Effects of soil drenching of water soluble potassium silicate on commercial avocado (*Persea americana* Mill.) orchard trees infected with Phytophthora root rot on root density, canopy health, induction and concentration of phenolic compounds

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Abstract.

Avocado root rot, caused by *Phytophthora cinnamomi* Rands, remains a major constraint to avocado production worldwide. In the current study effects of successive soil drench applications of soluble potassium silicate on canopy health and root density of thirteen-year-old *Persea americana* Mill. trees infected with *P. cinnamomi* were investigated. Soil drenching with 20 l per tree of a 20 $ml.l^{-1}$ soluble potassium silicate solution (20.7% silicon dioxide) resulted in significantly higher root density when compared to untreated control trees, and trees injected with potassium phosphonate (Avoguard®) during most but not all evaluation dates. Three successive drenches of soluble potassium silicate resulted in the most significant increase in root density. A similar effect was seen on canopy health. In general, total soluble phenolic concentrations were significantly higher between March 2005 and Jan 2006 in those trees drenched three times with soluble potassium silicate per growing season

(up to 72.62 µg.l^{-1}) compared to trees injected twice with potassium phosphonate per growing season (up to 68.77 µg. l^{-1}) and untreated control trees (51.62 µg. l^{-1}). This evidence suggests that multiple or even continuous applications of soluble potassium silicate to avocado trees will be required to effectively suppress *Phytophthora cinnamomi* over the entire growing season.

Keywords:, *avocado root rot,* Phytophthora cinnamomi, *potassium silicate.*

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Root rot of avocado, caused by *Phytophthora cinnamomi* Rands, poses a threat to avocado production worldwide (Hardy *et al.* 2001, Pegg *et al.* 2002, Zentmyer *et al.* 1994). Currently, avocado root rot management relies heavily on rootstock tolerance (Coffey, 1987) and chemical control (Hardy *et al.* 2001). Excessive reliance on phosphonate fungicides for control of the disease and the potential for the pathogen to develop resistance to the fungicides is a major concern for the avocado industry worldwide and every effort must be made to find alternatives. *In vitro* studies have shown that soluble silicon is capable of suppressing a range of plant pathogenic fungi of commercial significance (Bekker *et al.* 2006, Kaiser *et al.* 2011). *In vivo* studies have also demonstrated this effect on powdery mildews of grape leaves, cucumber, muskmelon, zucchini squash and mousseear cress (Bowen *et al.* 1992, Fauteux *et al.* 2006, Ghanmi *et al.* 2004, Menzies *et al.* 1991) as well as downy mildew and frog's eye spot in soybean (Nolla *et al.* 2006), post-harvest diseases of melons (Bi *et al.* 2006), avocado (Anderson *et al.* 2004, 2005) and root diseases such as *Pythium* spp. in cucumbers (Cherif *et al.* 1992a, 1994). Suppression of avocado root rot by soil drench application of potassium silicate was also demonstrated on avocado seedlings under greenhouse conditions (Bekker, 2007).

Regarding the mechanism by which soluble silicon inhibits plant diseases, several studies have reported *in vitro* inhibition of mycelial growth of phytopathogenic fungi by silicon (Abdel-Farid *et al.* 2009, Bi *et al.* 2006) and Qin and Tian (2005) also reported that Si inhibited spore germination and germ tube elongation of *Penicillium expansum* and *Monilinia fructicola in vitro*. Apart from direct *in vitro* inhibition of fungal growth by silicon, elicitor responses, or induced resistance have also been suggested as a mechanism of control in a range of crops (Cherif *et al.* 1992b, Remus-Borel *et al.* 2005, Rodriques *et al.* 2004). The correlation between disease resistance in plants and associated increased production of phenolic compounds was reviewed by Nicholson and Hammerschmidt (1992) and there are numerous reports on the matter (Cherif *et al.* 1994, Del Rio et al. 2003, Mandal *et al.* 2009). Indeed, Fawe *et al.* (1998) presented conclusive evidence that silicon fertilization results in increased resistance of cucumber to powdery mildew by activating low-molecular-weight metabolites, including the phytoalexin flavonol, aglycone rhamnetin. Similarly, Zhang et al (2013) reported on stimulation of phenolic metabolism by silicon contributing to rice resistance to sheath blight.

