EFFICACY OF WATER SOLUBLE SILICON FOR CONTROL OF *PHYTOPHTHORA CINNAMOMI* ROOT ROT OF AVOCADO

by

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DECLARATION

I hereby certify that this seminar is my own work, except where duly acknowledged. I also certify that no plagiarism was committed in writing this thesis.

Signed _____

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ABSTRACT

Avocado production worldwide has been under great pressure due to *Phytophthora cinnamomi* Rands. root infection, which ultimately leads to tree death. In an attempt to find a viable alternative treatment for phosphonate fungicides against Phytophthora root rot of avocado, studies have been conducted to determine the effect of potassium silicate application on *P. cinnamomi* root rot development in both avocado nursery trees and trees in the field.

The direct inhibitory effect of potassium silicate was tested *in vitro*, and results indicated it to have a dose-related inhibitory effect on *P. cinnamomi* growth at concentrations as low as 5ml.l^{-1}. By means of greenhouse trials it was demonstrated that potassium silicate either stimulates root growth or imparts some form of protection to avocado roots if applied prior to *P. cinnamomi* inoculation, as inoculated, silicon treated trees resulted in the highest fresh and dry root mass compared to all other treatments. Potassium silicate application inhibited Phytophthora root rot in inoculated greenhouse trees effectively, and in all repetitions either resulted in similar, or better root rot ratings compared to the current control treatment potassium phosphonate. The beneficial effects of potassium silicate was however lost in treatments receiving only one silicate application, and reapplication of potassium silicate is essential. 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 nursery trees. Three potassium silicate soil drench applications resulted in significantly higher root densities compared to the control and potassium phosphonate (Avoguard®) treatments. These results correlated well with tree canopy ratings. All potassium silicate soil drench treatments resulted in lower disease ratings (canopy condition). Three applications (Si x 3) of soluble potassium silicate per season resulted in significantly higher phenolic root concentrations compared to the untreated control. Crude phenolic concentrations obtained in the Si x 3 treatment samples were similar to that of potassium phosphonate. These results indicate that potassium silicate application to avocado trees under *P. cinnamomi* infectious conditions increase total phenolic content of avocado root tissue, suggesting potassium silicate to have an indirect inhibitory effect on *P. cinnamomi* infection of avocado trees.

INTRODUCTION

The avocado (*Persea Americana* Mill.) is an evergreen, polymorphic tree species, originating in a broad geographic region from the eastern highland of Mexico to the pacific coast of central America (Knight, 2002). The genus *Persea* (Clus.) belongs to the family *Laureaceae* (Scora *et al.*, 2002), being amongst the families *Proteaceae* and *Magnoliaceae*, one of the oldest plant families on earth. Three distinct, ecologically separate sub-species of the avocado have been termed by Popenoe (1920) as Guatemalan, Mexican and West Indian or Antillean. Avocado production is limited to the tropical and subtropical regions of the world, and the fruit is exported worldwide (Knight, 2002). In South Africa avocado production is confined to the Limpopo and Mpumalanga provinces in the north and north-east of the country, and to a lesser extent to the frost-free lowland coastal belts and cooler midlands of KwaZulu-Natal (Lovegrove and Hooley, 2000).

Phytophthora root rot has been the main limiting factor to successful avocado production in countries such as Australia, South Africa and the USA. Phytophthora root rot, caused by the fungus *Phytophthora cinnamomi* Rands. (Hardy *et al*., 2001), is the most important and destructive disease of not only avocados worldwide, but over 1000 plant types (Zentmyer, 1980), including pineapple, macadamia, peach, pear, kiwi fruit, chestnut, eucalyptus, and many native Australian and South African plants (Pegg *et al*., 2002). In avocado, it attacks and kills trees of all ages, from nursery trees to large bearing trees through the destruction of feeder roots. Its reproduction, growth and spread are favoured by free soil-water. Movement of infected soil therefore plays an important role in the spread of this fungus (Hardy *et al.,* 2001). It has been postulated by Arentz and Simpson (1986) and Linde *et al*. (1997) that the fungus originated in Papua New Guinea, and was moved by the activities of people into other tropical and subtropical regions of the world.

Phytophthora cinnamomi causes rot of feeder roots, leading to the death of host plants (Anon, 2004). Infection is mostly limited to the feeder roots, which become black and brittle and eventually die off. Feeder roots may be difficult to find under trees with advanced root rot symptoms (Pegg *et al*., 2002), and this dieback of feeder roots may impose severe water stress on the tree, even in moist soils. Visible symptoms include

wilted and chlorotic foliage and eventually defoliation and dieback of branches, depending on root rot severity.

Numerous control measures have been implemented to control root rot, but a well-managed program is necessary to ensure disease suppression. Biological control of *P. cinnamomi* has been investigated by numerous authors (McLeod *et al.*, 1995; Duvenhage and Kotze, 1993; Casale, 1990; Pegg, 1977) and shows promise for reducing root rot (Pegg *et al.*, 2002). Host resistance is an important method of reduction of Phytophthora root rot (Coffey, 1987), with some rootstocks expressing tolerance to root rot by the rapid regeneration of active feeder roots while in others the progress of infection in the root is inhibited (Phillips *et al.*, 1987). Cahill *et al.* (1993) reported increased levels of lignin and phenolics after inoculation with *P. cinnamomi*, suggesting phenolic compounds play a role in plant resistance to Phytophthora root rot. Wehner *et al.* (1982) and Brune and van Lelyveld (1982) reported on the sensitivity of pathogens to antifungal substances in avocado tissue. They concluded that some phenolics act as antioxidants during induced resistance and these phenolic antioxidants are present in plant lipophylic regions.

Chemical control however 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 *et al.*, 1995). It is believed to control *Phytophthora* spp. by a combination of direct fungitoxic activity and stimulation of host defence mechanisms (Guest *et al.*, 1995; Hardy *et al.,* 2001). Darvas *et al.* (1983, 1984) and Darvas (1983) first reported on the use of a trunk injection method obtaining “outstanding control” of *P. cinnamomi* by fosetyl-Al. This remains to date the most effective application method of phosphonate fungicides in avocado. Subsequently, Duvenhage (1994) was the first to report 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 affected by fosetyl-Al and H_3PO_3 *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 industry.

Research on the role of silicon in plant physiology depended on the advent of the solution culture technique (Epstein, 1999). Numerous functions have been attributed to silicon including improvement of mechanical properties (soil penetration by roots, stature, resistance to lodging, exposure of leaves to light), enhancement of growth and

yield, resistance to salinity, reduction of transpiration and resistance to drought stress. As discussed in more detail later, a number of studies demonstrated suppression of a range of diseases by means of silicon application. Mechanisms include induction of plant enzymes and increased resistance arising from the deposition in amorphous silica, or accumulation of phytotoxic phenolic compounds (Fauteux *et al*., 2005).

The present study was initiated to determine whether the application of potassium silicate to *P. cinnamomi* infected trees would suppress the disease. The objectives of the study were:

- To determine whether potassium silicate has a direct effect on fungal growth *in vitro*.
- To investigate the possibility of potassium silicate having an indirect effect on disease development, through the alteration of the plant's biochemical composition.
- To establish if the application of potassium silicate to avocado nursery and field grown trees suppresses root rot development and spread in avocado roots.
- To determine, if suppression is observed, the concentrations, dosage rates and timing of potassium silicate applications to avocado orchards for *P. cinnamomi* suppression.
- To investigate the biochemical composition of plants with specific reference to phenolic concentrations in avocado root tissue to ascertain if potassium silicate leads to an increase or alteration of the phenolic content of plant cell content.

The primary applied objective of these investigations was to develop an alternative control strategy for the avocado industry to alleviate the stress of resistance to phosphonate fungicides as the only chemical control method currently used.

