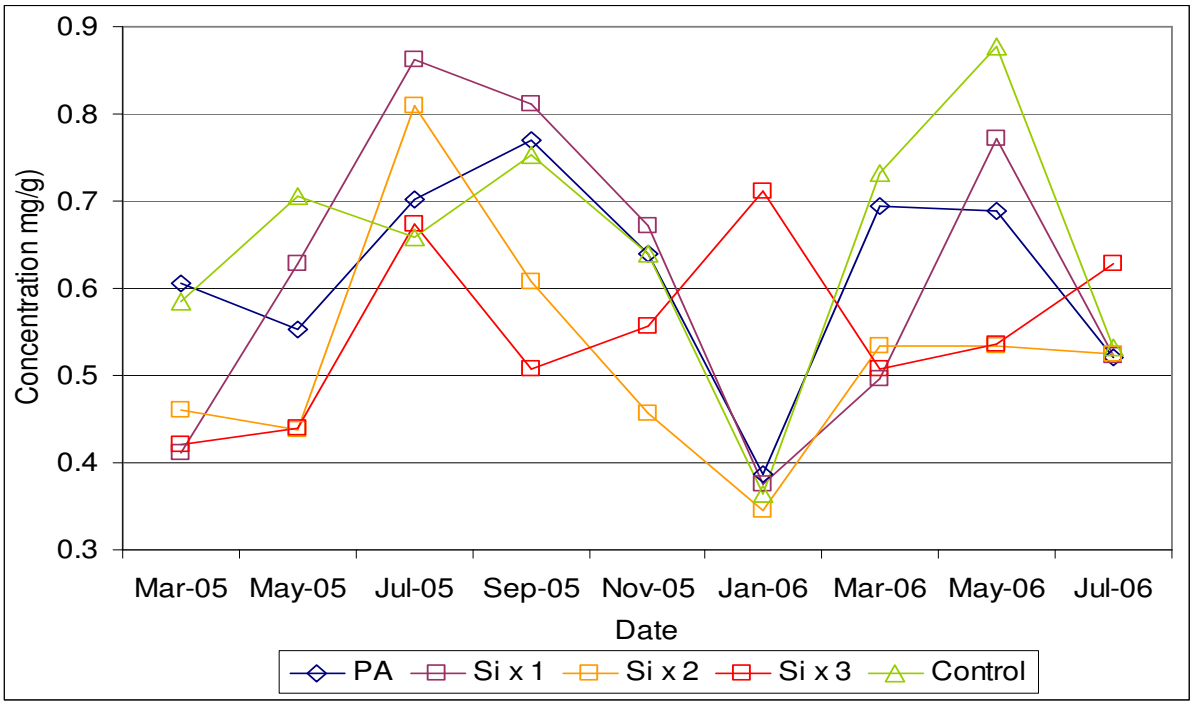
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	67.77b	57.26bc	1.94a	15.81ab	53.94b	68.77b	49.07a	59.46a	11.25a
Si x 1	45.42a	37.82a	1.66a	25.34b	62.94c	40.42a	108.23c	69.64b	10.61a
Si x 2	63.38b	65.19c	2.93a	15.77ab	57.56bc	63.08b	110.25c	61.62ab	11.94a
Si x 3	65.32b	72.62c	2.5a	23.18b	54.8bc	65.32b	94.61b	67.98b	17.92a
Control	46.34a	51.62b	7.7a	10.41a	31.94a	46.34a	133.66c	109.08c	12.28a

Figure 5.1: Total soluble phenolic content of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA). Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.



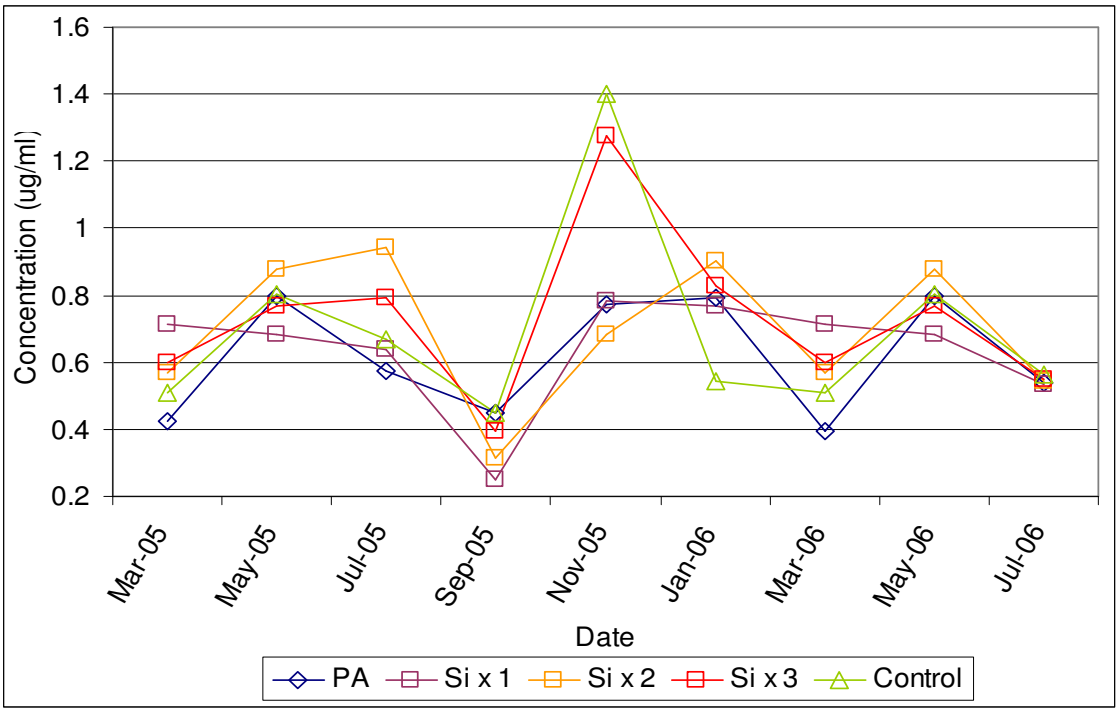
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	1.09b	1.16ab	0.59ab	0.34a	1.12b	1.27ab	1.09b	1.09a	0.79a
Si x 1	0.95ab	1.39b	0.49a	0.21a	0.82ab	1.08a	0.90b	1.54b	0.84a
Si x 2	0.65a	1.23ab	1.05b	0.37a	0.50a	0.92a	0.46a	1.21ab	0.75a
Si x 3	0.59a	1.60b	0.93b	0.26a	1.35b	1.72b	1.29b	1.72b	0.97a
Control	0.67a	0.95a	0.54ab	0.51a	1.05b	1.06a	0.49a	0.89a	0.99a

Figure 5.2: Total concentration of glucoside bound phenolic acid after hydrolysis of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA). Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.



