Efficacy of water soluble silicon against *Phytophthora cinnamomi* root rot of avocado: A progress report

T F Bekker[1], C Kaiser[1] and N Labuschagne[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 world avocado industry is very reliant on phosphorous acid for the control of Phytophthora cinnamomi *root rot of avocado. The threat of fungal resistance necessitated investigation into alternative methods of disease suppression. In a field trial conducted in Tzaneen, South Africa, soluble potassium silicate was applied as a soil drench to mature 'Hass' trees on 'Duke 7' rootstock, to determine whether silicon has disease suppressive properties under field conditions. Silicon application resulted in higher root densities compared to that of potassium phosphonate (Avoguard®) injections.These results were also related to canopy ratings, as trees that received frequent silicon applications, showed better canopy conditions compared to control treatments. One (Si x 1) and two (Si x 2) silicon applications per season resulted in significantly higher root densities under moist conditions compared to the control treatment, but this effect was negated under conditions of drought stress. Three (Si x 3) silicon applications resulted in significantly higher root densities throughout the season, compared to the control and potassium phosphonate (Avoguard®) treatments. Canopy conditions followed a similar trend to that of root density. Under conditions of limited drought stress, tree canopies showed less symptoms of disease stress. However, during dry conditions, canopy condition deteriorated dramatically. This was alleviated when rainfall returned during December 2005. All silicon treatments resulted in better canopy conditions (viz. Si x 3 rendering a canopy rating of 2.75 and Si x 1 giving a rating of 3.25) compared to that of the control (4.25) and potassium phosphonate (Avoguard®) (4) treatments. Drought stress however influenced this trend. Due to the fact that phenols are formed in reaction to pathogen infection, and literature indicate silicon application to enhance phenol expression, total phenolic content of avocado leaves and roots were determined. No significant differences were observed in total leaf phenolic content between treatments. However, the effect of silicon application on phenol expression in avocado roots in the presence of* Phytophthora cinnamomi *was evident. Significantly higher levels of phenols occurred with Si x 3 treatments compared to the control. The effect of silicon on phenol concentration in avocado roots is similar to, or exceeds that of potassium phosphonate (Avoguard®). These preliminary results indicate silicon to have both a direct mechanism of action as well as an indirect action by elevation of phenolic levels in the plant.*

INTRODUCTION

Phytophthora cinnamomi is an aggressive plant pathogen causing extensive root rot in avocados (*Persea americana* Mill.). On average the disease leads to an annual loss of 10% of the world avocado crop, which amounts to several million US$ worldwide (Zentmeyer & Schieber, 1991).

Numerous control measures have been implemented to control root rot, including biological control (McLeod, Labuschagne & Kotze, 1995; Duvenhage & Kotze, 1993; Casale, 1990) and induction of host resistance (Phillips, Grant & Weste, 1987). However, chemical control remains the most important control measure, and to this end, phosphate-based fungicides play a major role. Phosphonate fungicides, including fosetyl-Al (Aliette®) and its breakdown product, phosphorous acid, are highly mobile in plants (Guest, Pegg & Whiley, 1995) and are believed to control *Phytophthora* spp. by a combination of direct fungitoxic activity and stimulation of host defence mechanisms (Guest *et al*., 1995; Hardy Barrett & Shearer, 2001).

Duvenhage (1994) first reported on the possibility of resistance to fosetyl-Al and H_3PO_3 and found that isolates of *P. cinnamomi* obtained from trees treated with fosetyl-Al or H_3PO_3 were less sensitive to these compounds *in vitro*, compared to isolates obtained from untreated trees. He concluded that the possibility of resistance does exist (Duvenhage, 1999), which would pose a serious threat to the avocado industry.

To determine whether potassium silicate is a viable alternative treatment against *Phytophthora* root rot of avocado, studies have been conducted to determine the effect of potassium silicate application on *Phytophthora cinnamomi* root rot development in avocado plants.

The suppressive effects of silicon on plant diseases have been indicated by numerous authors (Ma & Takahashi, 2002; Epstein, 1999). Mechanisms of suppression include increased mechanical barriers (Datnoff, Deren & Snyder, 1997), plant enzymes (Samuels, Glass, Ehret & Menzies, 1993) and fungi-toxic compounds (Fawe, Abou-Zaid, Menzies & Bélanger, 1998).

