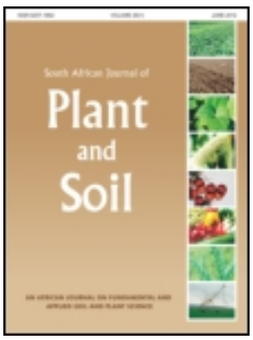

South African Journal of Plant and Soil

ISSN: 0257-1862 (Print) 2167-034X (Online) Journal homepage: http://www.tandfonline.com/loi/tjps20

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

Theo F Bekker, Nico Labuschagne, Terry Aveling, Thierry Regnier & Clive Kaiser

To cite this article: Theo F Bekker, Nico Labuschagne, Terry Aveling, Thierry Regnier & Clive Kaiser (2014) Effects of soil drenching of water-soluble potassium silicate on commercial avocado (Persea americana Mill.) orchard trees infected with Phytophthora cinnamomi Rands on root density, canopy health, induction and concentration of phenolic compounds, South African Journal of Plant and Soil, 31:2, 101-107, DOI: 10.1080/02571862.2014.912687

To link to this article: http://dx.doi.org/10.1080/02571862.2014.912687

South African Journal of Plant and Soil 2014, 31(2): 101–107
Printed in South Africa — All rights reserved

SOUTH AFRICAN JOURNAL OF PLANT AND SOIL
ISSN 0257-1862 EISSN 2167-034X
http://dx.doi.org/10.1080/02571862.2014.912687

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

Theo F Bekker[1], Nico Labuschagne[2]*, Terry Aveling[2], Thierry Regnier[2,3] and Clive Kaiser[2,4]

[1] *Department of Applied Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa; current address: Westfalia Technological Services, Westfalia Fruit Estate, Tzaneen, South Africa*
[2] *Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa*
[3] *Current address: Department of Biotechnology and Food Technology, Tshwane University of Technology, Pretoria, South Africa*
[4] *Current address: Horticulture Extension Faculty, Umatilla County, Oregon State University, Milton-Freewater, Oregon, USA*
* *Corresponding author, e-mail: Nico.Labuschagne@up.ac.za*

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 13-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 January 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

Introduction

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 (Si) 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 mouse-ear 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 Si inhibits plant diseases, several studies have reported *in vitro* inhibition of mycelial growth of phytopathogenic fungi by Si (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 Si, 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; Rodrigues 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 Si fertilisation 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 Si 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 Si 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 and Alexander 2004) and of rice blast, caused by *Magnaporte grisea* on rice plants (Rodrigues et al. 2004). In a recent review van Bockhaven et al. (2013) proposed five Si-induced regulatory mechanisms that might account for broad-spectrum plant disease resistance. One of these is the concept of Si 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 Si applications is also compared to that of potassium phosphonate.

Materials and methods

A 13-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 (23°43′60″ S, 30°10′0″ E). 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 randomised 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 Si soil drench (designated SilX1) applied at a rate of 20 l per tree using a 20 ml l^{-1} soluble potassium silicate solution (20.7% silicon dioxide) applied evenly to 20 m^2 (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 Si soil drenches (designated SilX2) each applied at the same rate as in treatment 1, applied four months apart; (3) three consecutive applications of soluble Si 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 metre of canopy diameter (industry standard) applied at the end of the spring flush (August 2004 and August 2005) and again at the end of the summer flush (January 2005 and January 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 bimonthly from the beginning of the study. Root density was recorded bimonthly according to the method described by Bekker (2007). Briefly, a 0.5 m^2 soil surface area was demarcated 1 m 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 megapixels, 35–420 mm 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 bimonthly 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 120 h. Freeze-dried materials were ground with an IKA® A11 basic grinder (IKA Werke, Staufen im Breisgau, Germany) to a fine powder.