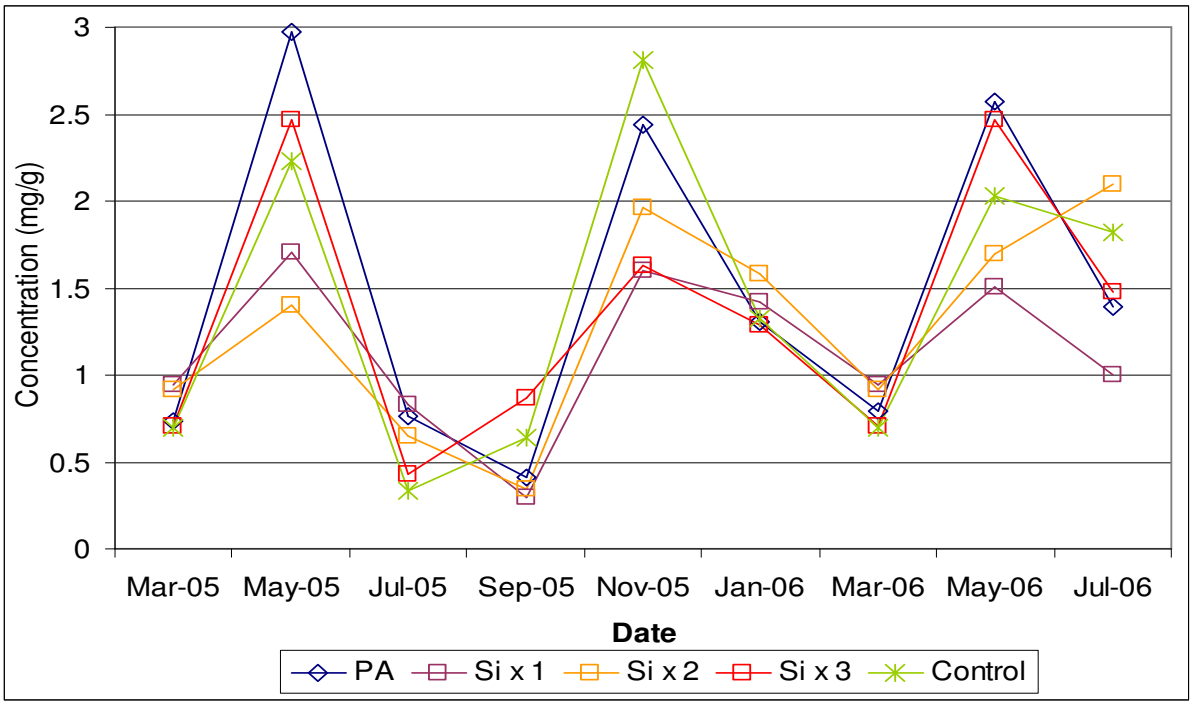
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	0.61b	0.55ab	0.70ab	0.77b	0.64b	0.39a	0.69b	0.68ab	0.52a
Si x 1	0.41a	0.63b	0.86b	0.81b	0.67b	0.38a	0.49a	0.77b	0.52a
Si x 2	0.46ab	0.44a	0.81ab	0.61ab	0.46a	0.35a	0.53ab	0.53a	0.52a
Si x 3	0.42a	0.44a	0.67a	0.51a	0.56ab	0.71b	0.51a	0.54a	0.63a
Control	0.58ab	0.71b	0.66a	0.75b	0.64b	0.36a	0.73b	0.88b	0.53a

Figure 5.3: Total concentration of cell wall bound phenolic acid after hydrolysis of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA). Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.



	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	0.42a	0.79a	0.57a	0.45a	0.77a	0.79a	0.39a	0.79a	0.54a
Si x 1	0.72a	0.68a	0.64a	0.25a	0.78a	0.77a	0.72a	0.68a	0.54a
Si x 2	0.57a	0.88a	0.94a	0.31a	0.68a	0.90a	0.57a	0.88a	0.54a
Si x 3	0.59a	0.77a	0.79a	0.39a	1.27a	0.83a	0.59a	0.77a	0.55a
Control	0.51a	0.80a	0.67a	0.45a	1.40a	0.54a	0.51a	0.80a	0.56a

Figure 5.4: Total concentration of ester bound phenolic acid after hydrolysis of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA. Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.



	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	0.73a	2.98b	0.76a	0.41a	2.44ab	1.31a	0.79a	2.58b	1.39ab
Si x 1	0.94a	1.71ab	0.83a	0.29a	1.60a	1.42a	0.94a	1.51a	0.99a
Si x 2	0.91a	1.39a	0.64a	0.35a	1.96ab	1.58a	0.91a	1.69ab	2.09b
Si x 3	0.70a	2.46b	0.43a	0.86a	1.62a	1.28a	0.70a	2.46b	1.48ab
Control	0.69a	2.23ab	0.34a	0.63a	2.80b	1.33a	0.69a	2.03ab	1.82ab

Figure 5.5: Total concentration of non-conjugated phenolics acid after hydrolysis of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA). Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.