A previous study conducted on root and hypocotyl infection of cucumber plants by *Pythium ultimum* (Cherif *et al.* 1992b) demonstrated the inhibitory effects on *P. ultimum* attack where soluble silicon applications alone resulted in a simultaneous accumulation of an electron-dense phenolic-like material, which resulted in damage to the invading pathogen hyphae in infected host tissues. Similar observations were reported for powdery mildew on *Arabidopsis thaliana* (Ghanmi *et al.* 2004) and of rice blast, caused by *Magnaporte grisea* on rice plants (Rodriguez *et al.* 2004). In a recent review Van Bockhaven et.al. (2013) proposed five silicon–induced regulatory mechanisms that might account for broad spectrum plant disease resistance. One of these is the concept of silicon priming the plant's own battery of

defence mechanisms, resulting in rapid deployment of these only when attacked by a pathogen.

The current study was initiated to investigate the effects of soil drench applications of soluble potassium silicate on root density and tree canopy conditions of avocado trees in a commercial orchard infected with *P. cinnamomi* in relation to elevation of a range of different phenolic compounds and their concentrations in avocado roots. In this context, efficacy of silicon applications is also compared to that of potassium phosphonate.

Materials and Methods

A thirteen-year-old 'Hass' on 'Duke 7' rootstock avocado orchard in a summer rainfall area growing in a sandy-clay loam was selected for the study. The orchard was established in a warm subtropical area at an altitude of 847 m above sea level on a south-facing slope in the Tzaneen area, South Africa (latitude 23° 43' 60S; longitude 30°10'0E). Trees were planted at a density of 204 trees.ha^{-1} and were heavily infested with *Phytophthora cinnamomi* root rot at the outset of the trial. The presence of *P. cinnamomi* in the soil was confirmed using the citrus leaf baiting technique (Grimm *et al.* 1973) and pathogen virulence was verified on avocado nursery trees before the trial was initiated in July 2004. At the commencement of the trial, all trees were similar in their canopy disease ratings, being between 3.3 and 3.5 according to the Ciba-Geigy disease rating scale of 0 to 10 with 0 = healthy-looking tree and 10 = dead tree (Darvas *et al.* 1984).

The trial consisted of five treatments with 10 trees per treatment laid out in a completely randomized block design comprising five blocks and two replicates per treatment per block with each tree representing one replication. Standard management practices, including irrigation, fertigation and understory weed management, were performed in the orchard.

The five treatments were as follows: 1) one application of a soluble silicon soil drench (designated SilX1) applied at a rate of 20L per tree using a 20 ml.L^{-1}soluble potassium silicate solution (20.7% silicon dioxide) applied evenly to 20 m² (5 m in the row by 2 m on either side of the tree) soil surface under the tree canopy; 2) two consecutive applications of soluble silicon soil drenches (designated SilX2) each applied at the same rate as in treatment 1, applied four months apart; 3) three consecutive applications of soluble silicon soil drenches (designated SilX3) each applied at the same rate as in Treatment 1, applied every four months from July 2004 to correspond with the growing season (see Table 1 for timings); 4) a total of two consecutive stem injections with potassium phosphonate (Avoguard®) at a rate of 10 g. L^{-1} per meter of canopy diameter (industry standard) applied at the end of the spring flush (Aug 2004 and Aug 2005) and again at the end of the summer flush (Jan 2005 and Jan 2006); and 5) untreated control trees. Data were collected from January 2005 to July 2006.

Canopy disease condition, using the Ciba Geigy rating scale, was rated bi-monthly from the beginning of the study. Root density was recorded bi-monthly according to the method described by Bekker (2007). Briefly, a 0.5m^2 soil surface area was demarcated 1m from the trunk of each tree and this area was covered with 10 sheets of newspaper mulch. Subsequently, feeder root growth underneath this mulch was photographed every second month with a Konica Minolta Dimage Z5 camera (5 megapixel, 35-420mm lens), and the total root surface area was determined by means of the computer software ImageJ 1.33u (Wayne Rasband, National Institutes of Health, USA). Root samples were taken bi-monthly on the northern side of the tree and transported to the laboratories at the University of Pretoria under refrigerated conditions where they were freeze-dried for 120h. Freeze-dried materials were ground with an IKA® A11 basic grinder (IKA Werke, GMBH & Co., KG, D-79219 Staufen) to a fine powder.

Table 1: Effects of soil drench applications of soluble potassium silicate on root density and canopy condition of avocado trees infected with *Phytophthora cinnamomi* in the field over two growing seasons (Jul 2004 to Jul 2006), compared to trees injected with potassium phosphonate (Avoguard®).

