Accumulation of total phenolics due to silicon application in roots of avocado trees infected with *Phytophthora cinnamomi*

T F Bekker[1], N Labuschagne[2], T Aveling[2], C Kaiser[1] and T Regnier[2]

[1]Department of Plant Production and Soil Science,
[2]Department of Microbiology and Plant Pathology,
University of Pretoria, Pretoria 0002, South Africa

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 (133.66 µg.l^{-1}; 109.08 µg.l^{-1}) resulted in higher crude phenolic levels compared to Si x 3 (94.61 µg.l^{-1}; 67.98 µg.l^{-1}). Significantly higher crude phenolic concentrations in avocado roots were obtained in Si x 3 during March and May 2006 (94.61 µg.l^{-1}; 67.98 µg.l^{-1}) when compared to potassium phosphonate (Avoguard®) (49.07 µg.l^{-1}; 59.46 µg.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 (0.71 µg.l^{-1}) than the control (0.36 µg.l^{-1}). Three silicon treatments per season resulted in significantly lower cell wall bound phenols during May, July and Sept 2005 and May 2006 compared to Si x 1, with higher concentrations obtained in Si x 3 only during Jan 2006 (0.71 µg.l^{-1} vs. 0.38 µg.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.

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-hen-eicosa-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, con-

centration (Karni *et al.,* 1989; Prusky *et al.,* 1991). Exposure of avocado fruit to CO_2 for 24 h 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 µg.g^{-1}), Pinkerton, Fuerte, Duke 7 and Edranol (0.4 µg.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.

MATERIALS AND METHODS

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).

Experimental layout

An avocado orchard (latitude 23° 43' 60S; longitude 30° 10' 0E) at an altitude of 847 m was selected in the Tzaneen area, South Africa. Trees consisted of thirteen-year-old "Hass" on "Duke7" seedling rootstocks planted at a density of 204 trees.ha^{-1} (7 x 7 m 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.

Treatments

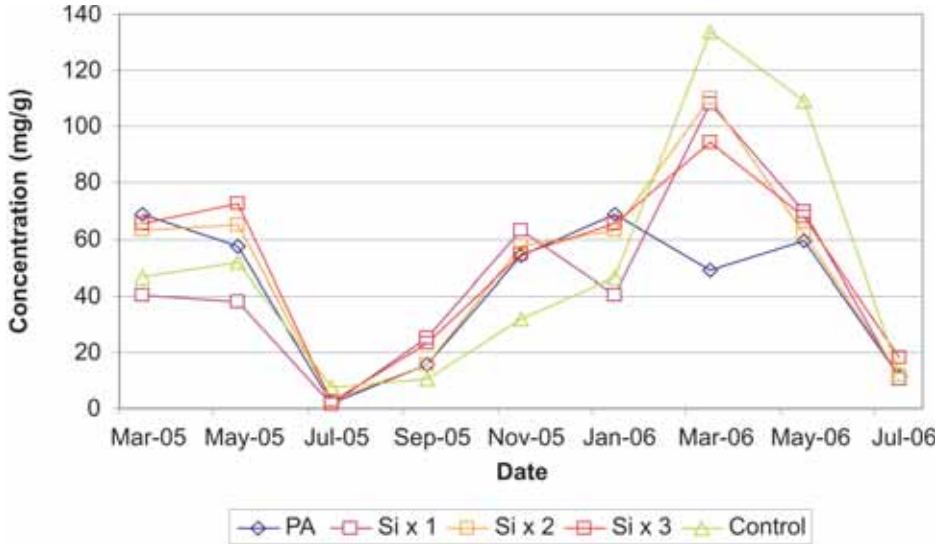
Treatments consisted of a soil drench with a 20 litre solution of 20 ml.l^{-1} soluble potassium silicate (20.7% silicon dioxide) (Bekker *et al*, 2006) per tree either once, twice or three times in a growing season. Trees injected with potassium phosphonate (Avoguard®) were incorporated as a standard 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.