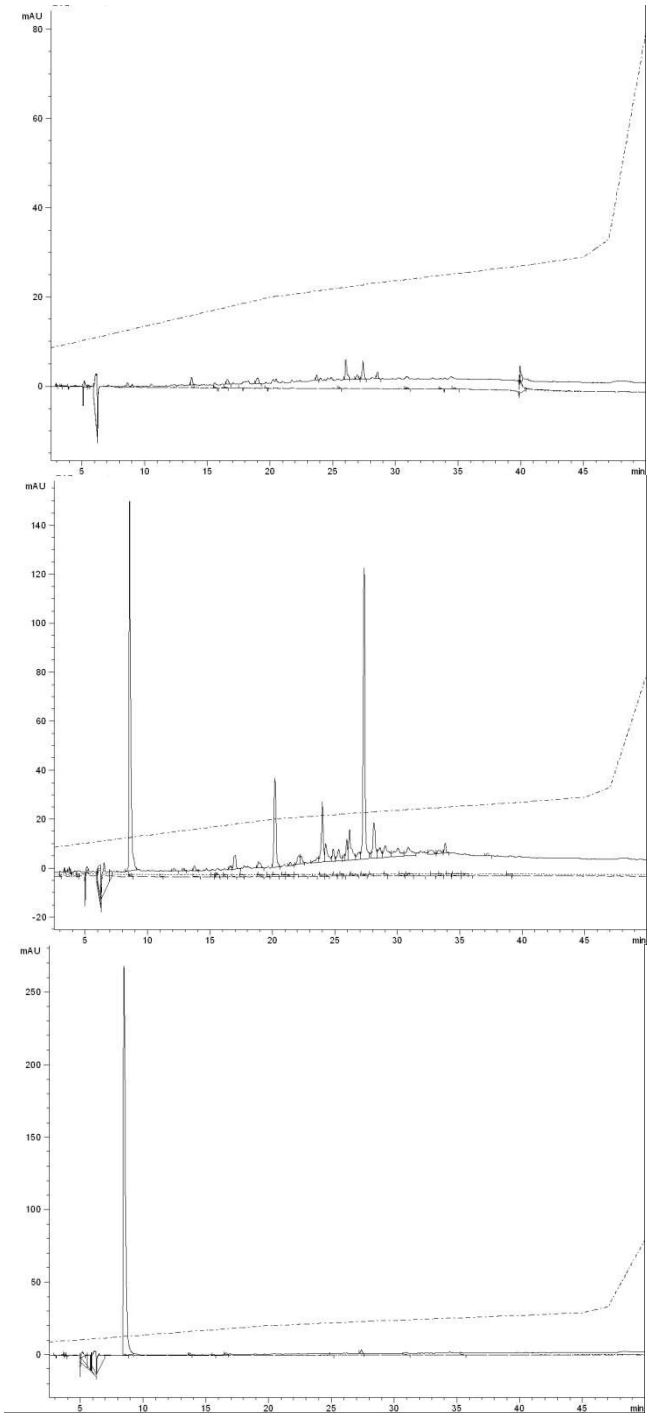


Figure 5.6: Chromatographs of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees. Treatments consisted of trees receiving no treatment as a control treatment (a), trees injected with potassium phosphonate (PA) (b) or three (Si x 3) potassium silicate applications per season (a).

GENERAL DISCUSSION AND CONCLUSIONS

The avocado (*Persea americana* Mill.) is a tropical fruit produced in almost all tropical and subtropical climatic regions, and exported worldwide (Knight, 2002). In South Africa, avocado production is confined to the Limpopo and Mpumalanga provinces in the north and north-east, and to a lesser extent in the frost free lowland coastal belts and cooler midlands of KwaZulu Natal (Lovegrove and Hooley, 2000). Currently the area planted with avocado trees in South Africa amounts to 12400ha, with approximately 3015000 trees in production, which could amount to more than 50 thousand tons, of which 36thousand tons (9 million cartons) are destined for the export market (Retief, 2007).

Phytophthora root rot, caused by the fungus *Phytophthora cinnamomi* Rands, is the most important pre-harvest disease of avocado trees and attacks trees of all ages, including nursery trees, leading to tree death by destruction of the feeder roots (Hardy *et al.,* 2001). *Phytophthora* root rot has been the main economic factor limiting successful avocado production in countries such as Australia, South Africa and the USA (Coffey, 1987).

The prevention of *Phytophthora* root rot relies on limited non-chemical practices including implemented cultural practices (Ohr and Zentmyer, 1991), biological control with the use of *Trichoderma* isolates (Duvenhage and Kotze, 1993; Casale, 1990; Pegg, 1977), and host resistance (Coffey 1987). Chemical control however remains the most important control measure, and to this end, phosphate-based fungicides play a major role. Duvenhage (1994, 1999) however concluded that the possibility of resistance against fosetyl-Al, the most commonly used fungicide, does exist, which would pose a serious threat to the avocado industry.

The need therefore exists to find a viable alternative to phosphonate fungicides. The suppressive effects of silicon on plant diseases have been indicated by various authors (Ma and Takahashi, 2002; Epstein, 1999). Potassium silicate was therefore investigated as a viable alternative treatment against *P. cinnamomi* infection of avocado trees. The objective of this study was to determine whether the application of soluble silicon from potassium silicate to *P. cinnamomi* infected trees would suppress the disease.

The first objective was to assess the affect of potassium silicate *in vitro* on fungal growth to establish if potassium silicate has any direct effect on fungal growth. In the current study potassium silicate (20.7% SiO_2) induced a total inhibition of *P. cinnamomi*

mycelial growth at all concentrations tested, with effective inhibition at 5ml.l^{-1}. This was however not true for all fungi investigated, and a total inhibition for all fungi tested (*Alternaria solani, Colletotrichum gloeosporioides, Curvularia lunata, Drechslera* sp., *Fusarium oxysporum, Fusarium solani, Glomerella cingulata, Lasiodiplodia theobromae, Mucor pusillus, Natrassia* sp., *Pestalotiopsis maculans, Phomopsis perniciosa, Phytophthora capsicii, Phytophthora cinnamomi, Pythium* F-group, *Sclerotinia sclerotiorum, Sclerotium rolfsii, Stemphylium herbarum* and *Verticillium tricorpus*) was only attained at a concentration of 40ml.l^{-1} and higher. Soluble potassium silicate, having a pH of 12.7, raised the pH of agar from 5.6 to 10.3 and 11.7 at concentrations of 5 and 80ml.l^{-1} agar respectively. Fungal growth was however only partially inhibited at these high pH values. Clearly, potassium silicate has an inhibitory effect on fungal growth *in vitro* and this was mostly fungicidal rather than attributed to a pH effect.

The effect of such a high concentration of silicon, as those required to suppress fungal growth *in vitro*, on beneficial micro-organisms in the soil is yet to be determined, and a concentration of 20ml.l^{-1} consequently led to a suppression of root rot development in both greenhouse and field trials. If silicon inhibits spore formation, inoculum concentration in soils may be reduced to such an extend that lower fungicidal rates will be necessary, resulting in an overall reduction in production costs and a reduced pressure on resistance development.