	Treatment	Date													
		Jul-04	Aug-04	Nov-04	Jan-05	Mar-05	May-05	Jul-05	Aug-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
	Potassium Silicate Soil drench[1]	SilX1		SilX2		SilX3		SilX1			SilX2		SilX3		
	K Phos[3]		End of Spring Flush (X1)		End of Summer Flush (X2)				End of Spring Flush (X1)			End of Summer Flush (X2)			
Canopy Disease Rating[2]	**Control**	-	-	-	4.10 a	4.05 a	3.50 a	5.10 a	-	6.10 a	5.55 a	4.30 a	3.15 a	3.50 a	3.15 a
	K Phos[3]	-	-	-	3.90 a	3.00 b	3.10 ab	4.35 b	-	5.85 b	5.35 a	4.35 a	2.90 a	2.70 b	2.95 ab
	SiX1	-	-	-	3.35 ab	3.30 b	3.15 ab	3.85 bc	-	4.35 c	5.05 a	3.60 b	2.45 ab	2.85 ab	2.55 ab
	SiX2	-	-	-	2.95 b	3.00 b	2.85 ab	3.60 c	-	4.1 c d	5.15 a	3.2 b c	2.15 b	2.70 b	2.40 b
	SiX3	-	-	-	2.80 b	2.95 b	2.55 b	2.90 d	-	3.40 d	4.15 b	2.80 c	2.35 ab	2.50 b	2.55 ab
Root Density (%)	**Control**	-	-	-	-	2.35 b	1.39 a	1.12 a	-	0.26 b	0.38 c	5.66 c	6.37 c	5.38 c	1.06 b
	K Phos[3]	-	-	-	-	2.16 b	2.65 a	3.22 b	-	0.20 b	0.31 c	5.04 c	8.38 b	6.85 bc	1.60 b
	SiX1	-	-	-	-	2.30 b	1.93 a	4.12 b	-	1.09 a	1.30 b	5.49 b	7.32 bc	7.39 b	2.48 a
	SiX2	-	-	-	-	4.45 a	2.46 a	3.16 ab	-	0.28 b	0.48 c	5.90 b	10.18 a	7.33 b	2.49 a
	SiX3	-	-	-	-	5.54 a	2.52 a	3.93 b	-	0.93 ab	3.98 a	9.62 a	10.82 a	9.65 a	3.06 a

1. SilX1= one soil drench application of silicon; SilX2= two successive soil drench applications of silicon and SilX3= three successive soil drench applications of silicon at a rate of 20 l per tree of 20 ml.l^{-1} soluble potassium silicate [20.7% silicon dioxide (W/V)].

2. Canopy condition was determined according to the Ciba-Geigy disease rating scale of 0-10 with 0=healthy tree and 10=dead tree.

3. K Phos = potassium phosphonate trunk injection at a rate of 10 g.L^{-1} per meter of canopy diameter at the end of the spring flush and again at the end of the summer flush.

Values in each column followed by different symbols indicate significant differences at P = 0.05 for canopy rating and root density respectively as determined by Duncan's Multiple Range Test .

Three separate extractions were performed on each sample. One milliliter of a cold mixture of methanol (MERCK analytical grade): acetone (MERCK analytical grade): water (Millipore Milli Q) (7:7:1, v:v:v) solution was added to 0.05g powdered plant sample, ultrasonicated for 5 min in a VWR ultrasonic bath, and centrifuged at 24000 g for 1 min. No antioxidants (ascorbic acid or $Na_2S_2O_5$) were added, as these would have interfered with total phenol determination (Regnier, 1994). This extraction procedure was performed twice, and the supernatant fractions pooled. Insoluble materials left in Eppendorf tubes after the two extractions were retained 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 30s followed by centrifugation for 30s at 3000 g. The organic solvent mixture was evaporated in a laminar flow cabinet at room temperature, after which the residue was dissolved in 1 ml distilled water. Crude samples were stored in a refrigerator at 4°C until extraction (Regnier 1994). Extraction of non-conjugated-, glycoside bound-, ester bound- and cell wall-bound phenolic acids were done according to the method described by De Ascensao and Dubery (2003).