Extraction and quantification of total phenolic compounds

Root samples were freeze dried for 120 h. 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 0.05 g powdered plant sample, ultrasonicated for 5 min by means of a VWR ultrasonic bath, and centrifuged at 24 000 g for 1 min. 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 0.5 ml chloroform to the supernatant, shaking it for 30 s and thereafter centrifuging it for 30 s. The organic solvent mixture was evaporated in a laminar flow cabinet at room temperature, whereafter the residue was dissolved in 1 ml distilled water. Crude samples were stored at 4°C until extraction.

Non-conjugated phenolic acids

An aliquot of 0.25 ml from the crude sample for total soluble phenolic determination was acidified by addition of 25 µl 1M HCl before extraction with 1 ml anhydrous diethyl ether. The ether extract was reduced to dryness at 4°C and the resulting pre-



	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 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.

cipitate was resuspended in 0.25 ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

Glycoside-bound phenolic acids

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

Ester-bound phenolic acids

Extraction of soluble ester-bound phenolics took place after hydrolysis under mild conditions. To an aliquot of 0.25 ml for total soluble phenolic determination, 0.1 ml 2M NaOH was added and the solutions were allowed to stand in the Eppendorf tubes for 3 h at room temperature. After hydrolysis 40 µl 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 0.25 ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

Cell wall-bound phenolic acids

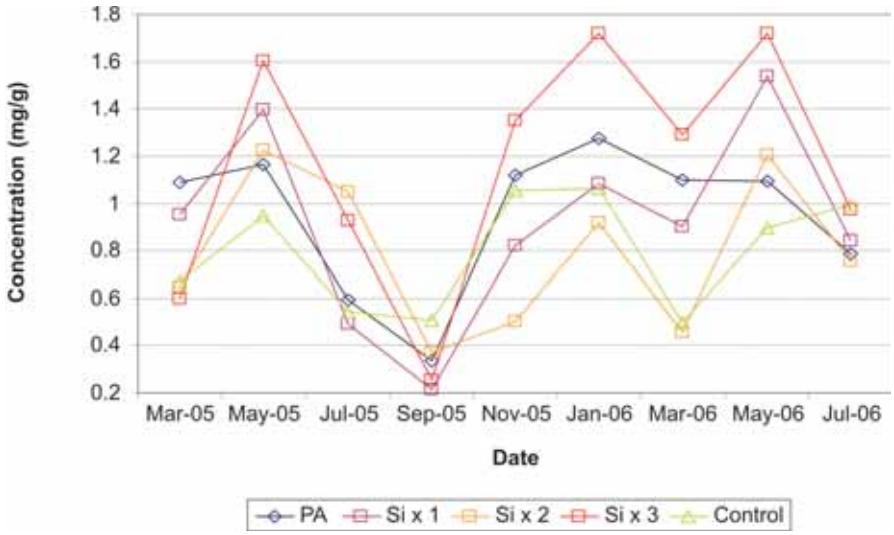
The solid material left in the Eppendorf tube after extraction was dried, weighed and resuspended in 0.5M NaOH for 1 h at 96°C. Cell wall esterified hydroxycinnamic acid derivatives were selectively released under these mild saponification conditions. The supernatant was acidified to pH 2 with HCl, centrifuged at 12 000 g for 5 min and then extracted with anhydrous diethyl ether. The extract was reduced to dryness and the precipitate was resuspended in 0.25 ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

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 – 1000 µg.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: 170 µl distilled water, 5 µl standard or plant extract sample, 50 µl 20% (v/v) Na_2CO_3 and 25 µl folin-ciocalteau reagent. After incubation at 40°C for 30 min the absorbance was read at 720 nm 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.