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CHAPTER 1

LITERATURE REVIEW

1.1 THE PLANT: *PERSEA AMERICANA*

1.1.1 History and Distribution

The avocado (*Persea americana* Mill.) is a polymorphic tree species that originated in a broad geographical region stretching from the Pacific coast of Central America through Guatemala to the eastern and central highlands of Mexico (Popenoe, 1920). Three distinct and separate taxa or sub-species now termed the Guatemalan, Mexican and West Indian or Antillean races have been selected over millennia (Knight, 2002). Although little is known or recorded about the introduction of avocado to South Africa, it is accepted that the first trees were West Indian race-seedlings planted on the coastal strip around Durban in the late 19th century (Landman, 1930). Fruits from these trees were inferior with regards to storability and it was only until the mid-1920's that budded trees of Mexican, Guatemalan and hybrid origin were imported from California, which were more adapted to South African climatic conditions (Malan, 1957).

Avocados are now widely distributed throughout South Africa, although production is predominantly in the Limpopo and Mpumalanga provinces in the north and northeast, and to a lesser extent in the frost-free lowland coastal belts and cooler midlands of KwaZulu Natal. South African avocado production focuses mainly on two cultivars viz. 'Fuerte' and 'Hass', and volumes have increased more than 11-fold from 4700 to 53 800t export-based annually in the years 1961 to 1996 (Knight, 2002). Currently 12400ha are planted with avocado trees in South Africa, with approximately 3015000 trees in production, which could amount to more than 50 thousand tons, of which 36 thousand tons (9 million cartons) are destined for the export market (Retief, 2007).

1.1.2 Plant Morphology and Physiology

1.1.2.1 Carbon Partitioning

Tree performance is ultimately measured by yield and quality. Average yields of avocado trees are determined by numerous factors including cultivar, rootstock, environmental factors, tree size, shape and age. However, the ultimate factor controlling yield is seasonal photosynthetic efficiency, and in particular the harvest index, i.e. the measure of photosynthate proportioned to fruit (Wolstenholme, 1987).

Consequently, the effect of Phytophthora root rot on photosynthate accumulation and storage is of major importance. Physiological effects of *Phytophthora cinnamomi* Rands. on avocado trees are severe and infected trees have lower water potential, reduced stomatal openings, and reduced water and nutrient uptake (Sterne *et al.,* 1977, 1978; Whiley *et al*., 1986).

Reports by Davies *et al.* (1986) indicated that stomata close even when leaves are not experiencing water stress, provided plant roots are stressed. Under optimal conditions for fungal growth, avocado roots become severely infected by *P. cinnamomi*, leading to severe root death, and thus a loss in water and nutrient uptake. This leads to a drop in photosynthesis resulting in a reduction of carbon partitioning to fruit (Pegg *et al*., 2002). nutrient

1.1.2.2 Root System

The most important function of plant roots, apart from anchoring the plant, is the uptake of water and nutrient elements. Roots also supply the plant with growth hormones, being major sources of cytokinins and gibberellins (Van Staden and Davey, 1979; Hedden and Kamiya, 1997; Lovegrove and Hooley, 2000), and act as storage organs of carbohydrate reserves (Wolstenholme, 1981). By their very nature, roots must continue to grow and be replaced to perform their absorption functions. This is only effectively achieved by white unsuberized rootlets, as avocado roots do not have root hairs (Wolstenholme, 1981). Environmental factors influencing root growth play a major role and include soil moisture and aeration, soil CO_2 content, soil pH, nutrient element availability, salt concentration, and soil temperature (Pegg *et al*., 2002).

Wolstenholme (1987) described the avocado tree root system as relatively inefficient, with low hydraulic conductivity and few mychorrizal associations. Furthermore, Pegg *et al*. (2002) stated that the system is relatively shallow, and does not spread beyond the tree canopy. Bergh (1992) postulated that evolutionary aspects have shaped avocado roots and listed them as being primarily influenced by good frequent rains, as found in its indigenous habitat. Secondly, rapidly draining soils, as exemplified by the high oxygen requirement of avocado roots and their sensitivity to poor drainage, has an effect on root evolutionary development. The tendency of healthy feeder roots to grow into a decomposing litter layer in the presence of rich surface organic mulch confirms Bergh's (1992) beliefs.

The studies of Whiley *et al*. (1987) on surface feeder roots, and Whiley (1994) on roots in deep red soils have shown considerable feeder root growth as deep as 1m although the majority of these white, unsuberized feeder roots were found in the top 0.6m of soil (Pegg *et al*., 2002). Vesicular arbuscular micorrhizal associations are formed, and Menge *et al*. (1980) found that addition of *Glomus* spp. isolates to sterilized growth media improved avocado seedling growth and nutrition. These fungi are associated with mulch in avocado orchards, and Broadbent and Baker (1974) were the first to recognise the inhibitory effect of mulch on *P. cinnamomi*, with mulch layers inducing healthy feeder root growth.

True to the phenological model, avocado roots display rhythmic growth, termed flushes, alternating with quiescent periods (Wolstenholme, 1981). Whiley (1994) found pronounced attrition of feeder roots coincident with flowering in spring, which, together with loss of photosynthetic capacity due to winter photo-inhibition, reduced the capacity of the feeder roots to supply water, metabolites and nutrients to setting fruit (Pegg *et al*., 2002). Consequently, a balance exists between root and shoot mass that must always be maintained. This balance is affected, not only by phenological root growth fluctuations, but even more severely by Phytophthora root rot. Wood and Moll (1981) reported on the effects of staghorning (severe pruning of avocado trees to a height of between 1-1.5m) on the recovery of *P. cinnamomi* infected avocado trees, and in particular root recovery. They concluded that staghorning is effective in re-establishing the root: shoot balance, but stated that staghorned trees need to be treated with an effective fungicide to inhibit further decline.

1.2 THE DISEASE: PHYTOPHTHORA ROOT ROT

1.2.1 Introduction

P. cinnamomi is a soilborne *psuedofungus* of the Class Oomycetes in the Kingdom Chromista (Hardy *et al.*, 2001). It is the most important and destructive disease of avocado worldwide (Pegg *et al*., 2002). It attacks trees of all ages, from nursery trees to large bearing trees, killing them by destroying the fine feeder roots. Reproduction, growth and spread of the fungus are favoured by free soil water. Consequently, movement of infected soil plays an important role in the spread of this fungus (Hardy *et al.,* 2001). *P. cinnamomi* was first described by Rands as the causal organism of a stem canker of cinnamon trees in Sumatra in 1922, and it was first reported in 1929 on

avocado in Puerto Rico where it caused severe root rot (Tucker, 1929). Its presence has now been reported in over 1000 plant species (Zentmyer, 1980), and hosts include pineapple, macadamia, peach, pear, kiwi fruit, chestnut, blue-gums, and many native Australian and South African plants (Pegg *et al*., 2002).

It has been postulated by Arentz and Simpson (1986) and Linde *et al.* (1997) that the fungus originated in Papua New Guinea, and was moved by the activities of people into other tropical and subtropical regions of the world.

Phytophthora root rot has been the main economic factor limiting successful avocado production in countries such as Australia, South Africa and the USA. In the US, where it is estimated that up to 70% of commercial orchards are affected, the annual loss attributed to the disease has been estimated at US$ 30 000 000 (Coffey, 1987). The loss in South Africa due to Phytophthora root rot of avocado trees amounts to R45 000 000.