Concentration of phenolic compounds in the various extracts was determined using Folin-Ciocalteau reagent (Merck) (Regnier 1994). The reaction volumes were reduced to enable use of 96-well ELISA plates for the quantification of phenolics. A dilution series (10 – 1000 $\mu g.ml^{-1}$ methanol) was used to prepare standard curves for ferullic 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 25 µl Folin-Ciocalteau reagent. After incubation at 40°C for 30 min the absorbance was read at 720 nm using an ELISA plate reader [Multiskan Ascent VI.24354 – 50973 (version 1.3.1) Ascent system software, Thermo Fisher Scientific Inc, Johannesburg, South Africa] . Spectrometric measurement of phenolic concentrations in

the various extracts was calculated from a standard curve ($y = 0.0013x + 0.0177$, $r^2 = 0.9982$) and expressed as μg gallic acid equivalent.g^{-1} (dry weight).

All data were analyzed using Genstat® 4.23 DE for Windows®. A General Analysis of Variance was performed for each data set and means compared using Duncans Multiple Range Test where appropriate. Standard errors of the means and LSDs at the 5% confidence level were also calculated.

Results and Discussion

Soil drenching with soluble potassium silicate resulted in an increase in root density (Table 1) when compared to Control trees and trees treated with potassium phosphonate, at most but not all, the assessment events. Multiple applications of silicate were more effective than a single application. The SilX3 and SilX2 treatments had significantly healthier canopies than those of trees injected with potassium phosphonate or untreated Control trees in January 2005 (Table 1). It was found that in the absence of any *Phytophthora* root rot treatment, significantly less healthy canopies were recorded after eight months. Evidence for this can be seen where untreated Controls (Rating 4.05) were significantly less healthy in March and July 2005 than all other treatments (Table 1). This effect was also evident in September 2005 when canopy health of untreated Control trees was at its worst (Rating 6.1) and this corresponded with the lowest rooting densities recorded during both growing seasons. Thereafter, canopy health began to recover in all treatments, which corresponded with the spring and summer flushes. Canopy health of trees receiving the Sil X2 treatment were significantly better from Jan 2006 (Rating 3.2) through Jul 2006 (Rating 2.4) compared to untreated Control trees (Ratings 4.3 to 3.15, respectively). Canopy health of trees receiving the SilX3 treatment, although slightly better (i.e. lower disease rating), were not significantly different from those receiving the SilX2 treatment. In comparison to root densities, it appears

that canopy disease ratings were not as accurate a measure of overall tree health and vigor, nevertheless, significant cyclical trends were also seen during the two growing seasons of this trial.

In March 2005 trees that received the Sil X2 or Sil X3 treatments had significantly higher root densities than did those with the Sil X1, Control- or potassium phosphonate treatments (Table 1). Root densities then decreased between May 2005 and September 2005 in all treatments, dropping as low as 0.2% in the potassium phosphonate treated trees, but began to recover in November 2005, and trees with Sil X3 treatments had significantly higher root densities than trees with all other treatments (all $\leq$1.3%). This result continued into January 2006. Those treeswhich had received the SilX3 and the SilX2 treatments over the two-year period had the highest rooting densities in March 2006. These treatments had significantly higher root densities than those trees treated with potassium phosphonate, the SilX1 treatment or the untreated Control, which had the lowest root density of all. The SilX3 treatment resulted in significantly higher rooting densities in March of both 2005 and 2006 compared to all other treatments. Root densities of the latter trees decreased after these peaks and reached their lowest level at the time of fruit set in September of 2005 and then steadily declined through Jul 2006 when the study was terminated. Root densities of trees injected with potassium phosphonate, peaked in May 2005 (2.65%) and March 2006 (8.38%) but also declined significantly to lows of 0.2% in Sep 2005 and 1.6% in July 2006, respectively (Table 1). Clearly, avocado root densities in trees infected with *P. cinnamomi* are cyclical in nature, regardless of treatment differences and this is linked to tree phenology. Furthermore, the SilX3 treatment consistently resulted in the greatest rooting densities compared to the other treatments.

Table 2: Effect of soil drench applications of soluble potassium silicate and potassium phosphonate stem injections on total soluble phenolic content in roots from avocado trees infected with *Phytophthora cinnamomi* in the field.