Phytophthora root rot of avocado nursery trees can be inhibited successfully by potassium silicate application as seen from the glasshouse trials conducted. The effectiveness of silicon application, however, depends on repeated applications to infected trees. Samuels *et al*. (1993), using the powdery mildew-cucumber pathogen system, showed that within a short period of time after Si application ceased, prophylactic effects receded. Interruption of silicon application, or if applications are too far apart, may lead to reduced disease control and according to the mechanical barrier hypothesis, the protection against fungal haustoria penetration may expire. The timing of reapplication will be determined by, among other factors, soil structure, as silicon in solution leaches easily, rendering the applied silicon unreachable for plant root uptake. Sandy soils will therefore necessitate more regular applications of silicon to maintain the level of disease suppression in the host plant. Root rot suppression in silicon treated trees was comparable to, or even better than root rot suppression in inoculated, potassium phosphonate (Avoguard®) treated trees. These findings are of paramount importance as this implies that potassium silicate may

be proposed as a possible alternative control measure to inhibit the effects of *P. cinnamomi* on avocado nursery trees.

The application of silicon to nursery trees seems to impart some form of protection as inoculated, silicon treated trees rendered the highest fresh and dry root mass compared to all other treatments. This implies that silicon either stimulates plant growth or imparts some form of protection to avocado roots if applied prior to *P. cinnamomi* inoculation. This may be due to mechanical barriers (Chérif *et al.,* 1994; Datnoff *et al.,* 1997), induction of plant enzymes (Chérif *et al.,* 1992) and a hastened expression thereof (Remus-Borel *et al*., 2005); or the induction of fungitoxic metabolites in the plant (Fawe *et al*., 2001). Although worldwide research is being conducted on the effect of silicon on other crops, future research on avocado should focus on the possible mode of action that silicon imposes to defend avocado tissue from infection. The accumulation of amorphous tissue in roots, and especially around sites of infection, should be examined microscopically. Datnoff *et al.* (1997) stated that disease suppression is made possible by the increased silicification of the epidermal cells, as the link between silicon deposition and pathogen resistance stems from the fact that Si accumulates at sites of infection (Fauteux *et al.,* 2005).

The economic viability and effective implementation of a new method to suppress Phytophthora root rot is only proven during a trial on field grown avocado trees in full production. In the current study, higher root densities were obtained throughout the trial period with potassium silicate application comparing to that of potassium phosphonate (Avoguard®) injections and untreated control trees. Differences in root density between treatments were affected by the availability of soil moisture, i.e. rainfall received throughout the season, although seasonal growth flushes and timing of silicon application also played a role. To provide maximum protection, and therefore minimize disease development, Bowen *et al.* (1992) suggested silicon be applied continuously. Our results indicate this to be true, as found in the nursery tree trials; and as three applications of silicon resulted in the best disease suppression and stimulation of new root growth. These results correlated well with tree canopy ratings, as trees that received silicon frequently, showed better canopy conditions compared to the untreated control treatments. It is proposed that silicon application should be timed according to the phenological model (Kaiser, 1993) with the first application during the period of flowering and fruit set (September); the second to occur before the fruit drop

(November); and the third application to be applied before the root flush during February to March.

Phenological cycling, rather than rainfall, was the determining factor in canopy health. Under conditions of limited drought stress, tree canopies showed less symptoms of disease stress, whereas during dry conditions, canopy condition deteriorated dramatically. All potassium silicate soil drench treatments resulted in better canopy ratings over the 18 month period of data collection compared to the untreated control. This indicates that potassium silicate soil drench treatments reduced drought stress, apart from reducing disease stress. This was reiterated by Gong *et al.* (2005) who reported that silicon improves the water status of drought stressed wheat plants compared to untreated plants.

The effect of potassium silicate as a stem injection to control *P. cinnamomi* severity was not evident in differences in tree root densities, or canopy health ratings. Potassium silicate injections did not show any significant trends throughout the trial period.

Potassium silicate injections did not increased tree health to such an extent that it had an effect on canopy condition and no clear trends were observed. Anderson *et al.* (2004) injected avocado trees, with a disease rating of 5.5, with a 200ppm (0.74ml potassium silicate) solution. They reported stimulation of epicormic buds, with "an eventual significant increase in canopy density", and a 31% mean tree health improvement. In the current study, no epicormic bud bursts were observed, and no simultaneous increase in canopy density detected. No mention is made as to when epicormic bud bursts were observed with relation to phenological cycling, and it is proposed that the cycling observed by Anderson *et al.* (2004) was due to tree phenology, rather than due to a siliceous effect.

In the current study, the overriding factor of silicon seems to be its effect on disease suppression, and therefore canopy condition as an indicator of disease severity. Chérif *et al.* (1994) reported that although silicon had no effect on phenolic concentrations of plants in the absence of pathogen infection, significant differences can be seen in inoculated plants compared to uninoculated control cucumber plants.

The application of potassium silicate to avocado trees to suppress Phytophthora root rot seems to be most effective when applied as a soil drench. Menzies *et al.* (1992) reported that foliar applications of potassium silicate at 17mM Si are as effective as a 1.7mM root application. This will however most probably not be as effective in avocado trees as data from nutrient analysis suggests that silicon is not actively and efficiently translocated in avocado tissue. The possibility of physical barrier formation in roots will therefore be

limited, and the expression of phenolic and other fungitoxic compounds confined to plant parts receiving silicon. Application of Si to Si-deficient soils also creates the possibility of reducing fertiliser rates to be applied in successive years after Si application, and reduced fungicide applications (Seebold *et al*., 2004).

Although the current study's focus was not on the effect of silicon on post harvest disease incidence of avocado, data were collected to determine possible benefits of silicon, over and above that of inhibiting Phytophthora root rot. Anthracnose severity during the 2004/2005 season was lower in fruit from trees treated with silicon. No significant differences were seen during the 2005/2006 season with regards to anthracnose incidence between treatments. Results obtained by Anderson *et al*. (2004) however confirmed that silicon injection may be a possible preventative measure to control anthracnose incidence and severity in avocado fruit if applied separately from phosphorous acid treatments. This was reiterated by Anderson *et al.* (2005) who stated that a silicon-phosphorous acid mixed application lead to no control of anthracnose as a result of a lower silicon solubility rate at a lower pH, deeming silicon to be unavailable to plants.

