CHAPTER 3

THE INHIBITION OF PHYTOPHTHORA ROOT ROT OF AVOCADO WITH POTASSIUM SILICATE APPLICATION UNDER GREENHOUSE CONDITIONS

3.1 ABSTRACT

Phytophthora cinnamomi root rot causes extensive avocado tree root death. The use of phosphonate fungicides are currently the only effective post-infectional control method, and to this end, an alternative was sought to inhibit Phytophthora root rot. Four replications were conducted over a period of two years to determine the efficacy of potassium silicate in inhibiting Phytophthora root rot in avocado nursery trees. Treatments consisted of uninoculated and inoculated untreated trees; uninoculated, silicon treated trees; trees inoculated with *Phytophthora cinnamomi* inoculum treated once before or multiple times after inoculation with silicon; and inoculated, potassium phosphonate (Avoguard®) treated trees. Silicon treated, inoculated trees resulted in the highest fresh and dry root mass compared to all other treatments. This implies that silicon stimulates growth under infectious stress conditions if applied prior to *P. cinnamomi* inoculation. Silicon application did not have a significant effect on canopy condition under conditions of root infection. Root rot in trees treated with silicon was statistically comparable to root rot in uninoculated, untreated control trees, with higher ratings of root regeneration/ new root formation. Trees receiving one silicon application one day before inoculation, harvested 23 weeks after inoculation, did not prove to inhibit Phytophthora root rot effectively, as no significant differences were obtained when compared to the uninoculated, untreated control. Trees receiving one application of silicon but harvested 40 days later had less severe root rot compared to the uninoculated, untreated trees. This indicates the necessity of reapplication of silicon. Timing of reapplication will be determined by soil structure, as silicon leaches easily, deeming the applied silicon as unreachable for plant uptake. Sandy soil will therefore require more regular applications of silicon to maintain the level of resistance required in the host plant. Root rot rating of inoculated trees treated with silicon were in all experiments either statistically comparable to, or better than root rot rating in inoculated trees, treated with potassium phosphonate. These findings are of paramount importance as this implies that potassium silicate may be proposed as a possible alternative control to inhibit the effects of *P. cinnamomi* on avocado trees.

3.2 INTRODUCTION

Phytophthora cinnamomi Rands. is a plant pathogen of global significance as it affects wild and cultivated plants, and is a serious threat to the diversity and structure of natural ecosystems (Wills and Keighery, 1994). This aggressive fungus causes extensive root rot in avocados (*Persea Americana* Mill.), and on average leads to an annual loss of 10% of the world avocado crop, which amounts to several million US$ worldwide (Zentmyer & Schieber, 1991).

Although numerous strategies have been implemented to inhibit Phytophthora root rot including planting resistant rootstocks (Coffey, 1987; Cahill *et al*., 1993; Pegg *et al*., 2002) and biological control (Pegg, 1977; Casale, 1990; Duvenhage and Kotze, 1993), chemical control is still the determining factor to ensure effective inhibition of Phytophthora root rot. Phosphonate fungicides, including fosetyl-Al and its breakdown product phosphorous acid, are highly mobile in plants (Guest *et al*., 1995), and are believed to control *Phytophthora* spp. by a combination of direct fungitoxic activity and stimulation of host defense mechanisms (Guest *et al*., 1995; Hardy *et al.,* 2001). Duvenhage (1994) first reported on the possibility of resistance and found that isolates of *P. cinnamomi* obtained from trees treated with fosetyl-Al or H_3PO_3 were less affected by fosetyl-Al and H_3PO_3 *in vitro* compared to isolates obtained from untreated trees. They concluded that the possibility of resistance exists, and that the mode of action is to be determined to effectively prevent this tendency. It is therefore imperative to obtain new control methods to ensure alternative treatments to be implemented in an alternative control strategy to limit the possibility of resistance to develop.

It is commonly accepted that plants need 16 essential nutrient elements to complete their life cycle (Arnon and Stout, 1939). Epstein (1999) however termed silicon to be quasi-essential, as although plants can complete their life cycle without silicon, soluble siliceous materials impart numerous beneficial effects to plants. Soluble silicon in the soil solution is commonly found as monosilicic acid $Si(OH)_4$, which is easily taken up by plant roots (Epstein, 1994, 1999, 2001). Silicon occurs in living organisms as amorphous silica (SiO_2 nH_2O) and to a lesser extent, soluble silicic acid, the soluble form taken up by plant roots (Fawe *et al*., 1998; Chen *et al*., 2000). Although the physiological and nutritional role of silicon appears to be limited, evidence is accumulating that silicon absorption has numerous benefits for the plant, and in particular, plant protection. Inconsistent results have been found between

different studies on different species where prophylactic properties are concerned. Cucumber (*Cucumic sativus* L.), rose (*Rosa* spp.), sugarcane and rice (*Oryza* spp.) have however received much attention and have been shown to benefit from the application of soluble Si, which leads to disease protection and consequent higher yields (Bowen *et al.,* 1995).

The aim of this study was to evaluate whether the addition of soluble silicon as potassium silicate to *P. cinnamomi* inoculated avocado nursery trees would inhibit fungal infection, and possibly increase plant resistance by activating plant defence mechanisms.

3.3 MATERIALS AND METHODS

3.3.1 Chemicals

Silicon was obtained from Ineos Silicas (Pty) Ltd. and potassium phosphonate (Avoguard®) from Ocean Agriculture, Johannesburg, South Africa.