1.2.2 Symptoms

Phytophthora cinnamomi causes rot of fine feeder roots, leading to death of host plants (Anon, 2004). Invasion of larger roots has also been reported (Anon, 2004; Pegg *et al*., 2002) and may lead to brown lesion formation in the wood. This may result in symptomatic peeling of bark, or cause a weeping canker at the tree base, below the soil line, possibly extending up the trunk for 1m (Pegg *et al*., 2002). However, infection is mostly limited to the fine feeder roots, which become black and brittle and eventually die off. Feeder roots may be difficult to find under trees with advanced root rot. Beneath such trees soil tends to remain damp, as the absence of feeder roots prevents trees from absorbing moisture (Pegg *et al*., 2002).

Foliage becomes wilted and chlorotic, leaves fall and branches rapidly die back depending on root rot severity. New leaf growth is minimal, and if leaves form, they are small and pale green. Fruit set is usually limited in root rot affected trees, and fruit are small. Visible symptoms in the tree can also result form unnatural distribution of nutrients in plant tissue and interference with nutrient uptake. Because roots are unable to control salt uptake, chloride accumulates in leaves and may reach toxic levels, resulting in scorching of leaf margins and tips (Whiley *et al*., 1987). Labanauskas *et al.* (1976) reported that *Phytophthora* infection affects the distribution of nutrients within plant parts (Ploetz and Schaffer, 1989).

A moderate tolerance is often observed in avocado trees without degradation of aerial tree health (Ploetz and Parrado, 1988). Reduced photosynthesis, transpiration and stomatal conductance can however be detected in root rot affected trees before these visible aerial symptoms appear (Sterne *et al*., 1978; Ploetz and Schaffer, 1989).

1.2.3 Disease Cycle and Epidemiology

Zentmyer *et al.* (1994) reported Phytophthora root rot of avocado to be more severe and develop more rapidly in soils with poor drainage. The disease has a short generation time and high reproductive capacity and inoculum can increase from low, often undetectable levels, to high levels within days, particularly in warm, moist and well aerated soils, and if feeder roots are in abundance (Zentmyer, 1980). High soil moisture increases infection due to increased sporangial production and favourable conditions for zoospore release, motility and movement to feeder roots. Oospore production (Figure 1) can occur in less than 48h and thus are responsible for the rapid colonization observed during epidemics (Zentmyer and Mircetich, 1966). They are fragile, short-lived and only motile in soils for periods of minutes to hours, depending on energy reserves and factors affecting encystment (Zentmyer *et al*., 1994). Chlamydospores survive for considerable periods in root debris and soil. They germinate by producing several germ tubes at soil temperatures above 15°C. Oospores occur infrequently and, although they may survive for long periods of time, they probably do not play an important role in the disease cycle (Zentmyer, 1980). Disease development is optimal in wet soil at temperatures from 21-30°C, whereas little or no infection occurs above 33°C, or below 13°C (Zentmyer *et al*., 1994)

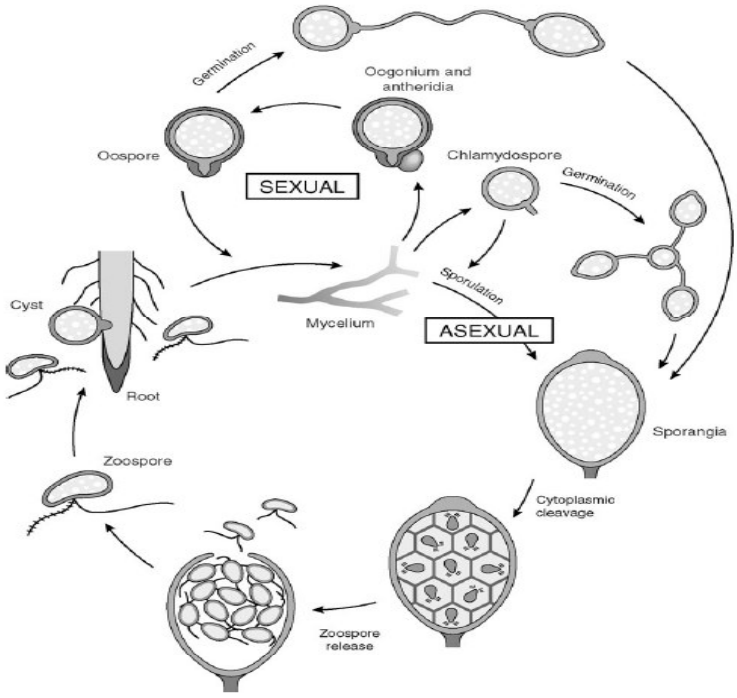

Figure 1.1: Generalized life cycle of *Phytophthora cinnamomi* (Anon, 2004)

Zentmyer and Mircetich (1966) reported the non-pathogenic stage of *P. cinnamomi* to be more significant than previously thought. Saprophytic tests indicate persistence of this fungus for long periods in the absence of a viable host, showing moderate mycelial growth through non-sterile soil, and appreciative invasion of dead organic matter, especially under moist conditions.

1.2.4 Physiology, Sporulation and Spore Germination

1.2.4.1 Sporangia Production

Phytophthora cinnamomi sporangia usually form near the air-substrate interface, and is a complex process involving numerous factors (Ribeiro, 1983). Relative humidity approaching 100% is highly conducive to sporangia formation (Ribeiro, 1983). The stimulatory effect of nitrogen (Ribeiro, 1983), a precise balance of K^+, Mg^+, Ca^{2+}, and Fe^{2+} (Halsall and Forrester, 1977), decreasing O_2 or increasing CO_2 concentrations from those normally found in the air (Mitchell and Zentmyer, 1971), colony age

(Ayers and Zentmyer, 1971) and pH (Zentmyer and Marshall, 1959) are all factors influencing sporangia production.

1.2.4.2 Germination of Sporangia

Sporangia germinate either by differentiation of the cytoplasm within the sporangium into discrete zoospores that are released through an exit pore (indirect germination), or by formation of a germ tube(s) that eventually grows to form a mycelium (direct germination) (Ribeiro, 1983; Pegg *et al*., 2002). Germination is affected by numerous factors including anion concentration (Gisi *et al*., 1977), light (Ribeiro *et al*., 1976), exogenous temperature (Ribeiro, 1983), and endogenous lipids (Bimpong, 1975). Zoospores undergo remarkable rearrangement that result in flagella loss, *de novo* synthesis of cell walls, and the emergence of a germ tube. This process is believed to be more dependent on exogenous nutrients than the motile state (Barash *et al*., 1965).

1.2.4.3 Chlamydospore Production

Chlamydospores are thin- or thick-walled asexual structures (Ribeiro, 1983), with numerous factors playing a role in their development including sterols, temperature and light (Englander and Turbitt, 1979). Unlike sporangia, chlamydospores can form readily on nutrient enriched media (Ribeiro, 1983; Pegg *et al*., 2002). Conflicting reports on chlamydospore formation in liquid media and the type of experiments that have been conducted, make it difficult to evaluate whether chlamydospore formation is due to the aeration effect, water potential, or both (Ribeiro, 1983). Data concerning the effect of pH on chlamydospore development is lacking (Ribeiro, 1983).

1.2.4.4 Chlamydospore Germination

Chlamydospores germinate by germ tubes that either continues to grow and form mycelium, or terminate in a sporangium. Continued germ tube formation is enhanced by asparagine- or glucose-amended soils (Mircetich *et al.,* 1968) and temperatures between 21 and 29°C (Chee, 1973). The formation of germ tubes, that terminate in sporangia, is enhanced by natural soils, low nutrient conditions (Mircetich *et al.,* 1968) and temperatures between 32 and 35°C (Chee, 1973).

1.2.4.5 Oospore Germination

Phytophthora cinnamomi is diploid in its vegetative state and outbreeding, possessing A1 and A2 mating types. Oospores form when these strains of opposite compatibility are paired (Pegg *et al*., 2002). Numerous factors influence oospore germination including light (Ribeiro *et al.*, 1976), temperature (Klisiewicz, 1970), nutrition (Banihashemi and Mitchell, 1976), culture growth media and enzymes (Ribeiro, 1983).