Three separate extractions were performed on each sample. One millilitre 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 0.05 g powdered plant sample, ultrasonicated for 5 min in a VWR ultrasonic bath, and centrifuged at 24 000 ×*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 30 s followed by centrifugation for 30 s at 3 000 ×*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 was 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 (10–1 000 μg 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

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 (July 2004 to July 2006), compared with trees injected with potassium phosphonate (Avoguard®). Values in each column followed by a different superscript letter indicates a significant difference at $P = 0.05$ for canopy rating and root density, respectively, as determined by Duncan's multiple range test

	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
Treatment	SilX1[1]	K Phos end of spring flush (×1)[2]	SilX2[1]	K Phos end of summer flush (×2)[2]	SilX3[1]		SilX1	K Phos end of spring flush (×1)[2]		SilX2	K Phos end of summer flush (×2)[2]	SilX3		
Canopy disease rating[3]														
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[2]	–	–	–	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^{cd}	5.15^{a}	3.2^{bc}	2.15^{b}	2.70^{b}	2.40^{b}
SiX3	–	–	–	2.80^{b}	2.95^{b}	2.55^{b}	2.90d	–	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[2]	–	–	–	–	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; SilX3 = three successive soil drench applications of silicon at a rate of 20 l tree^{-1} of 20 ml l^{-1} soluble potassium silicate (20.7% silicon dioxide [w/v])

[2] K Phos = potassium phosphonate trunk injection at a rate of 10 g l^{-1} per metre of canopy diameter at the end of the spring flush and again at the end of the summer flush

[3] Canopy condition was determined according to the Ciba Geigy disease rating scale of 0 to10 with 0 = healthy tree and 10 = dead tree

mixture comprised 170 µl distilled water, 5 µl standard or plant extract sample, 50 µl of 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, 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 micrograms gallic acid equivalent per gram (dry weight).

All data were analysed using GenStat® 4.23 DE for Windows® (VSN International, Hemel Hempstead). A general analysis of variance was performed for each data set and means compared using Duncan's multiple range test where appropriate. Standard errors of the means and least significant differences 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 with 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 January 2006 (rating 3.2) through July 2006 (rating 2.4) compared with 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 with root densities, it appears that canopy disease ratings were not as accurate a measure of overall tree health and vigour, nevertheless, significant cyclical trends were also seen during the two growing seasons of this trial.

In March 2005 trees that received the SilX2 or SilX3 treatments had significantly higher root densities than did those with the SilX1, 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 receiving the SilX3 treatment had significantly higher root densities than trees with all other treatments (all ≤1.3%). This result continued into January 2006. Those trees that had received the SilX3 and 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 2005 and then steadily declined through July 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 September 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.

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. 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 μg l^{-1}) and those trees receiving the SilX2 (63.38 μg l^{-1}) and SilX3 (65.32 μg l^{-1}) treatments when compared with roots from control trees (43.34 μg l^{-1}) or trees receiving the SilX1 treatment (45.42 μg 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 μg l^{-1}) in roots from the potassium phosphonate treatment compared with the control treatment (0.67 μg l^{-1}). Total soluble phenolic content was again significantly higher in roots of trees of the SilX2 and SilX3 treatments (65.19 and 72.62 μg l^{-1}) when compared with roots from control trees (51.62 μg 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 μg l^{-1}) when compared with the untreated control trees (0.95 μg l^{-1}). In contrast, cell wall-bound phenolics (Table 4) were significantly lower in roots of SilX3 and SilX2 treated trees when compared with 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 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 Si 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 the absence of significant differences in total phenolic content between any treatments in July 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 μg l^{-1}) in roots of SilX1 trees than those of the control (0.66 μg l^{-1}). There were no significant differences in any other phenolic extracts (Tables 3, 5 and 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 μg l^{-1}) than roots from SilX3 trees (23.18 μg 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 μg l^{-1}) than those in SilX3 trees (0.51 μg 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.

Roots from control trees again had significantly lower total phenolic contents (31.94 μg l^{-1}) than roots from all other treatments (all ≥53.94 μg 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 and 0.64 μg l^{-1}, respectively) in roots of the control than those from SilX2 trees (0.50 and 0.46 μg 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 μg l^{-1}) as well as in roots of SilX2- and SilX3-treated trees (63.08 and 65.32 μg l^{-1}, respectively) when compared to roots from control trees (46.34 μg l^{-1}) or those from SilX1-treated trees (40.42 μg 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

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. Values are total soluble phenolic content expressed as micrograms gallic acid equivalent per gram of dry weight. Values in each column followed by a different superscript letter indicates a significant difference at $P = 0.05$ as determined using Duncan's multiple range test

Treatment	Date								
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
Control	46.34^{b}	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 Phos[1]	67.77^{a}	57.26^{ab}	1.94^{a}	15.81^{ab}	53.94^{b}	68.77^{a}	49.07^{c}	59.46^{c}	11.25^{a}
SiX1[2]	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}
SiX2[2]	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}
SiX3[2]	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] Trees injected with 10 g l^{-1} potassium phosphonate (K Phos) per metre of canopy diameter at the end of the spring flush and again at the end of the summer flush