The aim of this study was to determine whether the application of soluble silicon from potassium silicate to *P. cinnamomi* infected trees would suppress the disease.

MATERIALS AND METHODS

Chemicals

Silicon was obtained from Ineos Silicas South Africa (Pty) Ltd, Germiston, South Africa. Potassium phosphonate (Avoguard®) was obtained from Ocean Agriculture. All solutions were prepared with distilled water.

Plant and fungal material

An avocado orchard (latitude 23° 43' 60S; longitude 30° 10' 0E) situated in Tzaneen, South Africa, at an altitude of 847 m, was used. Trees consisted of thirteen year old 'Hass' trees on 'Duke7' seedling rootstocks planted at a density of 204 trees.ha^{-1} (7 x 7 m spacing). The trees were on a southern facing slope. The presence of *Phytophthora cinnamomi* in the orchard soil and avocado tree roots was confirmed by isolation of PARPH medium (Jeffers & Martin, 1986) and subsequent identification. Fifty trees (n) were selected and 10 trees were randomly assigned per treatment in a randomised block design.

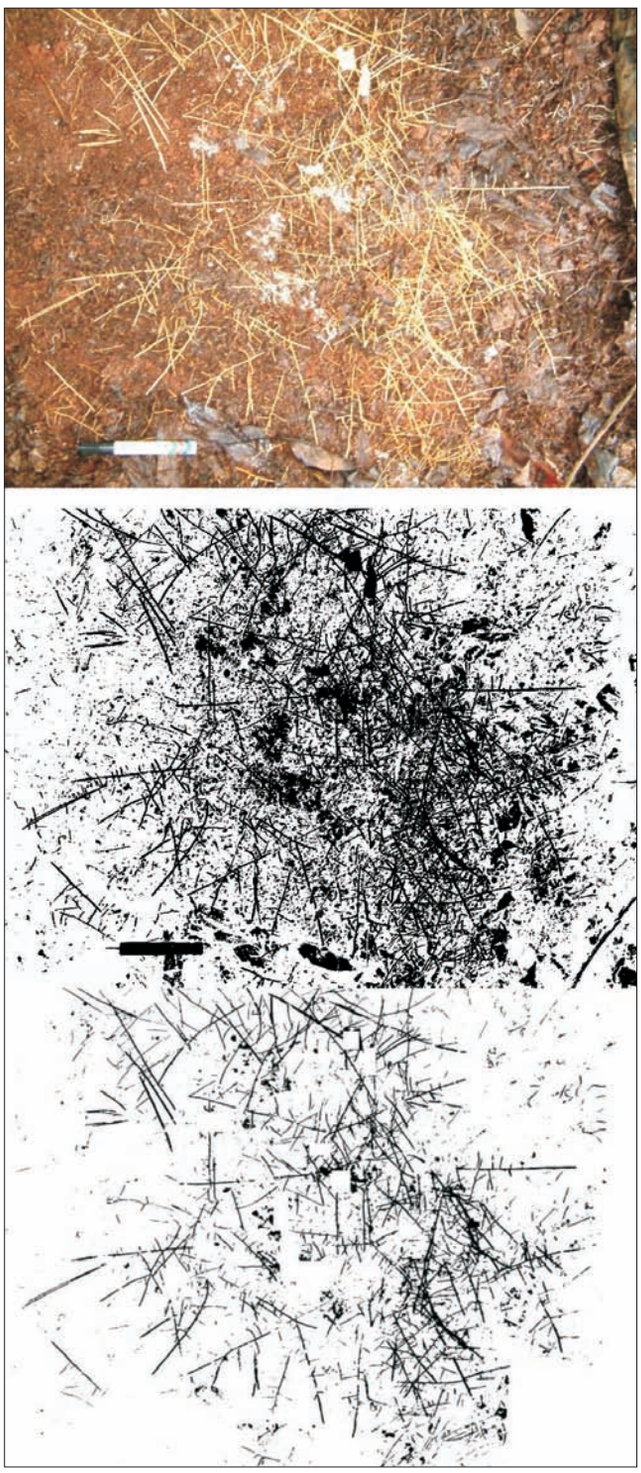

Figure 1. Photo preparation for root density determination by means of digital images which were analysed using ImageJ 1.33u software.
a) Normal photo of avocado roots on soil surface
b) Photo converted to black and white image
c) Pixels not related to roots (including leaf material and mulch litter) removed