3.3.2 Experimental Detail

Four replicate greenhouse experiments were conducted over a period of two years to determine the efficacy of potassium silicate in inhibiting Phytophthora root rot in avocado nursery trees. Avocado nursery trees used in the study were screened for the absence of *Phytophthora cinnamomi* by plating out randomly selected root tips on PARPH (Pimaracin-ampicillin-rifampicin-pentachloronitrobenzene-hymexazol) medium selective for *Phytophthora* (Jeffers & Martin, 1986) and identifying any fungal growth microscopically. Trees were thereafter sorted on greenhouse benches and treatments assigned according to a randomized block design.

3.3.2.1 Experiment 1

Twelve-month-old clonal 'Hass' on 'Edranol' seedling avocado rootstocks from Allesbeste Nursery (Duiwelskloof, South Africa) grown in composted pine-bark medium were replanted in 5l plastic pots in steam-sterilized soil acquired from the University of Pretoria experimental farm (Pretoria, South Africa) and allowed to re-establish for two months before the experiment was initiated. Soil texture was 64.9% coarse sand, 13.8% silt and 21.3% clay. The soil pH was 6.3 with 1500ohm resistance and the chemical composition was 4mg.kg^{-1} P, Bray I; 9703mg.kg^{-1} Ca; 533mg.kg^{-1} K; 2783mg.kg^{-1} Mg; 393mg.kg^{-1} Na; 9mg.kg^{-1} Cu; 83mg.kg^{-1} Fe; 459mg.kg^{-1} Mn;

2.163mg.kg^{-1} Zn. Experiment 1 differed from the other experiments with regards to treatment layout. Experiment 1 included a foliar application of a 1% phosphorous acid as a standard treatment with one application two weeks before inoculation and another, one week after inoculation with *P. cinnamomi*. The uninoculated and inoculated silicon treated trees were only treated twice, two weeks before and one week after inoculation.

3.3.2.2 Experiment 2

Eighteen-month-old Velvic avocado rootstocks from Schagen nursery (Schagen, South Africa) grown in composted pine bark were replanted in 5l plastic pots in the same soil as experiment 1 and allowed to re-establish for eight weeks before the experiment was initiated.

3.3.2.3 Experiment 3

Twelve-month-old seedling Duke 7 avocado seedling rootstocks grown in composted pine-bark medium were acquired from Westfalia Technological Services (Tzaneen, South Africa). These trees were replanted in 5l pots in steam-pasteurized soil acquired from a soil supplier and allowed to re-establish for four weeks before the experiment was initiated. The soil texture was 91% coarse sand, 4.4% silt, and 4.6% clay. The pH of the soil used was 5.2 with 1800ohm resistance and the chemical composition was 6mg.kg^{-1} P, Bray I; 198mg.kg^{-1} Ca; 41mg.kg^{-1} K; 54mg.kg^{-1} Mg; 23mg.kg^{-1} Na; 2mg.kg^{-1} Cu; 57mg.kg^{-1} Fe; 31mg.kg^{-1} Mn; 1mg.kg^{-1} Zn.

3.3.2.4 Experiment 4

Eighteen-month-old Velvic avocado rootstocks from Schagen nursery (Schagen, South Africa) grown in composted pine bark were replanted in 5l plastic pots in the same soil as experiment 3 and allowed to re-establish before the experiment was initiated.

3.3.3 Treatments

Treatments consisted of a) *P. cinnamomi* inoculated trees drenched with 20ml.l^{-1} soluble potassium silicate (20.7% silicon dioxide) at the rate of one litre per tree as a once off application; or b) multiple applications of potassium silicate (20.7% silicon dioxide) before and after inoculation (Bekker *et al*., 2006); c) trees treated with potassium silicate and not inoculated; d) inoculated trees treated with potassium phosphonate (Avoguard®); e) trees inoculated and untreated; f) and trees uninoculated and untreated (Table 3.1). Ten replicate trees were assigned to each treatment and

pots were sorted according to a randomized block design on greenhouse benches to ensure even growth. Trees were grown in controlled environment greenhouses (Data given in Appendix A) and watered manually every second day with 300ml water per pot.

3.3.4 Inoculation Procedure

An isolate of *P. cinnamomi* (freshly isolated from infected field grown trees) was obtained from Westfalia Technological Services (Tzaneen, South Africa) and grown on potato dextrose agar (PDA). Inoculum was prepared by soaking 300g red millet seed in 75ml water for 24h in 1L Erlenmeyer flasks, whereafter 75ml filtered V8 juice (Chen & Zentmyer, 1970; Cahill, Bennett, & McComb, 1993) was added to the flasks. Flasks were then autoclaved twice for 45min on two consecutive days, inoculated with twenty *P. cinnamomi* culture (5mm diameter) discs and incubated for three weeks at 25°C. Four equidistant cylindrical holes, 10mm in diameter and 80mm deep, were made in the soil in each pot, at a distance of 50mm from the stem of each tree. Subsequently, 20ml of *P. cinnamomi* millet seed inoculum was placed in each hole, which was then sealed with soil and watered thoroughly. This resulted in each tree receiving a total of 80ml inoculum.