[2] SiX1 = one soil drench application of silicon; SiX2 = two successive soil drench applications of silicon; SiX3 = three successive soil drench applications of silicon at a rate of 20 l $tree^{-1}$ of 20 ml l^{-1} soluble potassium silicate (20.7% silicon dioxide [w/v])

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. Values are glucoside-bound phenolic acid content expressed as micrograms gallic acid equivalent per gram of dry weight. Values in each column followed by a different superscript letter indicates a significant difference at $P = 0.05$ as determined using Duncan's multiple range test

Treatment	Date								
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
Control	0.67^{b}	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 Phos[1]	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}
SiX1[2]	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}
SiX2[2]	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}
SiX3[2]	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] Trees injected with 10 g l^{-1} potassium phosphonate (K Phos) per metre of canopy diameter at the end of the spring flush and again at the end of the summer flush

[2] SiX1 = one soil drench application of silicon; SiX2 = two successive soil drench applications of silicon; SiX3 = three successive soil drench applications of silicon at a rate of 20 l $tree^{-1}$ of 20 ml l^{-1} soluble potassium silicate (20.7% silicon dioxide [w/v])

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. Values are cell wall-bound phenolic acid content expressed as micrograms gallic acid equivalent per gram of dry weight. Values in each column followed by a different superscript letter indicates a significant difference at $P = 0.05$ as determined using Duncan's multiple range test

Treatment	Date								
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
Control	0.58^{ab}	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 Phos[1]	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}
SiX1[2]	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}
SiX2[2]	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}
SiX3[2]	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] Trees injected with 10 g l^{-1} potassium phosphonate (K Phos) per metre of canopy diameter at the end of the spring flush and again at the end of the summer flush

[2] SiX1 = one soil drench application of silicon; SiX2 = two successive soil drench applications of silicon; SiX3 = three successive soil drench applications of silicon at a rate of 20 l $tree^{-1}$ of 20 ml l^{-1} soluble potassium silicate (20.7% silicon dioxide [w/v])

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 μg l^{-1}, respectively) when compared with 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 SiX3 trees (1.29 and 1.72 μg l^{-1}, respectively) as compared with those of control trees (0.49 and 0.89 μg l^{-1}, respectively). Simultaneously, cell wall-bound phenolics (Table 4) were significantly lower in roots from SiX3 trees (0.51 and 0.54 μg l^{-1}, respectively) than the control trees (0.73 and 0.88 μg 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

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. Values are non-conjugated phenolic acid content expressed as micrograms gallic acid equivalent per gram of dry weight after hydrolysis. Values in each column followed by a different superscript letter indicates a significant difference at $P = 0.05$ as determined using Duncan's multiple range test

Treatment	Date								
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
Control	0.51[a]	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 Phos[1]	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]
SiX1[2]	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]
SiX2[2]	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]
SiX3[2]	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] Trees injected with 10 g l^{-1} potassium phosphonate (K Phos) per metre of canopy diameter at the end of the spring flush and again at the end of the summer flush

[2] SiX1 = one soil drench application of silicon; SiX2 = two successive soil drench applications of silicon; SiX3 = three successive soil drench applications of silicon at a rate of 20 l $tree^{-1}$ of 20 ml l^{-1} soluble potassium silicate (20.7% silicon dioxide [w/v])

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.

These data, together with previous findings of *in vitro* suppression of *P. cinnamomi* by potassium silicate (Bekker et al. 2006), support 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 Si treatment of cucumbers infected with *Pythium*, another oomycete (Cherif et al. 1992a, 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 and 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 Si-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 Si for suppression of fungal diseases have resulted in increasing phenolic acid concentrations in the plants (Carver et al. 1998; Menzies et al. 1991, 1992; Cherif et al. 1992a, 1994; Epstein 1999; 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 with control trees or trees injected with potassium phosphonate. Previous studies on cucumber infected with *Pythium* (Cherif et al. 1992a, 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 defence 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 Si treatments, resistance to pathogen attack and expression of plant defence mechanisms. More recently, van Bockhaven et al. (2013) proposed five potential mechanisms to explain how Si activates plant innate immune responses. One of these is the hypothesis that Si 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), Bekker (2007) and Kaiser et al. (2011) where *P. cinnamomi* mycelial growth was suppressed directly by potassium silicate, it is possible that Si has both a direct fungitoxic effect on the pathogen as well as an indirect effect through activation/priming of the host's defence system.