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, 340 nm). A Luna 3u C-18 (Phenomenex®) reverse phase column (250 mm length, 5 µm particle size, 4.6 mm inner diameter) was used. An excess injection volume of 50 µl of each sample was used in a 20 µl loop. A gradient elution was performed with water (pH 2.6 adjusted with H_3PO_4) and acetonitrile (ACN) as follows: 0 min, 7% ACN; 0 – 20 min, 20% ACN; 20 – 28 min, 23% ACN; 28 – 40 min, 27%, ACN; 40 – 45 min, 29%, ACN; 45 – 47 min, 33%, ACN; 47 – 50 min, 80%. The flow rate was 0.7 ml.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 10 min. The detector was programmed for peak detection at 280 nm, 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).



	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 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.

Statistical analysis

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

RESULTS AND DISCUSSION

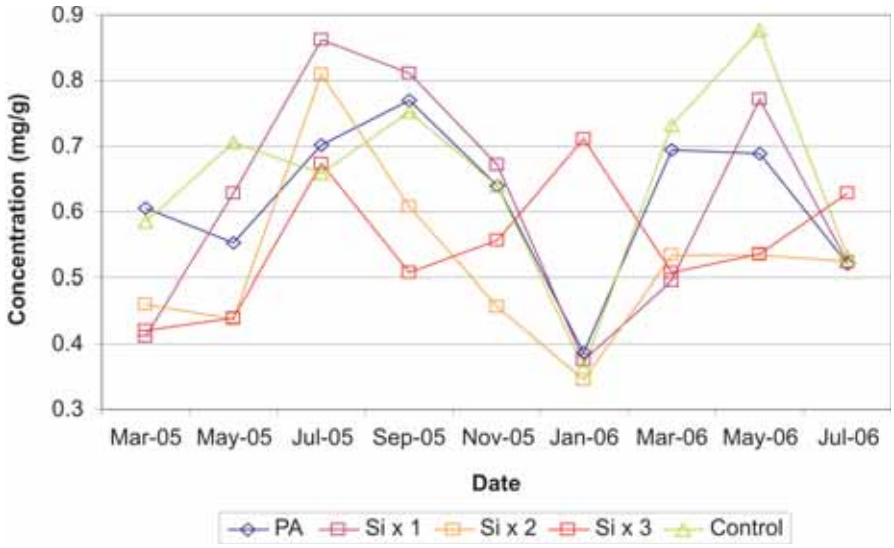
Extraction of phenolics in the present study

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 1**). From March to May 2006, the control treatment (133.66 µg.l^{-1}; 109.08 µg.l^{-1}) resulted in higher crude phenolic levels compared to Si x 3 (94.61 µg.l^{-1}; 67.98 µg.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 (94.61 µg.l^{-1}; 67.98 µg.l^{-1}) compared to potassium phosphonate (Avoguard®) (49.07 µg.l^{-1}; 59.46 µg.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 July 2006.

Glucoside bound phenolic acid concentrations (**Figure 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 treatments 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 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 (0.71 µg.l^{-1}) than the control (0.36 µg.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, July and Sept 2005 and May 2006 compared to Si x 1, with higher concentrations obtained in Si x 3 only during Jan 2006 (0.71 µg.l^{-1} vs. 0.38 µg.l^{-1}). No significant difference was obtained between Si x 1 and Si x 3, except during Jan 2006, when Si x 3 (0.71 µg.l^{-1}) resulted in higher cell wall bound phenols compared to Si x 2 (0.35 µg.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



	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 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.

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.