3.3.5 Harvesting and Evaluation

Trees were harvested after five (experiment 1) or 23 weeks (experiment 2,3 & 4) and intact roots and shoots were photographed for each plant. Root condition was assessed using a root rot rating scale of 1 to 5 (1 = roots completely rotten, with no root ball present; 5 = no root rot, with a healthy intact root ball) and a root regeneration rating scale of 1 to 5 (1 = no root regrowth; 5 = copious new root-growth) (Figure 3.2). Representative photographs were also taken of each treatment (Figure 3.3).

Re-isolation of P. *cinnamomi* from the trees after trial completion were only done for experiment 4. Ten root tips from each plant were excised, rinsed in sterile, distilled water and plated out on PARPH medium selective for *Phytophthora.* After incubation for seven days, the plates were examined microscopically and *P. cinnamomi* identified. Data of experiment 4 is presented in Table 3.2. Fresh mass was determined gravimetrically for both roots and shoots of each plant. All plant material was dried in a forced draught oven at 65ºC. Final dry mass was recorded for roots and shoots of

each plant and root: shoot mass ratios on a dry mass basis were subsequently determined.

3.3.6 Canopy Condition

The canopy condition of each tree was rated according to a compiled rating scale from 1 to 5 with 5 = healthy looking tree and 1 = completely wilted/ dead tree. Ratings were done independently by two parties, as well as from photographs taken during harvesting (Figure 3.1). Leaves were counted per plant and leaf area determined with a leaf area scanner (Licor1300, USA). Due to the nutrient solution being too concentrated leaves from these experiments 2 and 3 showed signs of leaf tip burn and in severe instances, leaf drop. Canopy ratings were therefore not done on theses trees in these two experiments.

3.3.7 Data Analysis

All data were analysed using Genstat® 4.23 DE for Windows®. A general analysis of variance was performed for each data set and means. Standard errors of the means and LSD's at the 5% confidence level were calculated.

3.4 RESULTS AND DISCUSSION

Isolation frequency of *P. cinnamomi* from uninoculated, untreated control trees and trees treated only with silicon were zero, indicating that the growth medium was free of pathogenic inoculum (Table 3.2). Root tips from inoculated, untreated trees had a 90% isolation frequency of *P. cinnamomi,* indication the virulence of the fungus as an inoculum. Root tips of inoculated and potassium phosphonate treated trees, inoculated, silicon treated trees and trees treated with silicon applied one day before inoculation had statistically similar infection rates, indicating the effect of silicon on root infection to be statistically similar to that obtained through potassium phosphonate treatment. Trees receiving silicon one day before inoculation tended to have a lower incidence of P. cinnamomi than that of inoculated and potassium phosphonate treated trees and inoculated, silicon treated trees, although this was not statistically significant.

3.4.1 Root Rot and Regeneration

In experiment 1, trees which were inoculated with *P. cinnamomi* and not treated with silicon or potassium phosphonate, had significantly more root rot than all other treatments (Table 3.3). *P. cinnamomi* inoculated trees treated with either 1% phosphorous acid (root rot rating = 4.67) or drenched with potassium silicate and inoculated with *P. cinnamomi* (root rot rating = 4.60) were statistically comparable to the uninoculated control (root rot rating = 5.00). Results indicated no significant differences in root regeneration between treatments in experiment 1.

In experiment 2, root rot in trees that received a silicon application one day before inoculation (root rot rating = 1.20) did not differ significantly from the inoculated untreated control (root rot rating = 1.00). However, root rot in these two treatments had significantly low ratings when compared to all other treatments. Roots from trees receiving only silicon (root rot rating = 4.20), and uninoculated, untreated tree roots (root rot rating = 3.90) had the lowest root rot rate. Root rot in inoculated, silicon treated trees (root rot rating = 3.30) were low, and comparable to root rot in uninoculated, untreated control roots. This rating was significantly better than for phosphorous acid-treated trees. Although there was no significant difference between treatments with regards to root regeneration, there was a trend in silicon treated trees as well as potassium phosphonate treated trees to have healthier/ uninfected roots compared to the inoculated, untreated tree roots.

Root rot in potassium phosphonate treated trees was more severe in experiment 3 (root rot rating = 1.50). Inoculated, silicon treated trees (root rot rating = 2.88), trees treated one day before inoculation (root rot rating = 2.30), and inoculated, untreated trees (root rot rating = 2.10) were statistically comparable with regards to root rot. Root rot of uninoculated, untreated trees (root rot rating = 3.50) corresponded to that of silicon treated trees (root rot rating = 3.20), and were the least affected by Phytophthora root rot. Although no significant differences were observed between treatments with regards to root regeneration, silicon treated trees tended to have healthier roots compared to other treatments.

There was not as marked a difference between treatments in experiment 4 with regards to root rot compared to other experiments. Both one (root rot rating = 1.80) and repeated silicon applications (root rot rating = 1.70) in conjunction with *P. cinnamomi* inoculation did not result in an inhibition of root rot development. Root rot in these treatments was statistically comparable to that of the inoculated, untreated

control (root rot rating = 1.66). Root rot in uninoculated, untreated trees (root rot rating = 3.44) and silicon treated trees (root rot rating = 3.80) were statistically comparable, and less pronounced compared to that of the inoculated, untreated trees. Regenerated roots were more pronounced in uninoculated, untreated (root regeneration = 4.00), inoculated and potassium phosphonate treated (root regeneration = 3.30) and silicon treated tree roots (root regeneration = 4.00) than the inoculated, untreated (root regeneration = 1.78), and inoculated, silicon treated trees (root regeneration = 1.70). Trees treated with silicon one day before inoculation with *P. cinnamomi* did not differ significantly from any treatment with regards to root regeneration.