Treatments

Treatments consisted of soil drenched with a watering can under the drip line of the tree with 20 litre 20 ml.l^{-1} soluble silicon (20.7% silicon dioxide) (Bekker, Labuschagne & Kaiser, 2005) per tree either once, twice or three times during a growing season. Trees injected with potassium phosphonate (Avoguard®) were incorporated as a standard treatment and applied at a concentration of 0.5 ml.l^{-1} water. Untreated trees were used as a control treatment and only drenched with water.

Data collection

Data was collected from January 2005 to January 2006. Digital photographs (described hereafter) and plant samples (root and leaf) were taken every second month on the northern slide of the tree, and fruit samples were taken at harvest.

Environmental factors

Temperature was measured every 30 min from January 2005 to July 2006 using a HOBO® H8 data logger (Onset computer Corporations, Bourne, MA, USA). Rainfall data was obtained by a rain gauge situated in the orchard. Tensiometers situated in the orchard at two different levels measured soil moisture content, and this was used as a measure to regulate additional water application through drip irrigation.

Tree health assessment

The condition of a tree canopy was rated according to a Ciba Geigy (Bezuidenhout, Darvas & Toerien, 1987) avocado tree rating from 0 to 10, with 0 = healthy looking tree and 10 = dead tree. Ratings were done independently by two parties, as well as from digital photographs taken in the field. This was done fortnightly.

Root health assessment

Ten sheets of newspaper were placed on top of each other under the drip line of each tree on the soil surface to cover a 0.5 m^2 area, and covered with leaf mulch. After two months, the mulch was carefully raked away and the newspaper was removed. A digital photo of the exposed roots was taken at a set height of 75 cm above the soil level with a Konika Minolta Dimage Z5 camera (5 megapixel, 35-420 mm lens). The newspaper was replaced with new sheets and covered with mulch. This was done fortnightly.

Photos were analysed using ImageJ 1.33u (Wayne Rasband, National Institutes of Health, USA) software. The photos were converted from a RGB colour type photo to an 8-bit image. A threshold (upper threshold 255, lower threshold level 170-195) was assigned to the foreground colour (the yellow/white avocado feeder roots) and the remaining pixels to the background colour (soil surface), where after the photo was converted to a black and white picture. Pixels not related to roots, including leaf material and mulch litter (background noise) in the photo, was deleted from the picture (**Figure 1**). Hereafter it was computer analysed, an area fraction determined and recorded as a percentage root area.

Extraction and quantification of total phenolic compounds

Samples were freeze dried for 72 h. The dried material was ground with an IKA® A11 basic grinder (IKA Werke, GMBH & Co., KG, D-79219 Staufen) to a fine powder. One millilitre of a methanol : acetone : water (7:7:1 on volumetric basis) solution was added to 0.05 g powdered sample, ultrasonified for 5 min using a VWR ultrasonic bath, and centrifuged at 13500 rpm

(24000 g) for 1 min. This extraction procedure was repeated twice.

Chlorophyll was removed from the leaf samples by addition of 0.5 ml chloroform to the samples, where after it was shaken for 30 s and centrifuged for 30 s. Supernatants were pooled and the organic solvent mixture was evaporated in a laminar flow cabinet at room temperature, where after the residue was dissolved in 1 ml water.

The concentration of phenolic compounds was determined using the Folin-Coicalteu reagent (Bray & Thorpe, 1954). The reaction mixture was scaled down to enable the use of 96 well ELISA plates. For the quantification of phenolic content a dilution series (10 – 1000 µg/ml methanol) were used to prepare standard curves for ferullic- and gallic acid. 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-Coicalteu reagent. After incubation at 40°C for 30 min the absorbance was read at 690 nm using an ELISA reader (Multiskan Ascent VI.24354 – 50973 [version 1.3.1]). Phenolic concentration was expressed as ferullic or gallic acid equivalents per gram dry root material (Regnier, 1994).