1.2.5 Motility, Taxis and Tropism

1.2.5.1 Zoospores and their Motility

Zoospores are biflagellate, being narrower at the anterior than posterior end, longer than they are wide, and flattened dorsoventrally (Allen and Newhook, 1973). *Phytophthora* zoospores often follow a helical pathway, rotating about their axis as they swim (Allen and Newhook, 1973). Newhook *et al.* (1981) determined a motility coefficient of about 0.004mm.sec^{-1} through a coarse sand medium, resulting in a total distance of 30-60mm from their source. They concluded that soil composition, although having an effect on total distance travelled, did not inhibit zoospore encystment.

1.2.5.2 Taxis of Zoospores

Cameron and Carlile (1977) reported that negative geotaxis helps to keep zoospores near the soil surface, where host rootlets are more abundant than at greater depths. Massive accumulation of zoospores in the zone of elongation of roots, just behind the root tip or at wounding sites have been reported (Carlile, 1983; Aveling and Rijkenberg, 1989). Zentmyer (1961) reported that attraction to these areas are not species-specific, and that *Phytophthora* species are usually attracted to a wide variety of nonhost as well as host plants, although *P. cinnamomi* appeared to respond better to hosts than nonhosts, and are less attracted to roots of resistant compared to susceptible avocado cultivars. This phenomenon was confirmed in avocado plants by Botha and Kotze (1989). Zoospores of *P. cinnamomi,* however, were only attracted to living avocado roots, and failed to be attracted to boiled roots or roots treated with propylene

oxide (Zentmyer, 1961). Root exudates contain a wide range of low molecular weight compounds, so it is likely that attraction to roots is due to positive chemotaxis to substances exudated from the roots, and diffusing through the aqueous phase in soil.

1.2.5.3 Encystment and Cyst Germination

Given that highly unfavourable conditions do not cause lyses, zoospores ultimately encyst (Reichle, 1969; Aveling and Rijkenberg, 1989). They travel shorter distances between turns, become sluggish. Motility ceases, flagella are withdrawn or shed (Reichle, 1969), rounding off occurs and the cyst wall is synthesized within ten minutes (Carlile, 1983). Ho and Hickman (1967) reported that the longer zoospores swim, the more sensitive they are to factors that induce encystment. These factors include root exudates, extreme temperatures or pH, and circumstances likely to cause collision.

Sing and Bartnicki-Garcia (1975) reported that zoospores become adhesive early in encystment, and if they come in contact with a solid surface, they firmly attach to it. However, adhesiveness is soon lost during encystment, and zoospores that failed to connect to a solid surface remain unattached (Carlile, 1975). In nature, unattached cysts are free to be moved to other sites by water currents or other agents, while those attached are facilitated to invade plant roots (Ho and Hickman, 1967). Zentmyer (1970) observed that when cysts located near avocado roots germinate, germ tubes emerge from the side nearest to the root and grow toward it. This could partially be due to chemotaxis to avocado root exudates.

Cysts may germinate either by producing germ tubes or releasing a secondary zoospore (Ho and Hickman, 1967). Germination of attached cysts begins within 30min after attachment and all cysts produce germ tubes within three hours. Germ tubes either penetrate avocado roots directly, or form appressoria-like swellings before penetration (Carlile, 1975).

1.2.6 Physical Factors affecting Development of *P. cinnamomi*

Numerous physical factors affect the behaviour of *P. cinnamomi* in the soil environment. This includes humidity and water potential (Duniway, 1983). The additive effects of flooding and Phytophthora root rot on stomatal conductance and photosynthesis of avocado may vary with soil type (Ploetz and Schaffer, 1987, 1988,

1989). Continuous flooding of healthy trees or infection of non-flooded trees by *P. cinnamomi* each reduced transpiration by 50% after 14 days (Ploetz and Schaffer, 1987; Schaffer *et al.*, 1992). Phytophthora root rot exacerbates the effects of flooding on the inhibition of transpiration (Schaffer *et al.*, 1992). Reduced transpiration of avocado following Phytophthora root rot infection and/or flooding may be due to decreased hydraulic conductivity based on observations that *P. cinnamomi* infection alone or in conjunction with flooding reduced water potential (ψ) compared to that of non-flooded or flooded, non-infected trees (Schaffer and Whiley, 2002). In trees with severe root rot, the ψ has been shown to mimic that of trees under sever water stress, even when soil moisture is adequate. In field studies, reduced ψ of *Phytophthora*-infected avocado trees was correlated with a six-fold decrease in transpiration (Sterne *et al.*, 1978).

Wolstenholme (2002) states that waterlogged conditions are optimal for infection of avocado trees with *P. cinnamomi*, but stress the detrimental effect of flooding and consequent lack of aeration, even in the absence of *P. cinnamomi* in avocado trees.

The extent to which *P. cinnamomi* suppresses the growth of avocado seedlings in soil is closely correlated with the effects of temperature on mycelial growth (Zentmyer, 1980).

1.2.7 Chemical Factors affecting Development

Compared to plants which require 10 macro-elements to grow and that assimilate these from inorganic salts, water and CO_2, fungi require an organic source of carbon, and may have additional demands for specific growth factors or compounds containing certain food sources (Cantino and Turian, 1959). The most widely used sugars as carbon sources are sucrose and glucose, followed by fructose, starch and maltose, while in general, hexoses are preferred (Clarke, 1966). Though several lipids have strong growth-promoting properties, the small amount needed to stimulate growth and influence reproduction, indicate that they do not act primarily as a carbon source, but belong to a general group of growth factors. Calcium has been identified as an essential element for *Phytophthora* growth with optimal concentrations ranging from 50 to 100mg.l^{-1} (Hohl, 1983). The only essential vitamin appears to be thiamine, although some authors (Cameron, 1966; Singh, 1975) suggest that ascorbic acid may play a role in fungal development.

Labanauskas *et al.* (1976) and Whiley *et al*. (1987) studied the effect of *Phytophthora* infection on the nutrient content of avocado plants. Although there is little consistency between the two studies with respect to the effects of individual nutrients (possibly due to varietal and/or physiological maturity differences between the two groups of trees), an increase in Cl in the top of trees was reported in both studies, suggesting that Cl uptake or translocation are altered by damaged roots. Phytophthora root rot infected trees tend to have lower leaf concentrations of nitrogen, phosphorous, sulphur, zinc and boron than healthy trees (Pegg and Whiley, 1987), and an increased chloride content of leaves.

HNO_2 and NH_3 produced at pH 6 and 8 respectively are responsible for the effective inhibition of *P. cinnamomi* in soils amended with high rates of urea or other organic nitrogen sources (Tsao and Zentmyer, 1979).

High NO_3^- concentrations reduce avocado root rot (Bingham *et al*., 1958) and high nitrogen organic residue in the form of alfalfa meal may act as an effective control measure (Zentmyer, 1963; Gilpatrick, 1969).

Overall, phosphorous appears to have less of an effect on disease development than other major chemical factors, although there are claims that P halts the spread of *Phytophthora* diseases (Newhook, 1970; Newhook and Podger, 1972) and may even lead to recovery of diseased plants (Hepting *et al*., 1945; Newhook, 1970). With the use of phosphorous-based fungicides, and especially with the use of injection techniques, accumulation of phosphite in avocado root tips was observed, with consequent reduction in root colonization by *P. cinnamomi* (De Villiers *et al.*, 1994).