In all experiments, root rot of the inoculated, untreated trees were significantly more severe than that of the uninoculated, untreated trees, indicating the successful infection of nursery trees after inoculation. Except for experiment one, root rot in trees treated with silicon were statistically similar to root rot in uninoculated, untreated control trees and these trees had similar or higher levels of root regeneration.

Soluble silicon polymerizes rapidly, resulting in insoluble silicon compounds (Epstein, 2001). For effective disease suppression silicon must therefore be applied continuously (Bowen *et al.*, 1995). This seems to be confirmed by results from the present study, as trees receiving one silicon application one day before inoculation did not exhibit improved resistance to Phytophthora root rot. Ghanmi *et al.* (2004) reported that although the application of silicon to *Arabidopsis thaliana* prior to *Erysiphe cichoracearum* D.C. inoculation did not prohibit fungal penetration and infection, the rate of disease development was altered.

In the current study, during the 23 weeks after inoculation, no significant differences were obtained between the inoculated, untreated control trees and those treated one day before inoculation with regards to root rot for experiments 2, 3 or 4. In experiment 1 where harvesting took place 40 days after inoculation, root rot was more severe in the inoculated, untreated trees. It could be that the disease developed slower in the once-off silicon treated trees, but this difference could not be detected 23 weeks after inoculation.

In the experiments conducted in the heavier soils (higher clay content) (experiments 1 & 2), inoculated, silicon treated trees showed statistically similar root rot ratings than the uninoculated controls. However, in sandy soils (experiments 3 & 4) the trend was

different, and inoculated, silicon treated trees had significantly higher levels of root rot (a lower root rating) compared to the uninoculated, untreated trees. This could be due to the cation exchange capacity related to the clay percentage in each soil type. The clay soil contained 21.3% clay compared to the sandy soil containing only 0.6% clay. Matichenkov and Bocharnikova (2001) reported that soluble silicon compounds form complexes with Al, Fe and organic compounds. However, if silicates from siliceous-based fertilizers are not bound by the soil, these soluble nutrients leach from the plant available horizons, deeming these elements unavailable for uptake (Tokunaga, 1991). In the present study, it is believed that the applied potassium silicate leached from the pots (in the sandy soils in experiment 3 & 4) limiting the available silicon for plant protection and uptake. The effect of applied silicates will therefore be more pronounced in soils with high clay content.

Phosphonate fungicides, including potassium phosphonate, fosetyl-Al and its breakdown product phosphorous acid are believed to control *P. cinnamomi* by a combination of direct fungitoxic activity and stimulation of host defence mechanisms (Guest *et al*., 1995; Hardy *et al.,* 2001) and is currently the preferred option of control of Phytophthora root rot in avocados (Hardy *et al.,* 2001). Silicon application inhibited Phytophthora root rot to levels similar to, or better than those obtained by potassium phosphonate applications. Wutscher (1989) reported that in young orange trees, silicon accumulates in young leaves and feeder roots, leading to protection of plant roots from infection. Root rot data in the present study however tends to reiterate the findings of Chérif *et al*. (1994) who stated that silicon deposited on the surface of roots makes plant cells less susceptible to enzymatic degradation by fungal pathogens. Application of silicon to partially resistant and susceptible rice cultivars to control leaf and neck blast led to a decrease in disease severity levels similar to those levels found in resistant cultivars not treated with silicon, or better than that of commercial fungicide treated plants (Seebold *et al*., 2000, 2004)

These findings are of paramount importance to the avocado industry as it implies that potassium silicate may be proposed as a possible alternative control for *P. cinnamomi* root rot on avocado nursery trees.

3.4.2 Canopy Condition

Velvic rootstock trees grown in sandy soils and inoculated with *Phytophthora cinnamomi* had lower canopy ratings (i.e. poorer canopy conditions) than the

uninoculated, untreated control trees (canopy rating = 4.89) and uninoculated, silicon treated (canopy rating = 4.7) trees (Table 3.4). Silicon and phosphonate treatments of inoculated trees could not improve tree canopy health relative to the uninoculated, untreated control. Root rot of all the inoculated treatments were more severe than the uninoculated, untreated control and uninoculated, silicon treated trees (Table 3.3). No significant differences could be seen between treatments with regards to leaf area or number of leaves per plant. There was, however, a trend present in terms of the number of leaves per tree as uninoculated treatments generally had higher number of leaves than the inoculated treatments. This corresponds with the report by Ploetz and Parrado (1988) who stated that a moderate tolerance to Phytophthora root rot is often observed in avocado trees where infection has occurred without degradation of aboveground tree health. Reduced photosynthesis, transpiration and stomatal conductance can however be detected in root rot affected trees before visible aboveground symptoms appear (Sterne *et al*., 1978; Ploetz and Schaffer, 1989). Foliage becomes wilted and chlorotic, leaves fall and branches rapidly die back depending on root rot severity (Ploetz and Parrado, 1988). Results from the current study confirm this as canopy health assessment correlated with root rot severity.