Acknowledgements — This research was funded in part by the National Research Foundation of South Africa and by the South African Avocado Growers Association.

References

Abdel-Farid I, Jahangir M, van den Hondel C, Kim H, Choi Y, Verpoorte R. 2009. Fungal infection-induced metabolites in *Brassica rapa. Plant Science* 176: 608–615.

Anderson J, Pegg K, Coates L, Dann E, Cooke A, Smith L, Dean J. 2004. Silicon and disease management in avocados. *Talking Avocados* 15: 23–25.

Anderson J, Pegg K, Dann E, Cooke A, Smith L, Willingham S,

Giblin F, Dean J, Coates L. 2005. New strategies for the integrated control of avocado fruit diseases. In: Proceedings of the New Zealand and Australia Avocado Growers' Conference 2005, 20–22 September, Tauranga, New Zealand. Available at http://www.avocadosource.com/journals/ausnz/ausnz_2005/andersonjm2005.pdf [accessed 30 April 2014].

Bekker T. 2007. Efficacy of water soluble silicon for control of *Phytophthora cinnamomi* root rot of avocado. MSc thesis, University of Pretoria, South Africa.

Bekker T, Kaiser C, Merwe R, Labuschagne N. 2006. *In-vitro* inhibition of mycelial growth of several phytopathogenic fungi by soluble potassium silicate. *South African Journal of Plant and Soil* 26: 169–172.

Bi Y, Tian S, Guo Y, Ge Y, Qin G. 2006. Sodium silicate reduces postharvest decay on Hami melons: induced resistance and fungistatic effects. *Plant Disease* 90: 279–283.

Bowen P, Menzies J, Ehret D, Samuels L, Glass ADM. 1992. Soluble silicon sprays inhibit powdery mildew development on grape leaves. *Journal of the American Society for Horticultural Science* 117: 906–912.

Carver T, Robbins M, Thomas B, Troth K, Raistrick N, Zeyen R. 1998. Silicon deprivation enhances localized autofluorescent responses and phenylalanine ammonia-lyase activity in oat attacked by *Blumeria graminis*. *Physiological and Molecular Plant Pathology* 52: 245–257.

Cherif M, Asselin A, Belanger R. 1994. Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology* 84: 236–242.

Cherif M, Benhamou N, Menzies JG, Bélanger R. 1992a. Silicon induced resistance in cucumber plants against *Pythium ultimum*. *Physiological and Molecular Plant Pathology* 41: 411–425.

Cherif M, Menzies JG, Benhamou N, Bélanger RR, 1992b. Studies of silicon distribution in wounded and *Pythium ultimum* infected cucumber plants. *Physiological and Molecular Plant Pathology* 41: 371–385.

Coffey MD. 1987. Phytophthora root rot of avocado. *Plant Disease* 71: 1046–1052.

Darvas J, Toerien J, Milne D. 1984. Control of avocado root rot by trunk injection with phosetyl-A1. *Plant Disease* 68: 691–693.

de Ascensao AR, Dubery IA, 2003. Soluble and wall-bound phenolics and phenolic polymers in *Musa acuminata* roots exposed to elicitors from *Fusarium oxysporum* f. sp. *cubense*. *Phytochemistry* 63: 679–686.

del Rio J, Baidez A, Botia J, Ortuno A. 2003. Enhancement of phenolic compounds in olive plants (*Olea europaea* L.) and their influence on resistance against *Phytophthora* sp. *Food Chemistry* 83: 75–78.

Epstein E. 1999. Silicon. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 641–664.

Fauteux F, Chain F, Belzile F, Menzies JG, Bélanger RR. 2006. The protective role of silicon in the *Arabidopsis*–powdery mildew pathosystem. *Proceedings of the National Academy of Sciences of the USA* 103: 17554–17559.

Fawe A, Abou-Zaid M, Menzies J, Bélanger R. 1998. Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. *Phytopathology* 88: 396–401.

Ghanmi D, McNally DJ, Benhamou N, Menzies JG, Bélanger RR. 2004. Powdery mildew of *Arabidopsis thaliana*: a pathosystem for exploring the role of silicon in plant-microbe interactions. *Physiological and Molecular Plant Pathology* 64: 189–199.