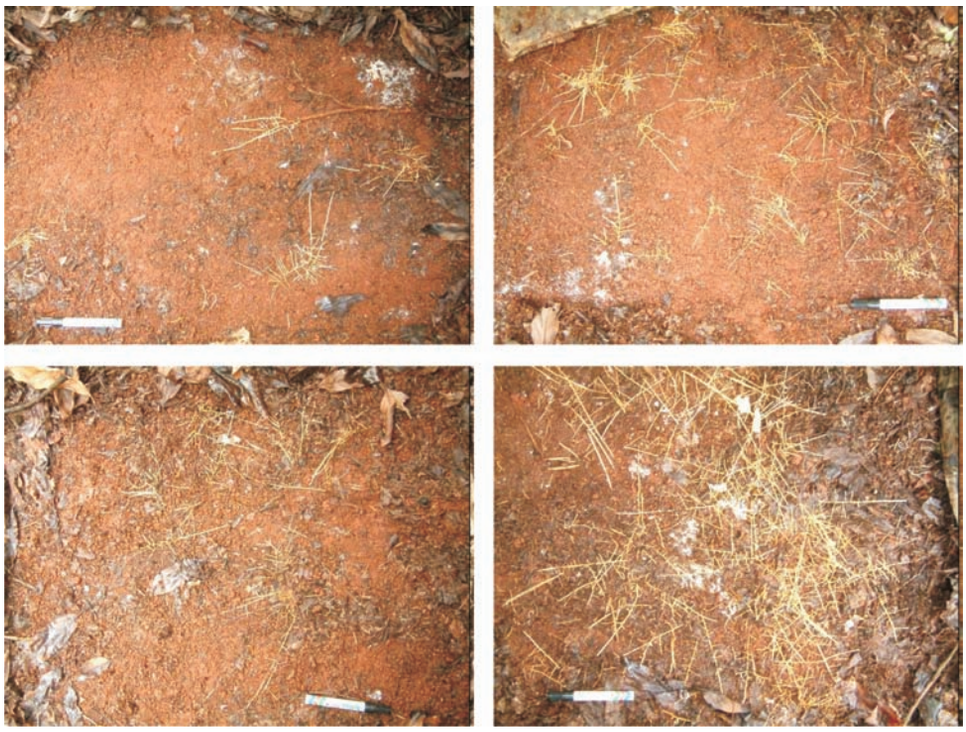

Figure 2. Digital images of avocado tree root densities after *P. cinnamomi* infected trees were subjected to the following treatments:
Top left: Control **Top right: Si x 1**
Bottom left: Potassium phosphonate (Avoguard®) **Bottom right: Si x 3**

RESULTS AND DISCUSSION

The application of silicon to avocado trees as a soil drench affected root density dramatically (**Figure 2**). Higher root densities were obtained with silicon application compared to that of potassium phosphonate (Avoguard®) injections. These results correlated well with tree canopy ratings, as trees that received silicon frequently, showed better canopy condition compared to the control treatments. Root density was, however, also affected by rainfall received throughout the season, although seasonal growth flushes and timing of silicon application also played a role. High rainfall in the first semester of 2005 resulted in higher densities compared to that of drier months in the second semester (**Figure 3**). One (Si x 1) and two (Si x 2) silicon applications per season resulted in significantly higher root densities under moist conditions compared to the control treatment, but this effect was negated under conditions of drought stress. Three (Si x 3) silicon applications resulted in significantly higher root densities compared to the control and potassium phosphonate (Avoguard®) treatments. It has been reported that soluble silicon polymerizes rapidly, resulting in insoluble silicon compounds, while diseases are effectively suppressed only if silicon is present in soluble form, Bowen, Menzies, Ehret, Samuels & Glass (1992). To provide maximum protection, and therefore minimize disease development, Bowen *et al.* (1992) suggested silicon to be applied continuously. Our preliminary results indicate this to be true, as three applications of silicon resulted in the best disease suppression and stimulation of new root growth.