3.4.3 Plant Mass and Root: Shoot Ratios

Numerous physiological processes are affected by phytopathogens. Infected plants usually grow slower than corresponding healthy plants and internodes are generally shorter. Once infected, most plants are less vigorous, have smaller root- and canopy systems than healthy plants and leaf development is usually delayed (Russell, 1981). Because pathogens affect physiological processes including photosynthesis, it is likely that changes in the amount of biomass and nutrients accumulated might also occur. Ishiguro (2003) reported up to 67% root loss and 55% aerial biomass loss due to *Phytophthora cinnamomi* infection of oak and chestnut species. Plant growth, and especially carbon partitioning between organs, is poorly understood and appreciable errors are made when estimating carbon partitioning as a result of photosynthesis alone. Numerous other factors play a role including plant health and nutrient content of plant material ranging between 5-20% of the dry mass (Farrar, 1993). Morikawa and Saigusa (2003) ascertained that if silicon was added as a soil drench to blueberry (*Vaccinum corymbosus* cv. bluecrop) cuttings, the silicon concentration in leaves of treated plants were 85 times higher than any essential element, with a mean

concentration of 60mg.g^{-1} dry weight. In the current study, experiment 2 was the only experimental repeat that resulted in significant differences between treatments with regards to root mass (Table 3.5). Root fresh mass, experiment 2, of the inoculated, untreated control (24.08g) was significantly lower compared to all other treatments. Fresh root masses of uninoculated, untreated trees (39.58g); inoculated, potassium phosphonate treated trees (40.98g); trees treated with silicon one day before inoculation (43.42g); and silicon treated trees (39.59g) were statistically similar to each other, but differed significantly from the inoculated, silicon treated (58.11g) trees with regards to fresh root mass, the latter having the highest average fresh root mass. Although not always statistically significant, results indicated the fresh root mass of inoculated, untreated trees to be the lowest compared to other treatments in all experiments except for experiment 3, where the inoculated, potassium phosphonate treated trees (59.46g) had the lowest root fresh mass. This was also true for root dry masses for all experiments except for experiment 3 where potassium phosphonate treated trees (15.50g) had the lowest dry root mass compared to the other treatments. Inoculated, silicon treated trees showed the highest average fresh and dry root mass compared to all other treatments for all four experiments although this difference was not always significant. This implies that silicon either stimulates growth or imparts some form of protection to avocado roots if applied prior to *P. cinnamomi* inoculation.

This protection has long been thought to be that of a physical barrier due to strengthening of the cell wall (Vance *et al*., 1980; Aist, 1983; Nicholson and Hammerschmidt, 1992). However, recent evidence points towards the activation of an induced systemic resistance (ISR) mechanism in the plant. Fawe *et al*. (1998) proposed that silicon stimulates phytoalexin formation in response to fungal attack. It could therefore be possible to further exploit this protection if soluble silicon is applied even earlier than 10 days before inoculation.

There were no significant differences between the uninoculated, untreated (8.96g) and inoculated, untreated controls (8.53g) with regards to leaf dry mass for experiment 1. These treatments did not differ from the uninoculated, silicon treated (11.26g) trees, but were significantly different to all other treatments. Inoculated, potassium phosphonate treated (12.61g), uninoculated, silicon treated (11.26g) and inoculated,

silicon treated (13.46g) trees did not differ with regards to leaf dry mass. In experiment 1, leaf dry mass of trees treated with silicon one day before inoculation (15.28g) was however significantly higher than all other treatments. In experiment 3, leaf fresh mass of inoculated, potassium phosphonate (29.27g) treated trees was statistically comparable to trees treated with silicon one day before inoculation (42.47g), but significantly lower than all other treatments.

Uninoculated, untreated control trees (214.40g) and inoculated, silicon treated trees (204.25g) in experiment 4 were significantly higher when compared to all the other treatments with regards to leaf fresh mass. With regards to root dry mass, trees (experiment 4) treated with silicon one day before inoculation (66.61g) were statistically similar to inoculated, potassium phosphonate treated trees (64.43g), but significantly different from uninoculated, untreated control (88.39g) and inoculated, silicon treated (78.63g) trees. Leaf dry mass of inoculated, untreated control (72.19g) and silicon treated (68.27g) trees did not differ from any treatment.

Although differences between treatments were not consistently significant, leaf fresh mass of experiments grown in sandy soil were the highest in uninoculated, untreated controls, whilst the inoculated, potassium phosphonate treated trees resulted in the lowest leaf fresh mass. For experiments grown in sandy soils, inoculated, potassium phosphonate treated trees had the lowest leaf dry mass compared to the other treatments.

In experiment 1, the root: shoot dry mass ratio of inoculated, silicon treated trees (1.77) was significantly higher that all other treatments (Table 3.6). There were no other significant differences between treatments with regard to root: shoot mass ratios between all treatments and in both soils. Root: shoot ratios were generally higher in sandy than in clay soils.

Sterne *et al*. (1977) reported the effect of soil structure on Phytophthora root rot disease development to be determinant of the level of disease severity. This in turn creates an imbalance in the source-sink relationship between plant parts. Higher root: shoot ratios indicate a healthy root system. In the current study, clay soils led to lower root compared to leaf masses, but in contrast, trees grown in sandy soils did not experience such a high level of root rot, leading to higher root: shoot ratios. These results corroborate the statements made by Sterne *et al*. (1977), suggesting a heightened disease combating strategy to be implemented in avocado orchards

situated in soils containing high clay percentages as clay soils have greater water retention properties which aid in the hastily spread of the disease.