A high calcium level is characteristic of a suppressive soil and reduces avocado root rot (Broadbent and Baker, 1974). Higher levels of $CaSO_{4\ have}$ been reported to induce better tree growth (Snyman and Darvas, 1982) and decrease susceptibility of avocado trees to *P. cinnamomi* root rot (Duvenhage and Kotze, 1991), whilst lower levels of $CaCO_3$ had the same effect (Snyman and Darvas, 1982).

Bingham and Zentmyer (1954) reported on the effect of pH on disease development, and concluded that avocado root rot can be controlled at a pH of 3. At higher pHs, disease development increased in roots, but at pH 8, disease development was again retarded. Growth of avocado is also adversely affected by a pH of 3. Snyman and Darvas (1982), however, indicated that soils planted with avocados in South Africa are generally acid, but application of dolomitic lime increases soil pH leading to increased tree yields, and decreased toxic effects of aluminium. Whiley *et al.*, (1984),

however, found the optimal pH for disease development in avocado trees to be pH 6.5. Conflicting reports therefore exist, and the optimum pH will therefore depend not only by yield expectancy, but also Al and other metal ion concentrations in the soil solution, and the disease severity of the production orchard (Broadbent and Baker, 1974).

Toppe and Thinggaard (2000) reported copper to be an effective inhibitor of disease development, and a possible component of disease management, as changes in copper concentration from 0.07 to 0.28ppm resulted in a decrease of *P. cinnamomi* incidence (92% to 8%) in ivy plants grown in nutrient solution.

1.2.8 Control of *P. cinnamomi* in Avocado

1.2.8.1 Cultural Practices

Site selection is of utmost importance and orchards should be established in soils that have good surface and internal drainage (Ohr and Zentmyer, 1991). *P. cinnamomi* free soils should be planted with healthy trees, while pathogen free nursery trees planted in infected soil gives tree establishment a head start. Balanced nutrient programmes should be implemented to aid in replacement of damaged roots, and in particular phosphorous, calcium, and boron should be within the recommended norms, as these elements aid in root growth (Wolstenholme, 1981).

Solarization of soil has been reported to be effective in controlling root rot. The effectiveness of this method is, however, linearly dependant on maximum daily temperatures (Lopez-Herrera *et al.,* 1997).

1.2.8.2 Chemical Control

Phosphonate fungicides, including fosetyl-Al (Aliette) and their breakdown product phosphorous acid, are highly mobile in plants (Guest *et al*., 1995). Translocation in association with photo-assimilates, in a source-sink relationship by both phloem and xylem, leads to a direct relationship between phosphite concentration in plant tissue and application rate (Hardy *et al.,* 2001). It is believed to control *Phytophthora* spp. by a combination of direct fungitoxic activity and stimulation of host defence mechanisms (Guest *et al*., 1995; Hardy *et al.,* 2001).

Phosphites (salts of phosphonic acid, H_3PO_3), also have direct effects on plants, resulting in phototoxic conditions in phosphate deprived plants (McDonald *et al.*,

2001). Phytotoxicity symptoms show a linear relationship with phosphite application rate, and are likely to occur in all instances where phosphite is applied, even at recommended rates. New growth is, however, not affected by the fungicide (Hardy *et al.,* 2001).

Application methods have ranged from soil drenches (Darvas, 1983) to trunk paints (Snyman and Kotze, 1983). Darvas *et al.* (1983, 1984) first reported the use of a trunk injection method by injecting 0.4g fosetyl-Al.m^{-2} canopy area and obtained "outstanding control" of *P. cinnamomi*. Trunk injections require a much lower chemical dosage than foliar sprays (Whiley *et al*., 1995), are longer lasting (Hardy *et al*., 2001), and are currently the preferred option.

Duvenhage (1994) first reported 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 was 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. There was an average decrease in sensitivity of 13% over a period of six years (1992-98) of isolates from phosphonate treated trees as compared to isolates from untreated trees (Duvenhage, 1999).

1.2.8.3 Biological Control

Biological control through modifying soils with amendments or applying effective biocontrol agents shows promise for reducing root rot (Pegg *et al*., 2002). The use of biological methods to control *P. cinnamomi* have been investigated by numerous authors (Pegg, 1977; Casale, 1990; Duvenhage and Kotze, 1993). McLeod *et al.* (1995) reported a reduction in *P. cinnamomi* populations of more than 50% with *Trichoderma* isolates. This control is thought to be the result of antibiosis, nutrient competition and competitive exclusion, among others (Korsten and De Jager, 1995).

1.2.8.4 Resistance

Host resistance is the best method for reducing Phytophthora root rot (Coffey, 1987). Some rootstocks express tolerance to root rot by the rapid regeneration of active feeder roots while in others the progress of infection in the root is inhibited (Phillips *et al.*, 1987). The moderate resistance expressed by existing "resistant" rootstocks is

still not adequate to give disease control under environmental conditions ideal for root rot. Cahill *et al.* (1993) reported increases in lignin and phenolic compounds in *Eucalyptus marginata* seedlings to be up to 94% higher in resistant eucalypt lines compared to susceptible lines after inoculation with *P. cinnamomi*. This suggests that phenolic compounds, and the ability of plants to produce sufficient amounts of phenolic compounds, play a role in plant resistance to Phytophthora root rot.

1.3 PLANT RESPONSES TO PATHOGENS

1.3.1 Introduction

All plant parts are in constant contact with pathogens, while every pathogen has evolved its own method to invade plants in a specific way (Salisbury and Ross, 1992). Activation of plant defense responses to pathogens includes a cascade of events, with the earliest steps of signal transduction involving plant membrane activities (Lebrun-Garcia *et al.*, 1999). Successful pathogen infection and disease only occur if preformed plant defenses are inadequate, if environmental conditions are favourable, and if either the plant fails to detect the pathogen or the activated defense responses are ineffective (Buchanan *et al*., 2000).

During infection the expression of many genes involved in normal housekeeping patterns, in both resistant and susceptible plants, are either altered or induced. As a consequence, a network of defense reactions are activated to ensure that local responses at the infection site are activated and that self-defense mechanisms are induced in adjacent tissues (Leone *et al.,* 2001). This rapid activation of defense reactions occurs within 24 hours and may lead directly or indirectly to localized tissue and cell death, and is termed a hypersensitive response (HR) (Buchanan *et al*., 2000). It has been suggested that silicon may activate this form of defense reaction, leading to phenol production and release at infection sites (Koga *et al*., 1988).

The elicitation of most of the defense proteins involves transcriptional activation and can either be confined to the wounding site, or can occur systemically throughout the infected plant (Bögre *et al*., 1997). Microorganism recognition by plant cells depends on the generation of elicitor molecules (including jasmonic acid (JA), salicylic acid (SA), ethylene, ABA, H_2O_2 and heavy metals) by pathogens (Blumwald *et al.*, 1998).

1.3.2 Phenols

Ward (1905) was the first to recognize the significance of pathogen inhibition after host penetration, and thus the dynamic nature of disease resistance. Bernard (1911) postulated that as a result of host-pathogen interaction, a second substance is produced that diffuses back to the fungus and inhibits subsequent growth. Although these papers by Ward (1905) and Bernard (1911) were mostly speculative and observational, they appear to represent the first statement of what has become the Toxin Theory of Disease Resistance (Cruickshank and Perrin, 1968).

For the purpose of defence, plants have evolved a multitude of chemicals and structures that are incorporated into their tissues. These constitutive defences can repel, deter, or intoxicate, with examples like leaf spines and hairs, resin-covered or fibrous foliage, resin-filled ducts and cavities, lignified or phenol-impregnated cell walls, and cells containing phenols or hormone analogues (Berryman, 1988).