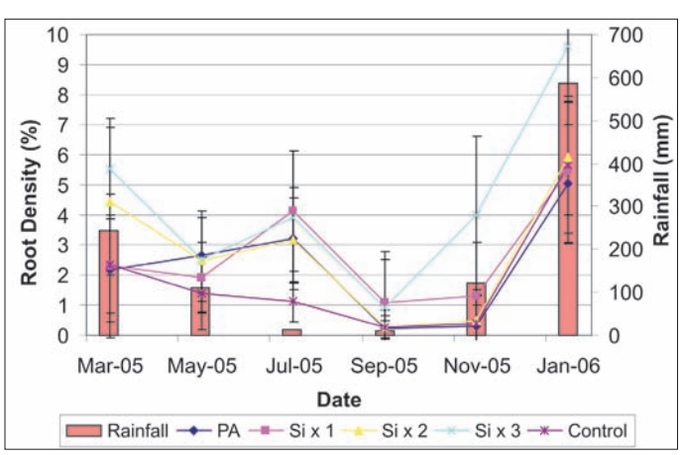

Figure 3. Avocado tree root density for a period of one year and bimonthly rainfall recorded in the orchard.

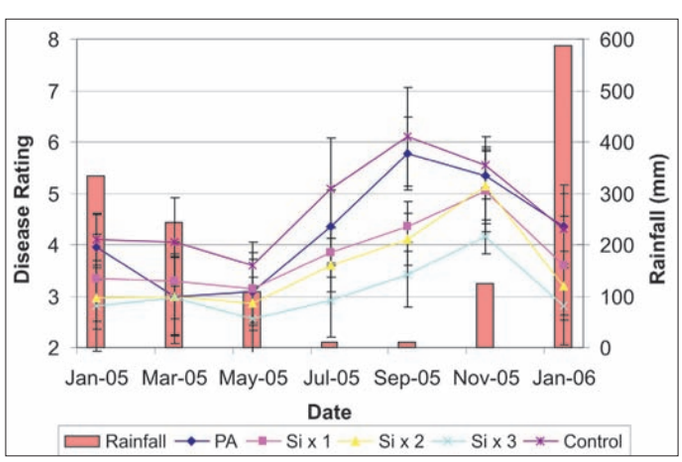

Figure 4. Avocado tree canopy rating according to the Ciba Geigy disease rating for a period of one year and bimonthly rainfall recorded in the orchard.

Canopy condition followed similar trends to that of root density over the period of data collection. Under conditions of limited drought stress, tree canopies showed less symptoms of disease stress. However, during dry conditions, canopy condition deteriorated dramatically. This was fortunately nullified when rainfall returned during December 2005 (**Figure 4**). All silicon treatments resulted in better canopy condition (viz. Si x 3 rendering a canopy rating of 2.75 and Si x 1 giving a rating of 3.25) compared to that of the control (4.25) and potassium phosphonate (Avoguard®) (4) treatments. This difference was, however, not significant for Si x 1 and Si x 2 applications under moist conditions as experienced in March. However, from July to November 2005, all silicon treatments resulted in significant differences compared to that of control and potassium phosphonate (Avoguard®) treatments. This indicates that the silicon treatments reduced drought stress, apart from reducing disease stress.

If excess water is lost during transpiration, stomata closes and a decrease in photosynthetic rate occurs. Transpiration mainly occurs through the stomata and partly through the cuticle. If Si is present in the plant, it is deposited beneath the cuticle forming a double layer (Si-cuticle), which limits transpiration through the cuticle. Especially in plants with thin cuticles, this can be a great advantage (Ma & Takahashi, 2002). Gong, Zhu, Chen, Wang & Zhang (2005) reported that silicon improved the water status of drought stressed wheat plants with regard to water potential and water content in leaf tissue of plants treated with silicon, compared to untreated plants. This also seems to be the case in silicon treated avocado plants.

However, in our study the overriding factor of silicon seems to be its effect on disease suppression, and therefore canopy condition as an indicator of disease severity. Chérif, Asselin & Belanger (1994) reported that although silicon had no effect on phenolic concentrations of plants in the absence of pathogen infection, significant differences can be seen in inoculated plants compared to uninoculated control plants with regards to the pathogen *Pythium ultimum*. Concentrations of phenolic compounds in inoculated plants were reported to be double that of uninoculated plants six days after inoculation. The difference seen in avocado canopy condition in our study can therefore be attributed to disease suppression by silicon, and not other external factors influencing tree health.