Treatment	Date								
	Mar-05	**May-05**	**Jul-05**	**Sep-05**	**Nov-05**	**Jan-06**	**Mar-06**	**May-06**	**Jul-06**
Control	46.34[b³]	51.62[b]	7.7[a]	10.41[b]	31.94[c]	46.34[b]	133.66[a]	109.08[a]	12.28[a]
K Phos²	67.77[a]	57.26a[b]	1.94[a]	15.81[ab]	53.94[b]	68.77[a]	49.07[c]	59.46[c]	11.25[a]
SiX1¹	45.42[b]	37.82[c]	1.66[a]	25.34[a]	62.94[a]	40.42[b]	108.23[a]	69.64[b]	10.61[a]
SiX2¹	63.38[a]	65.19[a]	2.93[a]	15.77[ab]	57.56[ab]	63.08[a]	110.25[a]	61.62[bc]	11.94[a]
SiX3¹	65.32[a]	72.62[a]	2.5[a]	23.18[a]	54.8[ab]	65.32[a]	94.61[b]	67.98[b]	17.92[a]

1. SilX1= one soil drench application of silicon; SilX2= two successive soil drench applications of silicon and SilX3= three successive soil drench applications of silicon at a rate of 20 l per tree of 20 $ml.l^{-1}$ soluble potassium silicate [20.7% silicon dioxide (W/V)].

2. Trees injected with 10 $g.L^{-1}$ potassium phosphonate (K Phos) per meter of canopy diameter at the end of the spring flush and again at the end of the summer flush.

3. Values are total soluble phenolic content expressed as µg gallic acid equivalent.g^{-1} of dry weight.

Values in each column followed by different symbols indicate significant differences at P = 0.05 as determined using Duncan's Multiple Range Test.

The allocation of soluble phenolics (free acids and esters) to the cell-wall is a mechanism enabling the plant to strengthen the hemicellulose matrix thereby decreasing the digestibility of the cells.

Table 3: Effect of soil drench applications of soluble potassium silicate and potassium phosphonate stem injections on Glucoside-bound phenolic acid content in roots from avocado trees infected with *Phytophthora cinnamomi* in the field.

Treatment	Date								
	Mar-05	**May-05**	**Jul-05**	**Sep-05**	**Nov-05**	**Jan-06**	**Mar-06**	**May-06**	**Jul-06**
Control	0.67^{b3}	0.95^{b}	0.54^{ab}	0.51^{a}	1.05^{a}	1.06^{b}	0.49^{b}	0.89^{b}	0.99^{a}
K Phos²	1.09^{a}	1.16^{ab}	0.59^{ab}	0.34^{a}	1.12^{a}	1.27^{ab}	1.09^{a}	1.09^{b}	0.79^{a}
SiX1¹	0.95^{ab}	1.39^{a}	0.49^{b}	0.21^{a}	0.82^{ab}	1.08^{b}	0.90^{a}	1.54^{a}	0.84^{a}
SiX2¹	0.65^{b}	1.23^{ab}	1.05^{a}	0.37^{a}	0.50^{b}	0.92^{b}	0.46^{b}	1.21^{ab}	0.75^{a}
SiX3¹	0.59^{b}	1.60^{a}	0.93^{a}	0.26^{a}	1.35^{a}	1.72^{a}	1.29^{a}	1.72^{a}	0.97^{a}

1. SilX1= one soil drench application of silicon; SilX2= two successive soil drench applications of silicon and SilX3= three successive soil drench applications of silicon at a rate of 20 l per tree of 20 ml.l^{-1} soluble potassium silicate [20.7% silicon dioxide (W/V)].

2. Trees injected with 10 g.L^{-1} potassium phosphonate (K Phos) per meter of canopy diameter at the end of the spring flush and again at the end of the summer flush.

3.Values are Glucoside-bound phenolic acid content expressed as µg gallic acid equivalent.g^{-1} of dry weight . Values in each column followed by different symbols indicate significant differences at P = 0.05 as determined using Duncan's Multiple Range Test.

Apart from total soluble phenolic content determination (Table 2), four targeted extractions were performed to obtain glycoside-bound phenolic acids (Table 3), cell wall-bound phenolic acids (Table 4), and non-conjugated phenolic acids (Table 5). There were no significant differences between any of the ester-bound phenolic acids; therefore, the latter results are not presented. 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 Zhou *et al.* 2004.