Although some level of inhibition of stem end rot was obtained in fruit from trees receiving silicon as a soil drench, results were not consistent, and fruit from silicon injected trees did not differ significantly from the control. This confirms results obtained by Anderson *et al.* (2005) who found that stem end rot incidence and severity thereof in fruit from silicon injected trees did not differ significantly from fruit harvested from uninjected trees. There was however a decrease in vascular browning in fruit harvested from trees receiving two and three silicon applications.

There seems to be a link between the application of silicon to avocado trees and a decrease in severity and incidence of the two most important post harvest diseases threatening the avocado industry. Although no significant differences were observed between treatments, copper concentrations in avocado leaves in some instances exceeded the permissible standard by a factor eleven. Some authors (Boshoff *et al*., 1996; Schoeman and Manicom, 2002) have reported on the beneficial effects of copper sprays on post harvest disease incidence, in particular *Colletotrichum gloeosporioides*. This, however, leads to a build up of copper in not only soils, but also in avocado tissue, possible leading to toxic levels in plants. Future research should focus on the possible optimisation of silicon application to inhibit post harvest disease development in fruit. Silicon may be a valuable alternative to copper application, especially in the light of

copper build-up in soils, and residues on fruit being unfavourable for the European export market (Duvenhage, 2002).

The application of potassium silicate to trees as a soil drench led to higher yields compared to the control treatment. It is possible that improved tree condition due to a lower root rot severity led to lower flower/fruit drop resulting in higher yields compared to the control treatment. This was also true for the number of fruit per tree. Results from both total yield per tree and the number of fruit per tree indicate that Si x 3 is effective in, if not increasing yield and fruit number, sustaining tree health to a productive level. It is imperative to determine whether the timing at which the third application was employed with regards to the tree phenological model, is the determining factor in increasing yields and number of fruit per tree.

Silicon applications to the soil also appeared to affect nutrient concentrations in avocado trees.

Three silicon applications resulted in higher boron concentrations in leaves compared to all other treatments and it thus appears that silicon application increases the boron uptake of avocado plants. Whiley *et al*. (1996) reported that boron application may increase fruit set and quality. If this does indeed occur, it may result in additional benefits of silicon application to the avocado plant.

It does not appear as if three silicon applications per season to avocado trees as a soil drench increase the silicon translocation to avocado leaves, and contrary to the expected outcome, silicon concentrations were the lowest in avocado leaves from plants receiving three silicon applications during 2005, and only marginally higher during 2006. However, significantly higher levels of silicon were obtained in avocado roots from trees receiving three silicon applications. This indicates that avocado roots absorb silicon, but this silicon is not effectively translocated in the plant to leaf tissue. High levels of silicon were also obtained in potassium phosphonate (Avoguard®) treated root tissues. It would be interesting to note whether phosphorous acid treatment of avocado trees increase the plants uptake of silicon. The mode of action of potassium phosphonate (Avoguard®) may not only be a direct, fungitoxic and indirect, enzyme releasing function, but may also alter plant nutrient composition, implementing the numerous functions ascribed to silicon.

In addition to suppression of disease, potassium silicate application to avocado trees as a soil drench also leads to an increase in soil pH. This is an especially important additional benefit of silicon application as it is known that most avocado producing areas of South

Africa have low pH values partly due to high rainfall and low CEC (cation exchange capacity) of the soil in which avocado is cultivated.

The accumulation of phenols and phenolic polymers in *Persea americana* roots exposed to *P. cinnamomi*, and treated with water soluble potassium silicate, was investigated. Similar or higher total phenolic concentrations were obtained in avocado roots receiving three silicate applications compared to roots from potassium phosphonate (Avoguard®) treated trees. This was also true for glucoside bound phenolic concentrations in the Si x 3 treatment compared to the potassium phosphonate (Avoguard®) treatment. Results indicate that potassium silicate application leads to lower cell wall bound phenolics. It is possible that silicon replaces phenol-binding molecules, or is bound in the place of phenolics, resulting in lower cell wall bound phenols. Results indicated that silicon accumulation was subsequent to phenol appearance in infected tissue, challenging the physical barrier-hypothesis that silicon accumulation in plant cell walls in close contact with the pathogen confers resistance to fungal penetration by physical means.

Future research is paramount to effectively determine the role silicon application does play in disease suppression. Results from the *in vitro* study opens up a wide scope for research into the effect of application of silicon to suppress post harvest disease development. Biggs *et al.* (1997) reported a 65% *in vitro* fungal inhibition of the brown rot pathogen *Monilinia fructicola* (G. Wint) Honey of peach fruit, and attained similar growth inhibition of fungal colonies on fruit dipped in a calcium silicate solution. Although infection of avocado fruit with the anthracnose and stem end rot complexes occurs before fruit are harvested (Anderson *et al*., 2005), post-harvest dips may inhibit lesion development.

Future research should be focused on the effect of silicon application on soil micro-fauna, especially those involved in biological control of *P. cinnamomi* including *Trichoderma* isolates (McLeod *et al.,* 1995), as applied silicon may affect the effectiveness of such a control method. It is also imperative to determine whether silicon application only inhibits mycelial growth, or if it has an effect on the sexual reproduction of the fungus.

Molecular work on gene activation due to silicon application is to be undertaken. The activation of a gene regulation system by Si has long been proposed by Wingate *et al*. (1988). The presence or absence of these genes does however not determine resistance or susceptibility, but the magnitude and speed with which the gene information is expressed is important (Chérif *et al.,* 1992). The effect of silicon on the speed and magnitude of expression are to be considered.

Although fungitoxic metabolites such as borbonol (Zaki *et al.,* 1980; Wehner and Apostolides, 1981) and diene (Chang *et al*., 1975; Prusky *et al*., 1982) have been reported in avocado tissue, their presence and heightened expression have not been linked to Phytophthora root rot. The possibility of heightened expression of these and other toxic compounds due to silicon application needs to be examined.

Phytophthora cinnamomi inoculum is present in the majority of avocado producing soils throughout the world. The control of root rot is therefore imperative for the continual and economically viable production of an avocado crop. The application of silicon to avocado trees to inhibit root rot results in an effective decrease in disease severity and aid in the maintenance of a healthy canopy condition under diseased circumstances.