3.5 CONCLUSION

Potassium silicate application to *Phytophthora cinnamomi* infected trees resulted in effective inhibition of root rot, similar to levels obtained by commercial application of potassium phosphonate (Avoguard®). Potassium silicate application imparts protection to roots under infection pressure, and induces new root growth. The beneficial effect of potassium silicate is however dependant on reapplication, as these beneficial effects are lost if control is reliant on only one application. The timing of reapplication will be determined by, amongst other factors, the growth medium characteristics, as silicon leaches easily in media with low CEC, rendering the applied silicon as unavailable for plant uptake. Sandy soil will therefore necessitate more regular applications of silicon to maintain the level of disease suppression reached in the host plant.

Root rot of inoculated trees treated with silicon were, in all experiments, either statistically comparable to, or better than root rot in inoculated trees treated with potassium phosphonate (the standard commercial fungicide) implying that silicon does induce some form of resistance in the plant suppressing fungal penetration and infection. These findings are of paramount importance as this implies that potassium silicate may be proposed as an alternative control to inhibit the effects of *P. cinnamomi* on avocado trees.

Silicon treated trees had the highest fresh and dry root mass compared to all other treatments. This implies that silicon either stimulates growth or imparts some form of protection to avocado roots if applied prior to *P. cinnamomi* inoculation. Leaf fresh mass of inoculated, silicon treated trees was similar to that of uninoculated, untreated trees. For experiments grown in sandy soils, inoculated, potassium phosphonate treated trees resulted in the lowest leaf dry mass compared to all the other treatments.

Drawing on this knowledge, where *P. cinnamomi* infection is already prevalent in the field, it is expected that protection of large trees, as a result of drenching the soil with soluble silicon, would be incremental. In previous studies it has been proposed that silicon increases diffusive resistance, or decreases the effect of infection on diffusive resistance, and if therefore applied after infection, may lead to increased diffusive resistance over a longer period of time.

3.6 LITERATURE CITED

AIST, J.R., 1983. Structural responses as resistance mechanisms. In: J.A. Bailey & B.J. Deverall (Eds.), The Dynamics of Host Defence, Academic Press, Sydney, pp 33-70.

ANON, 2004. *Phytophthora cinnamomi*: Diagnostic protocols for regulated pests. *OEPP/EPPO Bulletin* 34, 201-207.

ARNON, D.I. & STOUT, P.R., 1939. The essentiality of certain elements in minute quantities for plants with special reference to copper. *Plant Physiol*. 14, 371-375.

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

BOWEN, P.A., EHRET, D.L. & MENZIES, J.G., 1995. Soluble silicon: It's role in crop and disease management of greenhouse crops. *Plant Dis.* 79(4), 329-336.

CAHILL, D.M., BENNETT, I.J. & McCOMB, J.A., 1993. Mechanisms of resistance to *Phytophthora cinnamomi* in clonal, micropropagated *Eucalyptus marginata*. *Plant Pathol*. 42, 865-872.

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.

CHEN, J., CLADWELL, R.D., ROBINSON, C.A. & STEINKAMP, R., 2000. Silicon: The estranged medium element. University of Florida, Florida Co-operative Extension Service, Florida.

CHEN, D.W. & ZENTMYER, G.A., 1970. Production of sporangia by *Phytophthora cinnamomi* in axenic culture. *Mycol.* 62, 397-402.

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.

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

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

EPSTEIN, E., 1994. The anomaly of silicon in plant biology. *Proc. Natl. Acad. Sci. USA 91*, 11-17.

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

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

FAWE, A., ABOU-ZAID, M., MENZIES, J.G. & BELANGER, R.R., 1998. Silicon mediated accumulation of flavanoid phytoalexins in cucumber. *Phytopathology* 88, 396-401.

FARRAR, J.F., 1993. Photosynthesis and Production in a Changing Environment, a Field and Laboratory Manual, Chapman & Hall, London.

GHANMI, D., McNALLY, D.J., BENHAMOU, N., MENZIES, J.G. & BĖLANGER, R.R., 2004. Powdery mildew of *Arabidopsis thaliana*: A pathosystem for exploring the role of silicon in plant-microbe interaction. *Physiol. Mol. Plant Pathol*. 64, 189-199.

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 phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Austr. Plant Pathol*. 30, 133-139.

ISHIGURO, K., 2003. Review of research in Japan on the roles of silicon in conferring resistance against rice blast. In: L.E. Datnoff, G.H. Snyder & G.H. Korndörfer (Eds.), Silicon in Agriculture, Elsevier, Amsterdam, pp 277-287.

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

MATICHENKOV, V.V. & BOCHARMIKOVA, E.A., 2001. The relationship between silicon and soil physical and chemical properties, In: L.E. Datnoff, G.H. Snyder, & G.H. Korndorfer (Eds.), Silicon in Agriculture, Elsevier Science.

MORIKAWA, C.K. & SAIGUSA, M., 2003. Mineral composition and accumulation of silicon in tissues of blueberry (*Vaccinum corymbosus* cv. Bluecrop) cuttings, *Plant and Soil* 258, 1-7.

NICHOLSON, R.L. & HAMMERSCHMIDT, R., 1992. Phenolic compounds and their role in disease resistance. *Annu. Rev. Plantpathol*. 30, 369-389.

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

PEGG, K.G., COATES, L.M., KORSTEN, L. & HARDING, R.M., 2002. Foliage, Fruit and Soilborne diseases. In: A.W. Whiley, B. Schaffer, & B.N. Wolstenholme (Eds.), Avocado: Botany, Production and Uses, CABI-Publishing, California, pp 432.