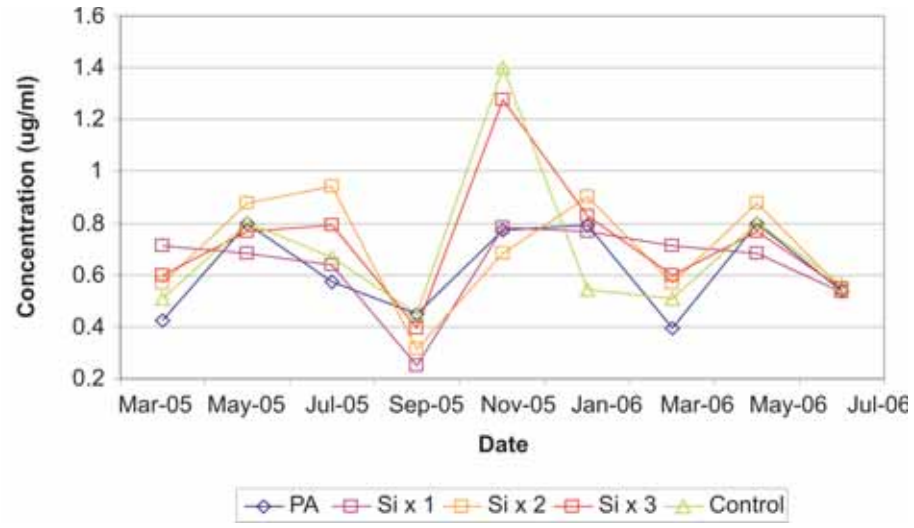
No significant differences were seen between treatments for ester bound phenolic concentrations throughout the duration of the trial (**Figure 4**).

Non-conjugated phenolic concentrations did not differ significantly between treatments during March, July and Sept 2005, and Jan and March 2006. Three silicon applications per season (1.62 $\mu g.l^{-1}$) and potassium phosphonate (Avoguard®) (2.44 $\mu g.l^{-1}$) resulted in significantly lower non-conjugated phenol concentrations compared to that of the control (2.80 $\mu g.l^{-1}$) only during Nov 2005 (**Figure 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 plant's 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 host's 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).



	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 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.

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 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).

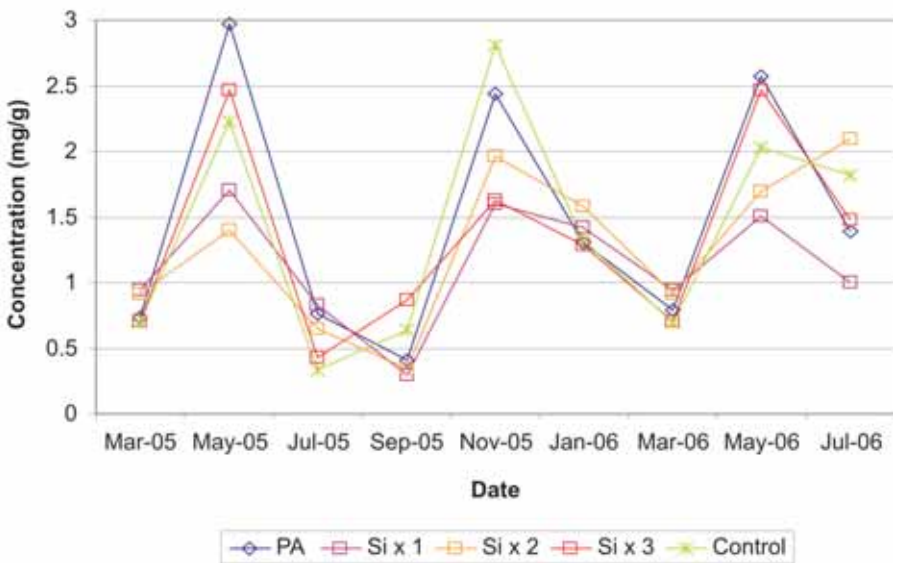
CONCLUSION

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.