Although flowering plants, ferns, mosses, liverworts and many microorganisms contain various amounts and kinds of phenolic compounds, the function of most phenolics, with important exceptions, are obscure (Salisbury and Ross, 1992). Current thought considers many of these chemicals as primary defensive compounds (Berryman, 1988). However, as early as 1935, Walker and Link (1935) suggested that the mere presence of phenolic compounds in a host plant does not warrant the conclusion that they play a role in the resistance of the host to a given pathogen. These compounds may be present in concentrations so low that their inhibitory effects on pathogens are negligible, or may even have a stimulatory effect if concentrations are low enough. The distribution of these compounds within the plant is also important with relation to the point of infection (Dixon and Paiva, 1995).

Phenolic compounds may be synthesised pre-infection in small quantities in the plant cell in which case it may act as a elicitor; de novo during a pathogen attack as part of a natural defence mechanism having antifungal properties; or may be produced after infection as part of a SAR strategy (Essenberg, 2001). These simple structures are synthesised from aromatic amino acids within the shikimic pathway and, within the phenylpropanoid pathway transformed into more complex biochemical structures acting as plant protection compounds, including phytoalexins and flavonoids (Nicholson and Wood, 2001).

Two small phosphorylated compounds are precursors of the amino acids phenylalanine, tyrosine and tryptophan, and many phenolic compounds, with a similar route of synthesis (Floss, 1986). The two compounds [erythrose-4-phosphate from the pentose-phosphate respiratory pathway and photosynthetic Calvin cycle, and Phosphoenolpyruvate (Phosphoenolpyruvic acid; PEP) from the glycolytic pathway of respiration], combine producing a seven-carbon phosphorylated compound that then forms a ring structure called dehydroquinic acid (Jensen, 1985). This acid is then converted by two reactions into a stable compound called shikimic acid (Figure 2) (Floss, 1986).

A wide range of phenolic compounds arises from the same shikimic acid pathway (Figure 2) and subsequent reactions, including the acids cinnamic, caffeic, ferulic, *p*-coumaric, chlorogenic, and gallic, of which the first four are of importance. They are derived entirely from phenylalanine and tyrosine, and are converted into several derivatives besides proteins, including phytoalexins, coumarins, various flavanoids, and lignin (Hammerschmidt and Kagan, 2001). Some phenols occur constitutively and function as preformed inhibitors (phytoanticipins) associated with non-host resistance, while others (phytoalexins) are formed in reaction to pathogen infection and their appearance is part of an active defence response (De Ascensao and Dubery, 2003).

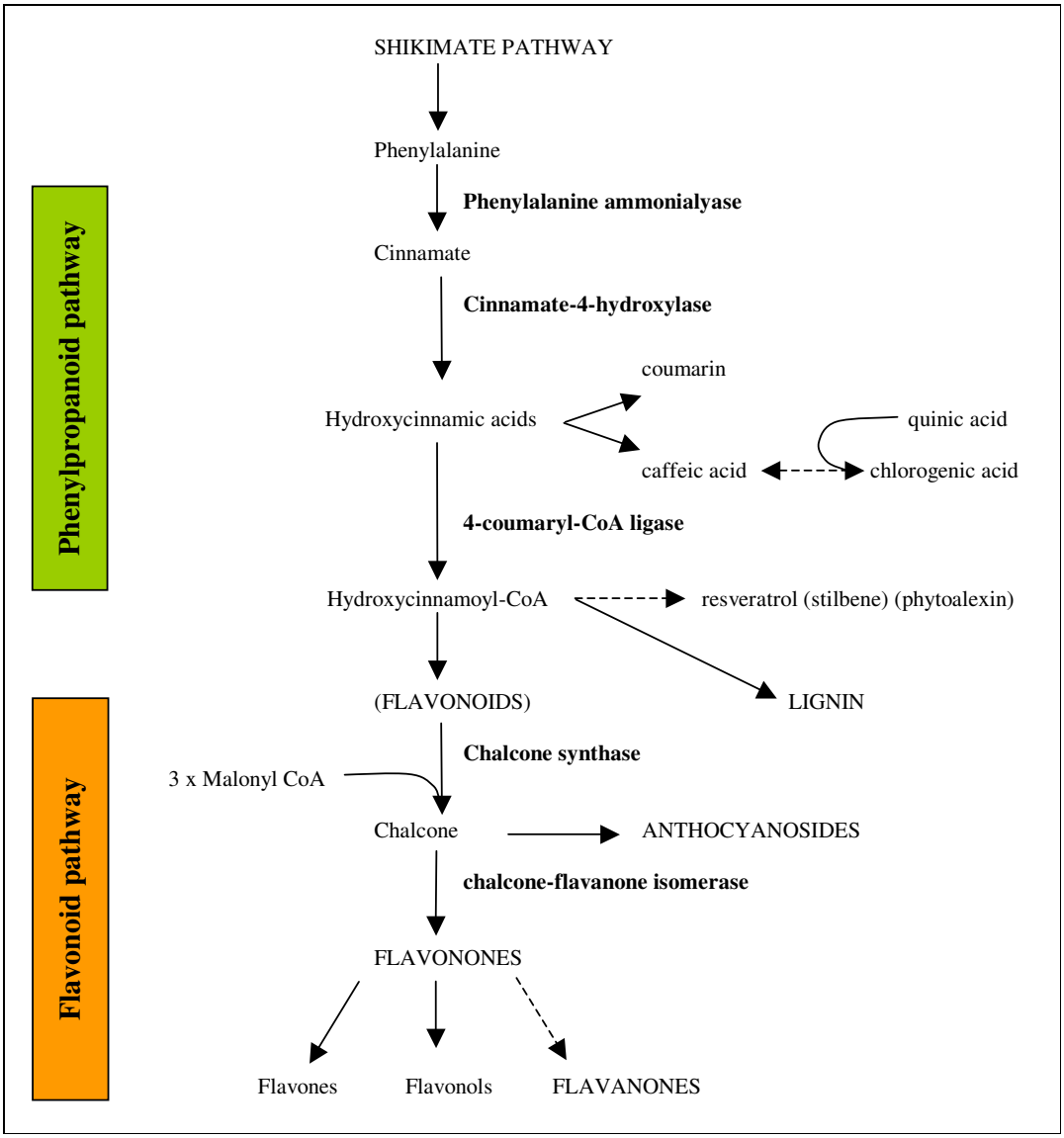

Figure 1.2: Overview of the biochemical pathway along which flavonoids and anthocyanocides are formed. Only key intermediates and enzymes of interest are indicated (Du Plooy, 2006).

1.3.2.3 Phytoalexins

Various antimicrobial compounds synthesized by plants after infection have been discovered since 1960 and are referred to as phytoalexins (Dixon and Paiva, 1995). Phytoalexins are in general more toxic to fungi than bacteria, and are primarily present in dicotyledonous plants (Essenberg, 2001). Most phytoalexin compounds are

phenolic phenyl-propanoids that are products of the shikimic acid pathway (Hammerschmidt and Kagan, 2001). Non-pathogenic fungi induce such high, toxic levels in the host that their establishment is prevented, while pathogenic fungi either induce only non-toxic phytoalexin levels or quickly degrade the phytoalexin (Macheix *et al*., 1990).

Several different kinds of compounds and viruses can induce phytoalexin production (Essenberg, 2001). These compounds, called elicitors, are polysaccharides produced by the plant either after attack from pathogenic fungi or bacteria on plant cell walls, or formed after degradation of fungal cell walls caused by plant enzymes that the fungus induces the plant to secrete (Dixon and Paiva, 1995). These elicitors are recognised by proteins in membranes, which then signal the plant to produce phytoalexins (Nicholson and Wood, 2001).