PLOETZ, R.C. & PARRADO, J.L., 1988. Quantisation and detection of *Phytophthora cinnamomi* in avocado production areas of South Florida. *Plant Dis.* 72, 981-984.

PLOETZ, R.C. AND SCHAFFER, B, 1989. Effect of flooding and *Phytophthora* root rot on net gas exchange and growth of avocado. *Phytopathol*. 79, 204-208.

RUSSELL, G.F., 1981. Disease and crop yield: the problems and prospects for agriculture. In: P.G. Ayres (Ed.), Effects of Disease in the Physiology of the Growing Plant, Cambridge University Press, London.

SEEBOLD, K.W. JR., DATNOFF, L.E., CORREA-VICTORIA, F.J., KUCHAREK, T.A. & SNYDER, G.H., 2000. Effect of silicon rate and host resistance on blast, scald and yield of upland rice. *Plant Dis.* 84 (8), 871-876.

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

STERNE, R.E., ZENTMYER, G.A. & KAUFMAN, M.R., 1977. The influence of matrix potential, soil texture, and soil amendment on root disease caused by *Phytophthora cinnamomi. Phytopahtthol*. 67, 1495-1500.

STERNE, R.E., KAUFMAN, M.R. & ZENTMYER, G.A., 1978. Effect of *Phytophthora* root rot on water relations of avocado: Interpretation with a water transport model. *Phytopathol.* 68, 595-602.

TOKUNAGA, Y., 1991. Potassium silicate: A slow release potassium fertilizer. *Fert. Res*. 30, 55.

VANCE, C.P., KIRK, T.K. AND SHERWOOD, R.T., 1980. Lignification as a mechanism of disease resistance. *Annu. Rev. Phytopathol.* 18, 259-288.

WILLS, R.T. & KEIGHERY, G.J., 1994. Ecological impact of plant disease on communities. *J. Royal Soc. West. Aust.* 77, 127–131.

WUTSCHER, H.K., 1989. Growth and mineral nutrition of young orange trees grown with high levels of silicon. *Hort. Sci.* 24 (2), 175-177.

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

Table 3.1: Treatments applied to avocado nursery trees grown in a greenhouse of three experiments to determine the effect of potassium silicate applications on *Phytophthora cinnamomi* root rot. Experiment 1 differed from the other experiments by having a foliar application of a 1% phosphorous acid as a standard treatment with one application two weeks before inoculation and another, one week after inoculation with *P. cinnamomi*. The uninoculated and inoculated silicon treated trees were only treated twice, two weeks before and one week after inoculation with potassium silicate.

	Week 1	**Week 2**	**Week 4**	**Week 7**	**Week 10**	**Week 13**	**Week 23**
Silicon 1day before inoculation	-	Silicon treated 1 day before Inoc.	-	-	-	-	Harvesting & Evaluation
Uninoculated, untreated control	-	-	-	-	-	-	Harvesting & Evaluation
Inoculated, untreated control	-	B	-	-	-	-	Harvesting & Evaluation
Inoculated & phosphorous acid	C	B	C	C	C	C	Harvesting & Evaluation
Silicon	A	-	A	A	A	A	Harvesting & Evaluation
Inoculated & Silicon	A	B	A	A	A	A	Harvesting & Evaluation

A Application of 1l of 20ml.l^{-1} soluble silicon/pot

B Inoculation with *P. cinnamomi*

C Soil drench with potassium phosphonate (In experiment 1 this was a foliar application of a 1% phosphorous acid)

Table 3.2: Incidence of *Phytophthora cinnamomi* in the roots of avocado nursery trees either uninoculated or inoculated and treated with soluble potassium silicate or potassium phosphonate of experiment 4.Values followed by the same letter do not differ significantly at 5% confidence interval.

Treatment	**Incidence***
Silicon 1day before inoculation	4.0b
Uninoculated, untreated control	0.0a
Inoculated, untreated control	9.0c
Inoculated & phosphorous acid	5.9b
Silicon	0.0a
Inoculated & Silicon	5.2b

* From the ten root pieces plated out, the number of root pieces rendering positive *P. cinnamomi* isolates

Table 3.3: Effect of treatments with silicon and potassium phosphonate on root rot and root regeneration of *Phytophthora cinnamomi* inoculated avocado nursery trees in the greenhouse. Values in each column followed by the same letter do not differ significantly at 5% confidence interval.

Treatment	Clay				Sandy			
	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Root rot*	Root ** regeneration	Root rot	Root regeneration	Root rot	Root regeneration	Root rot	Root regeneration
Silicon 1day before inoculation	3.83b	1.33a	1.20a	2.20a	2.30b	2.00a	1.80a	2.50ab
Uninoculated, untreated control	5.00c	2.67a	3.9cd	1.40a	3.50c	2.38a	3.44b	4.00b
Inoculated, untreated control	3.33a	1.67a	1.00a	0.89a	2.10b	2.00a	1.66a	1.78a
Inoculated & potassium phosphonate	4.67c	1.33a	2.40b	2.20a	1.50a	1.10a	2.40a	3.30b
Silicon	3.60ab	2.00a	4.20d	2.60a	3.20c	3.10a	3.80b	4.00b
Inoculated & Silicon	4.60c	1.17a	3.30c	1.60a	2.88b	2.38a	1.70a	1.70a

* Root rot assessed according to a rating scale of 1 to 5 (1 = roots completely rotten; and 5 = no root rot)

** Root regeneration assessed according to a rating scale of 1 to 5 (1 = no root regrowth; 5 = healthy new root formation)

Table 3.4: Effect of silicon and potassium phosphonate treatments on growth parameters of *Phytophthora cinnamomi* inoculated avocado nursery plants (cv. Velvic) in the greenhouse. Values in each column followed by the same letter do not differ significantly at 5% confidence interval.