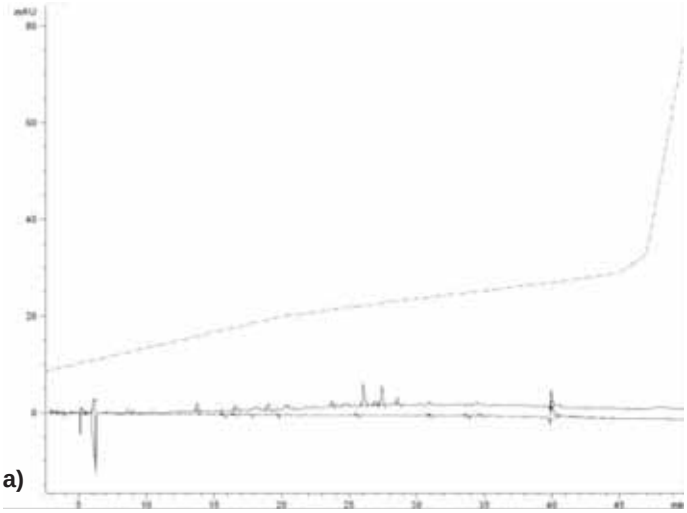
In this study the potassium silicate application lead to lower cell wall bound phenolics. The possibility that silicon replaces phenol-binding molecules is not fully understood. However, this study indicates that the accumulation of silicon was subsequent to phenol appearance in infected tissue; challenging 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.

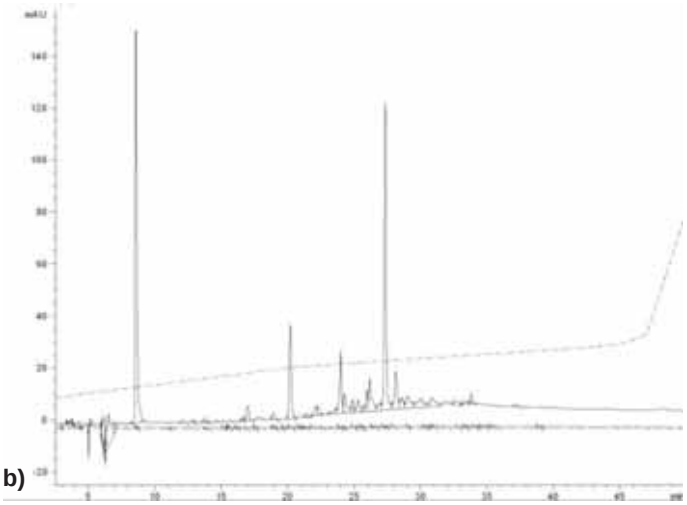


	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	0.73a	2.98b	0.76a	0.41a	2.44ab	1.31a	0.79a	2.58b	1.39ab
Si x 1	0.94a	1.71ab	0.83a	0.29a	1.60a	1.42a	0.94a	1.51a	0.99a
Si x 2	0.91a	1.39a	0.64a	0.35a	1.96ab	1.58a	0.91a	1.69ab	2.09b
Si x 3	0.70a	2.46b	0.43a	0.86a	1.62a	1.28a	0.70a	2.46b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

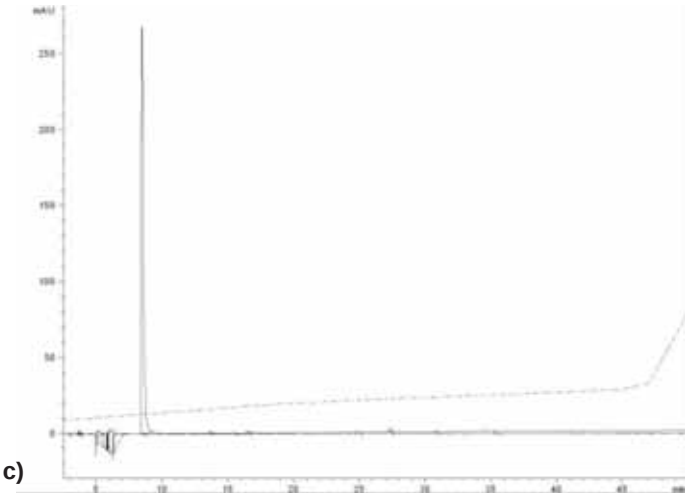
Figure 5: Total concentration of non-conjugated phenolics acid after hydrolysis of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 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.



a)



b)



c)

Figure 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 (c).

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