Grimm GR, Alexander AF. 1973. Citrus leaf pieces as traps for *Phytophthora parasítica* from soil slurries. *Phytopathology* 63: 540–541.

Hardy GESJ, Barrett S, Shearer B. 2001. The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Australasian Plant Pathology* 30: 133–139.

Kaiser C, Hamm PB, Gieck S, David N, Long L, Meland M, Christensen JM. 2011. In vitro fungicidal activity of calcium and potassium salts on several commercially significant plant pathogens. *HortScience* 46: 913–916.

Kaiser C, Wolstenholme B. 1993. Aspects of late hung Hass avocado (*Persea americana* Mill.) fruit in the Natal Midlands. II. Whole tree starch cycling. *South African Avocado Growers' Association Yearbook* 16: 46–55.

Mandal S, Mitra A, Mallick N. 2009. Time course study on accumulation of cell wall-bound phenolics and activities of defense enzymes in tomato roots in relation to Fusarium wilt. *World Journal of Microbiology and Biotechnology* 25: 795–802.

Menzies JG, Bowen P, Ehret D, Glass ADM. 1992. Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber, muskmelon, and zucchini squash. *Journal of the American Society for Horticultural Science* 117: 902–905.

Menzies JG, Ehret DL, Glass, ADM, Samuels AL. 1991. The influence of silicon on cytological interactions between *Sphaerotheca fuliginea* and *Cucumis sativus*. *Physiological and Molecular Plant Pathology* 39: 403–414.

Nicholson RL, Hammerschmidt R. 1992. Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology* 30: 369–389.

Nolla A, Korndörfer GH, Coelho L. 2006. Efficiency of calcium silicate and carbonate in soybean disease control. *Journal of Plant Nutrition* 29: 2049–2061.

Pegg KG, Coates LM, Korsten, L, Harding RM. 2002. Foliage, fruit and soilborne diseases. In: Whiley AW, Schaffer B, Wolstenholme BN (eds), *Avocado: botany, production and uses*. Wallingford: CABI Publishing. pp 432–456.

Qin GZ, Tian SP. 2005. Enhancement of biocontrol activity of *Cryptococcus laurentii* by silicon and the possible mechanisms involved. *Phytopathology* 95: 69–75.

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.

Regnier T, Macheix JJ. 1996. Changes in wall-bound phenolic acids, phenylalanine and tyrosine ammonia-lyases, and peroxidases in developing durum wheat grains (*Triticum turgidum* L. var. Durum). *Journal of Agricultural and Food Chemistry* 44: 1727–1730.

Remus-Borel W, Menzies JG, Bélanger RR. 2005. Silicon induces antifungal compounds in powdery mildew-infected wheat. *Physiological and Molecular Plant Pathology* 66: 108–115.

Rodrigues FÁ, McNally DJ, Datnoff LE, Jones JB, Labbé, Benhamou, N, Menzies JG, Bélanger RR. 2004. Silicon enhances the accumulation of diterpenoid phytoalexins in rice: a potential mechanism for blast resistance. *Phytopathology* 94: 177–183.

Sameuls AL, Glass ADM, Ehret DL, Menzies JG, 1991. Mobility and deposition of silicon in cucumber plants. *Plant, Cell and Environment* 14: 485–492.

van Bockhaven J, de Vleesschauer D, Höfte M. 2013. Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. *Journal of Experimental Botany* 64: 1281–1293.

Zentmyer GA, Menge JA, Ohr HD. 1994. *Phytophthora* root rot. In: Ploetz RC, Zentmyer GA, Nishijima WT, Rohrbach KG, Ohr HD (eds), *Compendium of tropical fruit diseases*. St Paul, Minnesota: American Phytopathological Society Press. pp 77–79.

Zhang G, Cui Y, Ding X, Dai Q. 2013. Stimulation of phenolic metabolism by silicon contributes to rice resistance to sheath blight. *Journal of Plant Nutrition and Soil Science* 176: 118–124.

Zhou Z, Robards K, Helliwell S, Blanchard C. 2004. The distribution of phenolic acids in rice. *Food Chemistry* 87: 401–406.

Received 2 October 2013, revised 4 March 2014, accepted 18 March 2014
Associate Editor: John Bower