The results of the current study support the hypothesis that silicon application, through an elevation of the total phenolic levels, causes an increase of resistance against *P. cinnamomi* root rot in avocados.

LITERATURE CITED

ANDERSON, J.M., PEGG, K.G., COATES, L.M., DANN, L., COOKE, T., SMITH, L.A. & DEAN, J.R., 2004. Silicon and disease management in avocados. *Talking Avocados* 15 (3), 23-25.

ANDERSON, J.M., PEGG, K.G., DANN, E.K., COOKE, A.W., SMITH, L.A., WILLINGHAM, S.L., GIBLIN, F.R., DEAN, J.R. & COATES, L.M., 2005. New strategies for the integrated control of avocado fruit diseases. New Zealand and Australia Avocado Grower's Conference 2005, Tauranga, New Zealand, session 3.

BIGGS, A.R., EL-KHOLI, M.M. & EL-NESHAWY, S., 1997. Effects of calcium salts on growth, polygalacturonase activity, and infection of peach fruit by *Monilinia fructicola*. *Plant Dis*. 81 (4), 399-403.

BOSHOFF, M., KOTZE, J.M. & KORSTEN, L., 1996. Effect of staggered copper sprays on pre-and post-harvest diseases of avocado in KwaZulu Natal midlands. *South African Avocado Growers' Association Yearbook* 19, 49-51.

BOWEN, P.A., MENZIES, J., EHRET, D., SAMUELS, L. & GLASS, A.D.M., 1992. Soluble silicon sprays inhibit powdery mildew development on grape leaves. *J. Amer. Soc. Hort. Sci.* 117(6), 906-912.

CASALE, W.L., 1990. Analysis of suppressive soils and development of biological control methods for *Phytophthora* root rot of avocado. *California Avocado Society Yearbook* 74, 53-56.

CHANG, C-F., ISOGAI, A., KAMIKADO, T., MURAKOSHI, S., SAKURAI, A. & TAMURA, S., 1975. Isolation and structure elucidation of growth inhibitors for silkworm larvae from avocado leaves. *J. Agric Biol. Chem*. 39, 1167-68.

CHÉRIF, M., ASSELIN, A. & BÉLANGER, R.R., 1994. Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathol.* 84(3), 236-242.

CHÉRIF, M., BENHAMOU, N., MENZIES, J.G. & BÉLANGER, R.R., 1992. Silicon induced resistance in cucumber plants against *Pythium ultimum*. *Physiol. and Mol. Plant Pathol.* 41, 411-425.

COFFEY, M.D., 1987. *Phytophthora* root rot of avocado: An integrated approach to control in California. *Plant Dis.* 71, 1046-1052.

DATNOFF, L.E., DEREN, C.W. & SNYDER, G.H., 1997. Silicon fertilisation for disease management of rice in Florida. *Crop Prot.* 16(6), 525-531.

DUVENHAGE, J.A., 1994. Monitoring the resistance of *Phytophthora cinnamomi* to fosetyl-Al and H_3PO_3. *South African Avocado Growers' Association Yearbook* 17, 35-37.

DUVENHAGE, J.A., 1999. Biological and chemical control of root rot. *South African Avocado Growers' Association Yearbook* 22, 115-119.

DUVENHAGE, J.A., 2002. Evaluation of new generation fungicides for control of *Cercospora* spot on avocado fruit. *South African Avocado Growers Association Yearbook* 25, 10-13.

DUVENHAGE, J.A. & KOTZE, J.M., 1993. Biocontrol of root rot of avocado seedlings. *South African Avocado Growers' Association Yearbook* 16, 70-72.

EPSTEIN, E., 1999. Silicon. *Ann. Rev. Plant Physiol. Plant Mol. Biol*. 50, 641-664.

FAUTEUX, F., RÉMUS-BOREL, W., MENZIES, J.G. & BÉLANGER, R.R., 2005. Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiol. Letters* 249, 1-6.

FAWE, A., MENZIES, J.G., CHÉRIF, M. & BÉLANGER, R.R., 2001. Silicon and disease resistance in dicotyledons. In: L.E. Datnoff, G.H. Snyder and G.H. Korndorfer (Eds.), Silicon in Agriculture, Elsevier Science. B.V., Amsterdam, pp 159-170.

GONG, H., ZHU, X., CHEN, K., WANG, S. AND ZHANG, C., 2005. Silicon alleviated oxidative damage of wheat plants in pots under drought. *Plant Sci.* 169, 313-321.

HARDY, G.E. St.J., BARRETT, S. & SHEARER, B.L., 2001. The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Austr. Plant Pathol*. 30, 133-139.

KAISER, C., 1993. Some physiological aspects of delayed harvest of 'Hass' avocado (*Persea americana* Mill.) in the Natal midlands. MSc thesis, Department of Horticulture, Pietermaritzburg, South Africa, pp 97.

KNIGHT, R.J. Jr., 2002. History, distribution and uses. In: A.W. Whiley, B. Schaffer, & B.N. Wolstenholme (Eds.), Avocado: Botany, Production and Uses, CABI-Publishing, pp 432.

LOVEGROVE, A. & HOOLEY, R., 2000. Gibberellin and abscisic acid signalling in aleurone. *Trends in Plant Sci*. 5(3), 102-110.

MA, J.F. & TAKAHASHI, E., 2002. Soil, fertilizer, and plant silicon research in Japan. Elsevier, Amsterdam, pp 4-105.

McLEOD, A., LABUSCHAGNE, N. & KOTZE, J.M., 1995. Evaluation of *Trichoderma* for biological control of avocado root rot in bark medium artificially infected with

Phytophthora cinnamomi. *South African Avocado Growers' Association Yearbook* 18, 32-37.

MENZIES, J., BOWEN, P.A., EHRET, D. & GLASS, A.D.M., 1992. Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber, muskmelon, and zucchini squash. *J. Amer. Soc. Hort. Sci.* 117 (6), 902-905.

OHR, H.D. & ZENTMYER, G.A., 1991. Avocado root rot. University of California Publication 2440.

PEGG, K.G., 1977. Soil application of elemental sulphur as a control of *Phytophthora cinnamomi* root rot of pineapple. *Aust. J. Exp. Agric. An. Husb*. 17, 859-865.

PRUSKY, D., KEEN, N.T., SIMS, J.J. & MIDLAND, S.L., 1982. Possible involvement of an antifungal diene in the latency of *Colletotrichum gloeosporioides* on unripe avocado fruits. *Phytopath*. 72 (12), 1578-1582.