In March 2005 total soluble phenolic content (Table 2) was significantly higher in roots of trees injected with potassium phosphonate (67.77 $\mu g.l^{-1}$) and those trees receiving the SilX2 (63.38 $\mu g.l^{-1}$) and SilX3 (65.32 $\mu g.l^{-1}$) treatments when compared to roots from Control trees (43.34 $\mu g.l^{-1}$) or trees receiving the SilX1 treatment (45.42 $\mu g.l^{-1}$). Further extraction of phenolics showed that significantly higher concentration of glucoside-bound phenolics (Table 3) accounted for the elevated phenolic concentration (1.09 $\mu g.l^{-1}$) in roots from the potassium phosphonate treatment compared to the Control treatment (0.67 $\mu g.l^{-1}$). Total soluble phenolic content was again significantly higher in roots of trees of the SilX2 and SilX3 treatments (65.19 $\mu g.l^{-1}$ and 72.62 $\mu g.l^{-1}$) when compared to roots from Control trees (51.62 $\mu g.l^{-1}$) or SilX1 trees in May 2005 (Table 2). These elevated concentrations were not significantly higher than those of roots from trees injected with potassium phosphonate. Further extraction of phenolics showed that glucoside-bound phenolics (Table 3) were significantly higher in roots of SilX3 treated trees (1.60 $\mu g.l^{-1}$) when compared to the untreated Control trees (0.95 $\mu g.l^{-1}$). In contrast, cell wall bound phenolics (Table 4) were significantly lower in roots of SilX3 and SilX2 treated trees when compared to untreated Controls and SilX1 treated trees.

In May 2005 the roots of SilX1 treated trees had significantly lower total soluble phenolic content (Table 2) than the untreated Control, but despite this, glucoside-bound

Table 4: Effect of soil drench applications of soluble potassium silicate and potassium phosphonate stem injections on cell wall-bound phenolic acid content in avocado trees, infected with *Phytophthora cinnamomi* in the field .

Treatment	Date								
	Mar-05	**May-05**	**Jul-05**	**Sep-05**	**Nov-05**	**Jan-06**	**Mar-06**	**May-06**	**Jul-06**
Control	0.58^{ab3}	0.71^{a}	0.66^{b}	0.75^{a}	0.64^{a}	0.36^{b}	0.73^{a}	0.88^{a}	0.53^{a}
K Phos²	0.61^{a}	0.55^{ab}	0.70^{ab}	0.77^{a}	0.64^{a}	0.39^{b}	0.69^{a}	0.68^{ab}	0.52^{a}
SiX1¹	0.41^{b}	0.63^{a}	0.86^{a}	0.81^{a}	0.67^{a}	0.38^{b}	0.49^{b}	0.77^{a}	0.52^{a}
SiX2¹	0.46^{ab}	0.44^{b}	0.81^{ab}	0.61^{ab}	0.46^{b}	0.35^{b}	0.53^{ab}	0.53^{b}	0.52^{a}
SiX3¹	0.42^{b}	0.44^{b}	0.67^{b}	0.51^{b}	0.56^{ab}	0.71^{a}	0.51^{b}	0.54^{b}	0.63^{a}

1. SilX1= one soil drench application of silicon; SilX2= two successive soil drench applications of silicon and SilX3= three successive soil drench applications of silicon at a rate of 20 l per tree of 20 ml.l^{-1} soluble potassium silicate [20.7% silicon dioxide (W/V)].

2. Trees injected with 10 g.L^{-1} potassium phosphonate (K Phos) per meter of canopy diameter at the end of the spring flush and again at the end of the summer flush.

3. Values are cell wall-bound phenolic acid content expressed as µg gallic acid equivalent.g^{-1} of dry weight. Values in each column followed by different symbols indicate significant differences at P = 0.05 as determined using Duncan's Multiple Range Test.

phenolics were significantly higher (Table 3). It is possible that elevated glucoside-bound phenolics and reduced cell wall-bound phenolics may play a role in increased rooting densities of avocado trees infected with *P.cinnamomi* and the former may well be a phytoalexin effect. However, in the current study, such a hypothesis was not consistently supported by each of the data points over the two year time period of the field trial and further investigations are warranted.

There were no significant differences in total phenolic content (Table 2) between any of the treatments in July 2005 (winter). In line with the concept of silicon priming the plant's own battery of defence mechanisms, resulting in rapid deployment of these only when attacked by a pathogen (Van Bockhaven *et. al.* 2013), a possible reason for this could be that *P.cinnamomi* was not active in the roots at that time. However, further extraction of phenolics showed that cell wall-bound phenolics (Table 4) were significantly higher (0.86 $\mu g.l^{-1}$) in roots of SilX1 trees than those of the Control (0.66 $\mu g.l^{-1}$). There were no significant differences in any other phenolic extracts (Tables 3, 5 & 6), so during July 2005 (winter) it seems feasible that *P.cinnamomi* was not significantly active, thereby not inducing phenolic production in the plant.