1.3.2.4 Storing of Phenols

Mace (1963) examined specialized 'tannin' cells randomly distributed throughout banana (*Musa acuminata* L.) root tissue. These cells contained a free o-dihydroxyphenol commonly known as dopamine (Swain *et al.,* 1979). This was an important finding, as phenolic compounds that are in a free state are normally oxidised and polymerized rapidly. Beckman (2000) however explained this stating that the major tonoplast protein in these cells is H^+-ATPase. The H^+ concentration in plant cell vacuoles is therefore orders in magnitude greater than that in the cytoplasm and serves to maintain the hydroxyl group of phenols in a non-ionized, reduced state within vacuoles (Wink, 1997). These phenol storing cells have a specialized distribution within plant tissues, which serves to synthesise phenols, and keep them compartmented and reduced in vacuoles, providing the means for their rapid decompartmetation and oxidation to occur (Beckman, 2000). Most phenolic compounds are stored as glycosides, in which form they are not toxic to plant cells (Mace, 1963).

1.3.2.5 Toxicity of Phenols

It is well established that simple phenolics are toxic to fungi *in vitro*. However, phenolic compounds vary widely in their toxicity. Flavonoids, querticetin, robinetin and catechin are not toxic to *Colletotrichum gleosporioides* (Penz.) Penz. & Sacc.,

although it was shown by Lulai and Corsini (1998) that anthocyanins, delphinidin, pelargonidin, petunidin and cyanidin inhibit fungal spore germination, with a 90% germination with delphinidin while only 5% with cyanidin. Numerous similar studies show comparable results (Campbell and Ellis, 1992; Beckman, 2000; Zdunczyk *et al*., 2002).

1.3.2.6 Pre-infection Phenols

Tannins were one of the earliest compounds to attract attention as a chemical substance that protects plants against fungal infection (Waterman and Simon, 1994), with tannin concentration related to tissue and cultivar resistance to pests (Bell *et al*., 1992). As early as 1911, Cook and Taubenhaus reported that the majority of fungi were retarded by a 0.1-0.6% tannin solution. The authors also reported that enzymes present in plant juices were responsible for tannin formation from gallic acid. However, even though tannins are present in plants in large amounts, fungi still readily attack them. It is therefore thought that the role tannins play in disease prevention may be an indirect one (Waterman and Simon, 1994). Numerous functions can be assigned to tannins including being antibiotics, anti-sporulants, feeding deterrents and enzyme denaturants (Bell *et al.*, 1992).

Chérif *et al.* (1992) and Waterman and Simon (1994) reported that increased resistance by exogenous applied silicon is not associated with accumulation of silicon at pathogen penetration sites, regardless of the plant organ investigated. Lulai and Corsini (1998) reported that the suberin phenolic matrix expressed in potato tubers does not offer any resistance to fungal infection. It is only after deposition of the suberin aliphatic domain within the first layer of suberized cells that total resistance is achieved.

1.3.2.7 Post-infection Phenols

Infectious symptoms in any plant part (roots, leaves, stems or inflorescence), apart from any visible fructifications of the pathogen, take the form of abnormal growth, local necrosis, vascular wilting and discolouration, or any combination of these (Dixon and Paiva, 1995). Phytotoxic compounds may be produced by fungi and released into infected plant tissue that could aid in the production of these symptoms (Darvas, 1983). In other cases, elicitors may not be phenols but interact with phenolic

compounds or cause the accumulation thereof in plant cells that may alter the typical disease symptoms (Waterman and Simon, 1994).

Numerous studies have concentrated on the accumulation of phenolic compounds in susceptible plant cells adjacent to those infected (Cahill and McComb, 1992; Bekkara *et al*., 1998; Goetz *et al*., 1999). Examples include increased chlorogenic acid and other polyphenolic concentrations in rice leaves infected with *Piricularia oryzae* Nishik and *Cochliobolus miyabeanus* (S. Ito & Kurib.) Drechsler ex Dastur, and in sweet potato tissue infected with *Helicobasidium compactum* (Boedijn) Boedijn (Darvas, 1983). Rapid and early accumulation of phenolic compounds at infection sites is a characteristic of phenolic-based defence responses. This accumulation of toxic phenols may result in effective isolation of the pathogen at the original site of penetration (De Ascensao and Dubery, 2003).

Fawe *et al.* (1998) reported the synthesis of a flavonoidal phenol identified as rhamnetin, a compound having fungitoxic abilities, after infection in cucumber plants treated with silicon and inoculated with powdery mildew. The compound was not present in uninoculated plants, or plants inoculated but not treated with silicon. The presence of the compound however subsided in samples six days after inoculation, and resumed an appearance similar to that observed in inoculated plants that were not treated with silicon. Because these compounds were not detected in plants not treated with silicon, it is suggested that they are essentially derived from neosynthesized conjugates (Epstein, 1999a).

Potato tubers deposit lignin more rapidly in resistant varieties than in susceptible ones when exposed to *Phytophthora infestans* (Mont.) de Bary. This lignin deposition is accompanied by browning of potato tissue due to oxidation and polymerization of phenolic compounds (Hammerschmidt, 1984). This may lead to an apparent failure of the fungi to penetrate into the tissue although some fungal growth was observed on the tuber surface (Rodriguez *et al.,* 2005).

1.3.2.8 Quantitative Changes

After *P. infestans* haustoria penetrate potato tuber cells, chlorogenic acid moves toward the site of infection (Hammerschmidt, 1984). Scopolin content of potato cells increase 10-20-fold and results in blue fluorescent zones around the infection site. The concentration of chlorogenic acid at the same site increases 2-3-fold (Darvas, 1983).

Increased phenolic synthesis is already measurable four hours after exposure of banana (*Musa acuminata* Colla) roots to elicitors from *Fusarium oxysporum* Schltdl. em. W.C. Snyder & H.N. Hansen f.sp. *cubense* (E.F. Sm) W.C. Snyder & H.N. Hansen, and reach a peak after 16h post-elicitation. Data indicates a 4.5-fold increase in total phenolics (De Ascensao and Dubery, 2003).

1.3.2.9 Qualitative Changes

As early as 1929, Tucker reported that gallotannins accumulate in healthy cell vacuoles adjacent to infected cells, but when fungi thrive in host tissue, tannins is formed in very small amounts, very slowly, and only in vacuoles in close proximity to the fungi (Hammerschmidt and Kagan, 2001). Recent studies of infected sweet potato have also shown an increase in chlorogenic acid, caffeic acid and their derivatives in sound parts of the sweet potato next to infected tissue, but found little antibiotic activity against fungi in culture and are therefore not considered to be the main cause of fungal inhibition (Lulai and Corsini, 1998).

1.3.2.10 Sources of Phenolic Variation

The pattern, by which phenolic concentration changes with tissue age, differs considerably within tissue types. Although phenolic concentration in stems and roots is low in the juvenile stage of plant growth, these concentrations normally increase with time through most of the life of the plant (Hunter, 1978; Bell *et al.,* 1992). In cotton, for instance, flavonol concentration is greatest in the young ball just after flowering, while in young leaves it is greatest just after the leaf unfolds (Bell *et al*., 1992)

Bell *et al.* (1992) reported that as a plant ages, each new leaf formed produces a greater flavonol concentration than the preceding leaf until approximately the tenth leaf, with this concentration maintained in successive leaves until a fruit load is developed, where after the concentrations may decline (Hammerschmidt and Kagan, 2001). The mean concentration of total leaf and terminal leaf will increase as the season progresses because of the effect of plant age on the phenolic concentrations (Darvas, 1983).

Solecka *et al.* (1999) reported a two-fold accumulation of anthocyanin in winter oilseed rape leaves grown in cold conditions (2°C) for three weeks. Ferulic and

sinapic acids accumulated in high concentrations under these conditions, while caffeic acid did not increase during the cold period, but increased by 70% when plants were removed from cold conditions. This could indicate a role for phenolics in acclimation of leaves to low temperatures (Hedden and Kamiya, 1997).