REMUS-BOREL, W., MENZIES, J.G. & BĖLANGER, R.R., 2005. Silicon induces antifungal compounds in powdery mildew-infected wheat. *Physiol. Mol. Plant Pathol*. 66, 108-115.

RETIEF, W., 2007. SAAGA technical data, unpublished.

SAMUELS, A.L., GLASS, A.D.M., EHRET, D.L. & MENZIES, J.G., 1993. The effects of silicon supplementation on cucumber fruit: Changes in surface characteristics. *Ann. Bot.* 72, 433-440.

SCHOEMAN, M.H. & MANICOM, B.Q., 2002. An evaluation of spray programs for the control of *Colletotrichum* spots of Hass and Pinkerton avocado. *South African Avocado Growers' Association Yearbook* 25, 6-9.

SEEBOLD, K.W. JR., DATNOFF, L.E., CORREA-VICTORIA, F.J., KUCHAREK, T.A. & SNYDER, G.H., 2004. Effects of silicon and fungicides on the control of leaf and neck blast in upland rice. *Plant Dis.* 88(3), 253-258.

WEHNER, F.C. & APOSTOLIDES, Z., 1981. Fungitoxic chemical substances in avocados. South African Avocado Grower's Association Yearbook 4, 92-94.

WHILEY, A.W., SMITH, T.E., WOLSTENHOLME, B.N. & SARANAH, J.B., 1996. Boron nutrition of avocados. *South African Avocado Growers' Association Yearbook* 19, 1-7.

WINGATE, V.P.M., LAWTON, M.A. & LAMB, C.J., 1988. Glutatione causes a massive and selective induction of plant defence genes. *Plant Physiol*. 87, 206-210.

ZAKI, A.I., ZENTMYER, G.A., PETTUS, J., SIMS, J.J., KEEN, N.T. & SING, V.O., 1980. Borbonol from *Persea* spp. - chemical properties and antifungal activity against *Phytophthora cinnamomi. Physiol. Plant Path*. 16, 205-212.

APPENDIX A

Greenhouse environmental data

Experiment 1

Trees were grown in a controlled environment greenhouse under diurnal temperature fluctuations of 7º to 32ºC and relative humidity between 30 and 90%.

Experiment 2 & 3

Table A 1: Environmental data presented as monthly averages collected at the height of the pot rim from the glasshouse for experiments 2 & 3.

	Av. Temp.	**Max temp**	**Min temp**	**RH**	**Dew point**	**Light intensity**
April	20.06	36.94	11.31	9.70	9.01	152.37
May	20.58	28.19	12.15	8.44	6.21	80.60
June	22.01	25.04	21.78	12.58	5.30	33.63
July	26.93	21.69	30.50	16.59	5.63	74.92
August	34.67	25.51	26.42	14.31	5.52	148.64
September	34.67	25.51	26.42	14.52	7.82	174.56
October	33.39	25.92	25.56	15.43	23.79	281.78
November	25.14	30.57	19.54	11.80	4.06	25.08

Experiment 4

Table A 2: Environmental data presented as monthly averages collected at the height of the pot rim the glasshouse for experiments 4.

	Av. Temp	**Max temp**	**Min temp**	**RH**	**Dew point**	**Light intensity**
April	21.60	26.83	18.28	9.49	8.86	30.60
May	20.45	23.35	16.71	12.26	7.79	29.28
June	21.08	26.34	14.56	9.69	6.12	47.08
July	26.93	28.72	16.44	9.56	5.63	74.92
August	34.67	30.55	16.34	9.27	5.52	148.64
September	34.67	30.55	16.34	9.27	5.52	148.65
October	33.29	30.00	17.10	9.60	5.46	144.92
November	23.73	29.30	18.24	10.36	5.58	150.86

APPENDIX B

D	E	A	B	A	C	D	B	C	E
C	B	D	E	B	E	A	C	D	A
B	D	C	D	A	E	A	B	E	C
B	E	B	D	D	C	A	C	E	A
B	D	B	A	C	A	E	D	E	C

A Potassium phosphonate (Avoguard®)

B Si x 1

C Si x 2

D Si x 3

E Control

Figure B 1: The randomized block resign of avocado trees treated with potassium silicate as a soil drench to inhibit *Phytophthora cinnamomi* disease severity. Treatments consisted of either one (Si x 1), two (Si x 2) or tree (Si x 3) potassium silicate soil drench applications; trees injected with potassium phosphonate (Avoguard®) (PA) and trees receiving no treatment (control)

C	B	D	A	A
A	D	B	C	B
D	A	C	D	C
B	C	A	B	D

A Potassium phosphonate (Avoguard®)

B KOH

C 0.74ml Si

D 20ml Si

Figure B 2: The randomized block resign of avocado trees treated with potassium silicate as a stem injection to inhibit *Phytophthora cinnamomi* disease severity.
Treatments consisted of biannual injections of either $0.74ml.l^{-1}$ or $20ml.l^{-1}$ potassium silicate solutions (20.7% silicon dioxide); a KOH solution at pH 10.35 or potassium phosphonate (Avoguard®) (PA).

APPENDIX C

Date	Product	Active ingredient		Dosage
17/02/2003	Demildex	copper oxychloride	0.3 kg	/100 L Hi
25/08/2003	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
7/11/2003	Avoguard 500SL	Phosphorous acid	0.05 ml	/ha
12/11/2003	Demildex	copper oxychloride	0.3 kg	/100 L Hi
2/02/2004	Demildex	copper oxychloride	0.3 kg	/100 L Hi
9/02/2004	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
4/08/2004	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
9/11/2004	Demildex	copper oxychloride	0.3 kg	/100 L Hi
29/11/2004	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
21/12/2004	Demildex	copper oxychloride	0.3 kg	/100 L Hi
21/01/2005	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
16/11/2005	Avoguard 500SL	Phosphorous acid	0.08 ml	/plant
21/01/2006	Demildex	copper oxychloride	0.3 kg	/100 L Hi
16/02/2006	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant

Table C 1: Standard spraying program implemented in the orchards used the a field trial to determine the efficacy of potassium silicate as either a sol drench or stem injection to inhibit *Phytophthora cinnamomi* disease severity.