Roots from Control trees had significantly lower total phenolic contents (10.41 $\mu g.l^{-1}$) than roots from SilX3 trees (23.18 $\mu g.l^{-1}$) in September 2005 (Table 2). However , at this time cell wall-bound phenolics (Table 3) were significantly higher in roots of the Control (0.75 $\mu g.l^{-1}$) than those in SilX3 trees (0.51 $\mu g.l^{-1}$) but there was apparently no corresponding change in rooting densities (Table 1). A possible explanation for this might be that Control trees were more subjected to environmental stress and therefore the low content of soluble phenolics compounds and the high content of cell wall-bound phenolics might be due to an active allocation of the secondary metabolites to the cell walls in order to strengthen the hemicellulose-lignin matrix.

Table 5: Effect of soil drench applications of soluble potassium silicate and potassium phosphonate stem injections on non-conjugated phenolic acid content in avocado trees, infected with *Phytophthora cinnamomi* in the field .

Treatment	Date								
	Mar-05	**May-05**	**Jul-05**	**Sep-05**	**Nov-05**	**Jan-06**	**Mar-06**	**May-06**	**Jul-06**
Control	0.51[a³]	0.80[a]	0.67[a]	0.45[a]	1.40[a]	0.54[a]	0.51[a]	0.80[a]	0.56[a]
K Phos²	0.42[a]	0.79[a]	0.57[a]	0.45[a]	0.77[a]	0.79[a]	0.39[a]	0.79[a]	0.54[a]
SiX1¹	0.72[a]	0.68[a]	0.64[a]	0.25[a]	0.78[a]	0.77[a]	0.72[a]	0.68[a]	0.54[a]
SiX2¹	0.57[a]	0.88[a]	0.94[a]	0.31[a]	0.68[a]	0.90[a]	0.57[a]	0.88[a]	0.54[a]
SiX3¹	0.59[a]	0.77[a]	0.79[a]	0.39[a]	1.27[a]	0.83[a]	0.59[a]	0.77[a]	0.55[a]

1. SilX1= one soil drench application of silicon; SilX2= two successive soil drench applications of silicon and SilX3= three successive soil drench applications of silicon at a rate of 20 l per tree of 20 $ml.l^{-1}$ soluble potassium silicate [20.7% silicon dioxide (W/V)].

2. Trees injected with 10 $g.L^{-1}$ potassium phosphonate (K Phos) per meter of canopy diameter at the end of the spring flush and again at the end of the summer flush.

3. Values are non-conjugated phenolic acid content expressed as µg gallic acid equivalent.g^{-1} of dry weight after hydrolysis.

Values in each column followed by different symbols indicate significant differences at P = 0.05 as determined using Duncan's Multiple Range Test.

Roots from Control trees again had significantly lower total phenolic contents (31.94 $\mu g.l^{-1}$) than roots from all other treatments (all $\geq$53.94 $\mu g.l^{-1}$) in November 2005 (Table 2). However, none of the further extracts of phenolics showed a similar reduction. In contrast, both glucoside-bound phenolics (Table 3) and cell wall-bound phenolics (Table 4) were significantly higher (1.05 $\mu g.l^{-1}$ and 0.64 $\mu g.l^{-1}$ respectively) in roots of the Control than those from SilX2 trees (0.50 $\mu g.l^{-1}$ and 0.46 $\mu g.l^{-1}$ respectively). This did not appear to have a detrimental impact on rooting densities (Table 1) as the effect of this treatment (0.48%) was not significantly different from the Control (0.38%).

Total soluble phenolic content was significantly higher during January 2006 in roots of trees injected with potassium phosphonate (68.77 $\mu g.l^{-1}$) as well as in roots of SilX2 and SilX3 treated trees (63.08 $\mu g.l^{-1,}$ and 65.32 $\mu g.l^{-1}$, respectively) when compared to roots from Control trees (46.34 $\mu g.l^{-1}$) or those from SilX1 treated trees (40.42 $\mu g.l^{-1}$). This would suggest that total phenolic concentrations may be elevated as a result of either multiple soil drenches of soluble potassium silicate or trunk injections with potassium phosphonate. On the other hand, in March and May 2006, this trend was confounded because phenolic concentrations in roots of the Control trees were the highest (133.66 and 109.08 $\mu g.l^{-1}$, respectively) when compared to all other treatments and this was significantly so in May 2006. However, in March and May 2006 glucoside-bound phenolic concentrations (Table 3) were significantly higher in roots of the SilX3 trees, (1.29 and 1.72 $\mu g.l^{-1}$, respectively) as compared to those of Control trees (0.49 and 0.89 $\mu g.l^{-1}$, respectively). Simultaneously, cell wall-bound phenolics (Table 4) were significantly lower in roots from SilX3 trees (0.51 and 0.54 $\mu g.l^{-1}$, respectively) than the Control trees (0.73 and 0.88 $\mu g.l^{-1}$, respectively). In this instance, this result seems to support the possibility that increased glucoside-bound phenolics and reduced cell wall-bound phenolics could be responsible for increased rooting densities. However, during July 2006 (winter) , there were no significant differences in any phenolic