Treatment	Canopy condition *	Av. Leaf area (cm^2)	No. of leaves per plant
Silicon 1day before inoculation	3.70a	3749.40a	30.80a
Uninoculated, untreated control	4.89b	3605.78a	41.11a
Inoculated, untreated control	3.78a	3526.00a	35.56a
Inoculated & K-phosphonate	3.70a	3178.60a	28.70a
Uninoculated, Silicon treated	4.70b	3889.80a	42.40a
Inoculated & Silicon	3.60a	3137.40a	32.30a

* Canopy condition assessed according to a rating scale (1 = permanently wilted leaves; 5 = healthy leaves, no signs of wilting)

Table 3.5: Effect of treatments with silicon and potassium phosphonate on root and shoot fresh (FM) and dry (DM) mass (g) of *Phytophthora cinnamomi* inoculated avocado nursery trees in the greenhouse. Values in each column followed by the same letter do not differ significantly at 5% confidence interval.

Treatment	Clay Soil						Sand Soil							
	Experiment 1		Experiment 2				Experiment 3				Experiment 4			
	Root	Shoot	Root		Shoot		Root		Shoot		Root		Shoot	
	DM	DM	FM	DM	FM	DM	FM	DM	FM	DM	FM	DM	FM	DM
Silicon 1day before inoculated	11.50a	15.28c	43.42b	14.00a	47.16a	18.15a	77.34a	23.33a	42.47ab	17.99a	118.84a	49.67a	173.54ab	66.61a
Uninoculated, untreated control	11.61a	8.96a	39.58b	17.89a	45.12a	18.32a	99.09a	27.75a	55.62b	20.52a	150.34a	64.29a	214.40b	88.39b
Inoculated, untreated control	9.78a	8.53a	24.08a	10.71a	46.69a	18.70a	65.24a	19.18a	50.16b	22.59a	113.60a	48.25a	172.23a	72.19ab
Inoculated & K-phosphonate	11.85a	12.61b	40.98b	14.41a	54.61a	22.24a	59.46a	17.50a	29.27a	15.33a	135.16a	56.40a	160.56a	64.43a
Uninoculated, Silicon treated	12.10a	11.26ab	39.59b	15.22a	43.34a	18.56a	82.71a	21.16a	44.09b	17.80a	128.07a	50.59a	163.35a	68.27ab
Inoculated & Silicon	12.83a	13.46bc	58.11c	17.95a	55.30a	22.59a	107.56a	28.06a	43.59b	17.69a	172.29a	69.75a	204.25b	78.63b

Table 3.6: Effect of treatments with silicon and potassium phosphonate on fresh and dry root: shoot (R:S) mass rations of *Phytophthora cinnamomi* inoculated avocado nursery trees in the greenhouse. Values followed by the same letter do not differ significantly at 5% confidence interval.

Treatment	Clay Soil				Sand Soil			
	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	R:S Fresh	R:S Dry	R:S Fresh	R:S Dry	R:S Fresh	R:S Dry	R:S Fresh	R:S Dry
Silicon 1day before inoculation	1.14a	0.96a	1.00a	0.80a	1.91a	1.30a	1.64a	0.72a
Uninoculated, untreated control	0.72a	0.81a	1.02a	0.99a	1.81a	1.32a	1.24a	0.86a
Inoculated, untreated control	1.41a	0.94a	0.58a	0.62a	1.41a	0.87a	1.31a	0.90a
Inoculated & K-phosphonate	1.18a	1.10a	0.76a	0.67a	2.61a	1.18a	1.35a	0.73a
Uninoculated, Silicon treated	1.37a	1.05a	1.31a	0.87a	2.41a	1.63a	1.52a	0.73a
Inoculated, Silicon treated	1.04a	1.77b	1.05a	0.87a	1.88a	1.17a	1.61a	0.66a

Figure 3.1: Representative trees from the various treatments illustrating the canopy condition of avocado trees inoculated with *P. cinnamomi* during experiment 4. From left to right: uninoculated, silicon treated tree(a); inoculated, potassium phosphonate treated tree (b); uninoculated, untreated tree (c); inoculated, untreated tree (d); tree treated one day before inoculation with silicon (e); and inoculated and silicon treated tree (f).

Figure 3.2: Root rot assessment of harvested avocado trees according to a root rot rating scale of 1 to 5 (1 = roots completely rotten, with no root ball present; 5 = no root rot, with a healthy intact root ball).

Figure 3.3: Representative samples of the root system of avocado trees inoculated with *P. cinnamomi* and subjected to various treatments from experiment 4. From left to right: uninoculated, untreated tree (a); inoculated, untreated tree (b); uninoculated, silicon treated tree (c); inoculated, silicon treated tree (d); inoculated, potassium phosphonate treated tree (e); and a tree treated one day before inoculation with silicon (f).