APPENDIX D

Figure D 1: Ciba Geigy avocado tree rating scale from 0 to 10 where 0 = healthy looking tree and 10 = dead tree.

EFFICACY OF WATER SOLUBLE SILICON FOR CONTROL OF *PHYTOPHTHORA CINNAMOMI* ROOT ROT OF AVOCADO

by

Theo Frederik Bekker

PROMOTOR	:	Prof. N. Labuschagne
CO-SUPERVISOR	:	Prof. T. Aveling
		Dr. J.H. v.d. Waals
		Dr. C. Kaiser
DEPARTMENT	:	Plant Production and Soil Science
DEGREE	:	MSc (Agric) Horticulture

RESUMÉ

In the current study potassium silicate (20.7% SiO_2) induced a 100% inhibition of *P. cinnamomi* mycelial growth at all concentrations tested. Total inhibition for all fungi tested (*Alternaria solani, Colletotrichum gloeosporioides, Curvularia lunata, Drechslera* sp., *Fusarium oxysporum, Fusarium solani, Glomerella cingulata, Lasiodiplodia theobromae, Mucor pusillus, Natrassia* sp., *Pestalotiopsis maculans, Phomopsis perniciosa, Phytophthora capsicii, Phytophthora cinnamomi, Pythium* F-group, *Sclerotinia sclerotiorum, Sclerotium rolfsii, Stemphylium herbarum* and *Verticillium tricorpus*) was attained at a concentration of 40ml.l^{-1} and higher. Although the high pH of potassium silicate solutions does contribute to the inhibition of fungal growth, the inhibitory effect of potassium silicate on fungal growth *in vitro* is mostly fungicidal rather than attributed to a pH effect. Phytophthora root rot of avocado nursery trees can be inhibited successfully by potassium silicate application. The effectiveness of potassium silicate application depends however on the repetition of applications. These findings are of paramount importance as this implies that potassium silicate may be a alternative control measure to inhibit the effects of *P. cinnamomi* on avocado nursery

trees. Silicon either stimulates plant growth or imparts some form of protection to avocado roots if applied prior to *P. cinnamomi* inoculation.

Potassium silicate applied as a soil drench resulted in higher root densities compared to that of potassium phosphonate (Avoguard®) injections and untreated control trees. Reapplication again resulted in the best disease suppression and stimulation of new root growth. These results correlated well with tree canopy ratings, as trees that received silicon frequently, showed better canopy conditions compared to the untreated control treatments. Potassium silicate application leads to effective inhibition of *Phytophthora cinnamomi* infection in avocado orchards.

Potassium silicate application resulted in an increase of crude phenols and phenolic polymers in avocado roots cells to similar levels to that obtained in roots from potassium phosphonate (Avoguard®) treated trees. Potassium silicate application leads to lower cell wall bound phenolics.

The results of the current study support the hypothesis that silicon application, through an elevation of the total phenolic levels, causes an increase of resistance against *P. cinnamomi* root rot in avocados.

EFFEKTIWITEIT VAN WATER OPLOSBARE SILIKON VIR *PHYTOPHTHORA CINNAMOMI* WORTEL VROT BEHEER IN AVOKADO

deur

Theo Frederik Bekker

LEIER	:	Prof. N. Labuschagne
MEDELEIERS	:	Prof. T. Aveling
		Dr. J.H. v.d. Waals
		Dr. C. Kaiser
DEPARTEMENT	:	Plantproduksie en Grondkunde
GRAAD	:	MSc (Agric) Tuinboukunde

SAMEVATTING

Kaliumsilikaat (20.7% SiO_2) induseer ‘n 100% inhibisie van *P. cinnamomi* groei by alle getoetste konsentrasies. Totale inhibisie van alle swamme getoets (*Alternaria solani, Colletotrichum gloeosporioides, Curvularia lunata, Drechslera* sp., *Fusarium oxysporum, Fusarium solani, Glomerella cingulata, Lasiodiplodia theobromae, Mucor pusillus, Natrassia* sp., *Pestalotiopsis maculans, Phomopsis perniciosa, Phytophthora capsicii, Phytophthora cinnamomi, Pythium* F-groep, *Sclerotinia sclerotiorum, Sclerotium rolfsii, Stemphylium herbarum* en *Verticillium tricorpus*) was verkry by ‘n konsentrasie van 40ml.l^{-1} en hoër. Alhoewel die hoë pH van kaliumsilikaat wel ‘n inhiberende uitwerking het op swamgroei, is die suksesvolle inhibisie van swamgroei grootliks toe te skryf aan die swamwerende effek van kaliumsilikaat op swamgroei *in vitro* eerder as ‘n pH effek.

Phytophthora wortelvrot van avokado kwekelinge kan suksesvol onderdruk word met kaliumsilikaat toediening. Die effektiwiteit van die toediening hang wel af van die hertoediening daarvan. Hierdie bevindinge is van kardinale belang aangesien dit impliseer dat kalium silikaat ‘n alternatiewe beheer middel is om *Phytophthora*

wortelvrot te inhibeer in avokado kwekelinge. Of silikon stimuleer plant groei, of dit induseer ‘n vorm van beskerming in avokado wortels voor infeksie plaasvind.

Kaliumsilikaat toediening as ‘n grond-benatter lei tot hoër worteldigthede in vergelyking met kaliumfosfaat (Avoguard®) staminspuitings en onbehandelde kontrole bome. Hertoediening lewer die beste resultate, maar drie toedienings per seisoen is voldoende. Worteldigdheid verhoging na silikaat toedienings korreleer goed met blaredak gesondheid, aangesien bome wat gereeld behandel is met silikon beter blaredekking getoon het in vergelyking met die kontrole bome. Kaliumsilikaat toediening lei to effektiewe inhibisie van *Phytophthora cinnamomi* infeksie in avokado boorde.

Kaliumsilikaat toediening lei tot ‘n toename in totale fenole en fenoliese polimere in avokado wortel selle tot soortgelyke vlakke soos gevind in avokado weefsel vanaf kaliumfosfaat behandelde bome. terselfdertyd lei silikaat toediening tot laer selwand gebinde fenole.

Hierdie resultate ondersteun die hipotese dat kaliumsilikaat toediening, deur die verhoging van oplosbare fenole in avokado wortelselle, die plant se weerstand verhoog, en die effek wat *Phytophthora* wortelvrot het op avokado plante inhibeer.