acid concentrations between any treatments or the Control, again, possibly due to *P.cinnamomi* being inactive or less active in the roots during winter.

Conclusions

The current study found that soil drenches with soluble potassium silicate applied to *P. cinnamomi* infected avocado trees in the field resulted in increased rooting density and an improvement in the canopy condition equal to or better than that obtained from two annual trunk injections with potassium phosphonate. Three consecutive soil drenches of soluble potassium silicate per season resulted in the highest rooting densities and healthiest canopies.

This data, together with previous findings of *in vitro* suppression of *P. cinnamomi* by potassium silicate (Bekker et. al. 2006) supports the hypothesis that Phytophthora root rot of avocado can be controlled by potassium silicate application under field conditions. These findings concur with those of other studies that reported suppression of root diseases with silicon treatment of cucumbers infected with *Pythium*, another oomycete (Cherif *et al.* 1992a, Cherif *et al.* 1994). The results of the current study also confirm that there are significant seasonal influences on rooting densities and associated canopy health in all trees infected with *P. cinnamomi.* Rooting densities of treated and untreated (control) trees were significantly lower during the dry periods (winter 2005 and 2006). This was most likely related to sink strength and expected higher carbohydrate demands within the tree during the winter months (Kaiser & Wolstenholme 1993). Furthermore, it appears that multiple applications of soluble silicates were more effective than a single application, suggesting that continuous applications would be even more effective. Sameuls *et. al.* (1991), in their study of silicon associated resistance to *Sphaerotheca fuliginea* in cucumber, concluded that the total Si present in plant tissue is not as important as the available, mobile Si present at the time of infection.

The current study also demonstrated that multiple soil drenches of soluble potassium silicate per growing season usually resulted in increased total phenolic concentrations in the roots of avocado trees infected with *P.cinnamomi.* Numerous other studies have indicated that application of soluble silicon for suppression of fungal diseases have resulted in increasing phenolic acid concentrations in the plants (Carver *et al.* 1998, Cherif *et al.* 1992a, Cherif *et al*. 1994, Epstein 1999, Menzies *et al*. 1991, Menzies *et al*. 1992, Ghanmi *et al.* 2004, Zhou *et al.* 2004, Remus-Borel *et al.* 2005). Increased total soluble phenolic concentrations in the current study were usually associated with both significantly higher concentrations of glucoside-bound phenolics and significantly lower concentrations of cell wall-bound phenolics. These effects were not cumulative over the two growing seasons when compared to control trees or trees injected with potassium phosphonate. Previous studies on cucumber infected with *Pythium* (Cherif *et al.* 1992a, Cherif *et al.* 1992b) showed that Si deposition does not appear to be the mechanism by which fungal growth and penetration of plant tissues are suppressed but rather that it is linked to the plant's defense mechanisms. Our study of Si treatment of *P.cinnamomi* infected avocado trees concur with the conclusions of Cherif *et al.* (1992b, 1994) and Zhang *et al* (2013) of a relationship between silicon treatments, resistance to pathogen attack and expression of plant defense mechanisms. More recently, Van Bockhaven (2013) proposed five potential mechanisms to explain how silicon activates plant innate immune responses. One of these is the hypothesis that silicon primes the plant's own defence repertoire, leading to a rapid deployment of natural defence mechanisms only when attacked by a pathogen.

Furthermore, in light of previous *in vitro* studies by Bekker *et al.* (2006, 2009) and Kaiser *et al*. (2011) where *P.cinnamomi* mycelial growth was suppressed directly by potassium silicate, it is possible that silicon has both a direct fungitoxic effect on the pathogen as well as an indirect effect through activation/priming of the host's defense system.

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