In the current study, no significant differences were seen in total leaf phenolic content between treatments throughout the period of sampling, except during March 2005, when both Si x 2 (136 mg equivalent gallic acid per gram of dry weight) and Si x 3 (185 mg.g^{-1}) were significantly different compared to the potassium phosphonate (Avoguard®) (113 mg.g^{-1}) and control (107 mg.g^{-1}) treatments (**Figure 5**). Si accumulates at sites of infection (Fauteux, Rémus-Borel, Menzies & Bélanger, 2005). Ma, Tamai, Ichii & Wu (2002) suggested an active transport system for silicon, at least in rice plants. Rodriguez, Jurick, Datnoff, Jones & Rollins (2005) promoted the hypothesis for an active participation of silicon in disease response. However, because *Phytophthora cinnamomi* attacks avocado roots, and silicon is applied as a soil drench, no transport of silicon is proposed. Whether the mode of action of silicon is direct or indirect, leaf phenolic content should not, and as our results indicate, is not affected by silicon application under conditions of disease stress.

The effect of silicon application on phenol levels in avocado roots in the presence of *Phytophthora cinnamomi* is evident from **Figure 6**. Significant differences between Si x 3 and the control groups were evident throughout the trial duration, except for November 2005 where Si x 3 (31 mg.g^{-1} gallic acid equivalent) were significantly lower compared to all other Si treatments and the potassium phosphonate (Avoguard®) treatment (54 mg.g^{-1}). However, the effect of silicon on phenol concentration in avocado roots is similar to, or exceeds that of potassium phosphonate (Avoguard®) presumably indicating a higher level of induced resistance expressed in plants.

Anderson, Pegg, Coates, Dann, Cooke, Smith & and Dean (2004) stated that analysis of avocado fruit from trees injected with silicon for the control of anthracnose indicated higher levels of Mn, which possibly could have contributed to disease suppression, as Mn is an important co-factor in synthesis of phenols and lignin necessary for plant defense. Manganese also inhibits the activity of pectolytic enzymes produced by fungi. This effect of silicon could also be true for avocado roots, but mineral analysis would be necessary to confirm this. Nonetheless, our observations confirm findings by Chérif *et al.* (1994) indicating the release of these fungitoxic metabolites as a result of silicon application, thereby contributing to the enhanced resistance of host plants to pathogens. This release and accumulation of phenol-like material (such as the phytoalexin rhamnetin in cucumber) is deleterious to fungal haustoria (Fawe, *et al.,* 1998) and thus limits infection. Similarly, silicon application limits disease development in avocado trees.

CONCLUSIONS

Effective control of *Phytophthora cinnamomi* root rot of avocado is of paramount importance to ensure the economical survival of this industry, not only in South Africa, but worldwide.

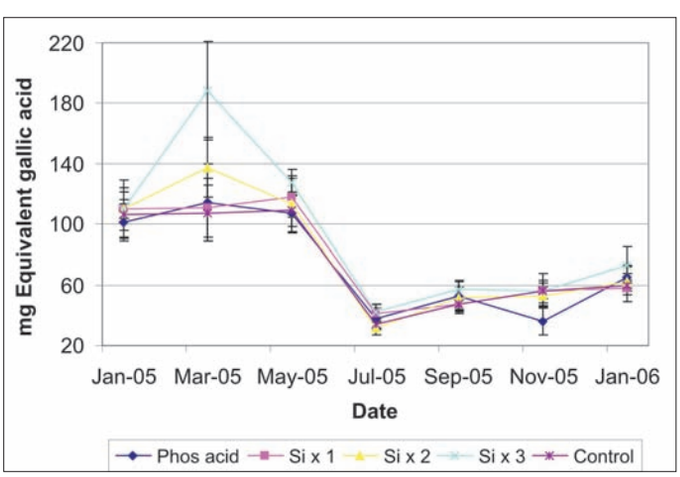

Figure 5. Concentrations of total phenolics in crude extracts from milled leaf samples collected from *Phytophthora cinnamomi* infected avocado trees, subjected to various treatments with soluble potassium silicate.

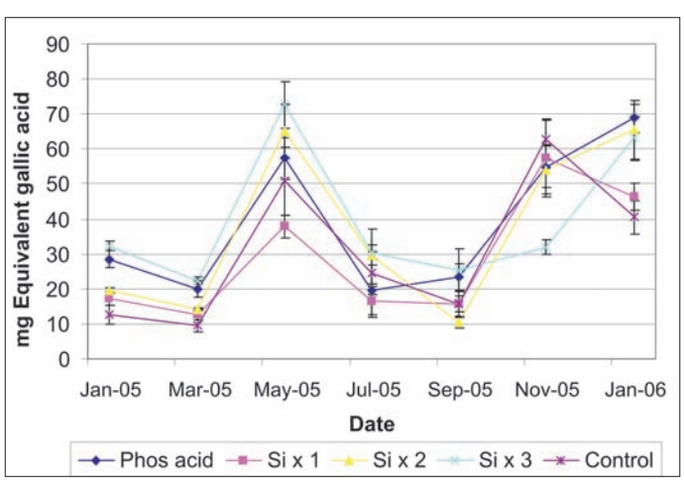

Figure 6. Concentrations of total phenolics in crude extracts from milled root samples collected from *Phytophthora cinnamomi* infected avocado trees, subjected to various treatments with soluble potassium silicate.

Our preliminary results indicate the application of water soluble silicon to be effective in suppression of avocado root rot if applied at the rate of 20 litres per tree (20 $ml.l^{-1}$ water) throughout the production season. Data also indicate silicon to have both a direct mechanism of action as well as an indirect action by inducing plant resistance through elevation of phenolic levels in the plant, indicating an induced resistance response.

LITERATURE CITED

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

BEZUIDENHOUT, J.J., DARVAS, J.M. & TOERIEN, J.C. 1987. Chemical control of *Phytophthora cinnamomi*, Proceedings of the First World Avocado Congress. *South African Avocado Growers' Association Yearbook* 1987, 10: 106-108.

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

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

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

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

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

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

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

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

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

FAWE, A., ABOU-ZAID, M., MENZIES, J.G. & BÉLANGER, R.R. 1998. Silicon Mediated Accumulation of Flavanoid Phytoalexins in Cucumber. *Phytopathol.,* 88(5): 396-401.

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

GUEST, D.I., PEGG, K.G. & WHILEY, A.W. 1995. Control of *Phytophthora* diseases of tree crops using trunk-injected phosphonates. *Hort. Rev*., 17: 299-330.

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

JEFFERS, S.N. & MARTIN, S.B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease,* 70: 1038-1043.

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

MA, J.F., TAMAI, K., ICHII, M. & WU, G.F. 2002. A rice mutant defective in Si uptake. *Plant Physiol*., 130: 211-2117.

McLEOD, A., LABUSCHAGNE, N. & KOTZE, J.M. 1995. Evaluation of *Trichoderma* for biological control of avocado root rot in bark medium artificially infected with *Phytophthora cinnamomi*. *South African Avocado Growers' Association Yearbook,* 18: 32-37.

PHILLIPS, D., GRANT, B.R. & WESTE, G. 1987. Histological changes in the roots of an avocado cultivar, Duke 7, infected with *Phytophthora cinnamomi*. *Phytopathol*., 77: 691-698.

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, pp. 43-45. Montpellier University, France.

RODRIGUEZ, F.A., JURICK II, W.M., DATNOFF, L.E., JONES, J.B. & ROLLINS, J.A. 2005. Silicon influence cytological and molecular events in compatible and incompatible rice-*Magnaporthe grisea* interactions. *Physiol. Mol. Plant Pathol*., 66: 144-159.

SAMUELS, A.L., GLASS, A.D.M., EHRET, D.L. & MENZIES, J.G. 1993. The Effects of Silicon Supplementation on Cucumber Fruit: Changes in surface Characteristics. *Ann. Bot.,* 72: 433-440.

ZENTMEYER, G.A. & SCHIEBER, E. 1991. *Persea* and *Phytophthora* in Latin America. World Avocado Congress II Proceeding, I: 61-66.