Primitive plant cultivars collected and used in propagation studies often do not flower or flower late in the season, and these photoperiodic stocks may lead to increased phenolic concentrations (Bell *et al.*, 1992).

Field grown plants are usually more resistant to diseases and insects than those grown in a greenhouse (Hedden and Kamiya, 1997). This may be due to the effect of light on phenol production. In a trial conducted by Bell *et al*. (1992) it was concluded that tannin concentrations in plants grown outside were two times higher than those plants grown in a greenhouse, with a variation of 150 to 300% in different cultivars.

Agricultural and Biocontrol Agents

Phenol concentrations can significantly be affected by plant growth regulators, insecticides and biocontrol agents. Parrot *et al.* (1983) found that the insecticide methomyl, that causes visible reddening of cotton foliage, is associated with significant increases in tannin and anthocyanin content of fully expanded leaves. In contrast, Yokohama *et al*. (1987) found that methyl parathion sprays cause tannin concentration decreases and increase protein concentrations in cotton. However, Zummo *et al*. (1984) found that the growth regulator epiquat chloride (PIX) significantly increases both terpenoid and tannin concentrations within 48h after application, and eventually increases both concentrations in leaves by about 20%.

1.3.2.11 Phenols in Avocado

Wehner *et al.* (1982) reported on the sensitivity of pathogens to antifungal substances in avocado tissue. They concluded that no consistent tendencies exist in the antifungal compound concentration in different cultivars, although marked differences were found between plant parts, with avocado leaves containing the highest levels, followed by fruit mesocarp, root, seed and skin extracts.

Upon penetration of the cuticle, invading fungi encounter a pectinatious barrier (Kolatuducky, 1985). To overcome this barrier, pathogens produce multiple forms of different pectic enzymes (Crawford and Kolatukudy, 1987), which macerate avocado tissue (Vidhyasekaran, 1997).

Some phenolics may act as antioxidants and induce resistance and are present in plant lipophylic regions. These phenols increase resistance of avocado fruit upon ripening and include epicatechin acting as a trap for free radicles (Vidhyasekaran, 1997) and diene (1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene) (Prusky *et al.,* 1982; Prusky *et al*., 1983).

Epicatechin inhibits lipoxygenase *in vitro*, and may act as a regulator of membrane-bound lipoxygenase. Epicatechin concentration in the fruit peel is inversely correlated with lipoxygenase activity and decreases significantly when lipoxygenase increases (Marcus *et al.,* 1998). Treatments with epicatechin and other antioxidants delayed the decrease of the antifungal diene (Prusky, 1988). This suggests that epicatechin takes part in induced resistance by inhibiting lipoxygenase, which may degrade fungitoxic diene into a non-toxic one. Antifungal diene decrease is regulated by lipoxygenase activity, which in turn is regulated by a decrease in the antioxidant epicatechin concentration (Prusky *et al*., 1988; Karni *et al*., 1989; Prusky *et al*., 1991). Diene has also been isolated from avocado leaves (Carman and Handley, 1999), and appears to accumulate in order of magnitude in Hass ($4.5\mu g.g^{-1}$), Pinkerton ($2.9\mu g.g^{-1}$), Fuerte ($2.5\mu g.g^{-1}$), Duke 7 ($0.9\mu g.g^{-1}$) and Edranol ($0.4\mu g.g^{-1}$).

In addition to diene, numerous other compounds are produced in avocado plants with fungitoxic characteristics (Domergue *et al*., 2000). Brune and van Lelyveld (1982) conducted studies on the biochemical composition of avocado leaves and its correlation to susceptibility to *P. cinnamomi* root rot. Detectible amounts of phenylalanine ammonia lysase (PAL) were found in all five cultivars tested. The non-oxidative deamination catalyzed by this enzyme is considered one of the initial steps for a variety of pathways leading to biosynthesis of lignin, flavonoids and pterocarpans involved in host-pathogen reactions (Friend, 1976; Albersheim and Valent, 1978). The majority of phenols detected in avocado plant material were either phenolic acid (C_6-C_1) or cinnamic acid derivatives (C_6-C_3), and the possibility exists that avocado plants may convert specific phenolics into coumarins, from which coumarin phytoalexins may be derived (Brune and van Lelyveld, 1982).

1.4 SILICON

1.4.1 History

Research on the role of silicon in plant physiology depended on the advent of the solution culture technique (Epstein, 1999a). Most soils contain high percentages of total Si, with the amount of Si exceeding all elements except oxygen, the mean values being 49% for oxygen and 31% for silicon. It was only until ultra low levels of nutrient elements could be detected in plant tissue that silicon could be studied (Epstein, 1999b).

Silicon has not been included in the list of essential elements, resulting from the definition of essentiality conceived by Arnon and Stout (1939): "an element is not considered essential unless (a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (b) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying the element; and (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavourable microbiological or chemical condition of the soil or other culture medium."

Several flaws have, however, hindered the near-universal acceptance of this definition. As for "(a)", many plants can complete their life cycle although quite deficient in a nutrient; "(b)" is superfluous, seeing that "(a)" excludes all others; and "(c)" assumes that assignment of an element as essential has to implicate knowledge of its direct participation in plant nutrition (Epstein, 1999b). As a result, Epstein (1999b) defined an element to be quasi-essential if: "It is ubiquitous in plants, and if a deficiency of it can be severe enough to result in demonstrable adverse effects or abnormalities in respect to growth, development, reproduction, or variability."

It has previously been shown that both non-accumulating and accumulating plants can mature without Si supply, but that their growth and yield are significantly reduced by Si deficiency (Epstein, 1999a). The effect of Si on a plant depends on the plant species and is usually more prominent in species accumulating a large amount of Si (Epstein, 1999b). The fact, however exists, that Si does fulfill certain functions in the plant (Ma and Takahashi, 2002). Although the earliest scientific work on the role of Si in plants, and especially plant protection dates from 100 years ago to the 1920-1930's (Germar, 1934), no actual physiological role for Si in plant growth has been discovered (Chérif *et al.,* 1994).

1.4.2 Silicon in Soil

Silicon is the most widely distributed element in the earth's crust and constitutes 40-70% in clay soils, and up to 90-98% in sandy soils as SiO_2 (Matichenkov *et al.,* 2000). As a soil constituent in most of these soils, Si is second only to oxygen; the mean values being O, 49% and Si, 31%. Two hundred to 800kg.ha^{-1} Si is removed annually from soil either through leaching or plant uptake in the form of monosilicic acid. Anderson and Snyder (1992) found the amount of silicon absorbed by plants to constitute 70-700kg.ha^{-1}. Most monomers taken up are transformed to amorphous silica in the epidermal tissue (Lanning and Eleuterius, 1992).

Most monosilicic acid in the soil profile is weakly absorbed and it migrates slowly through the soil profile (Matichenkov *et al.*, 2000). These authors reported that increased levels of monosilicic acid in the soil solution resulted in the transformation of plant-unavailable phosphates into plant available phosphates.

Monosilicic acids may react with Fe, Al and Mn, forming slightly soluble silicate substances (Lumsdon and Farmer, 1995). Monosilicic acid is also able to react with heavy metals to form soluble complex compounds and slightly soluble metal silicates (Matichenkov *et al.*, 2000), but at the same time, high monosilicic acid concentrations may lead to full precipitation of heavy metals resulting in formation of slightly soluble silicates.

Polysilicic acids form an integral component of the soil solution and essentially affect soil structure (Liang *et al.,* 1994). The mechanism of polysilicic acid formation is not clearly understood. Silicic acid polymerization is assigned to the type of condensable polymerization (Matichenkov *et al.*, 2000). Unlike monosilicic acid, polysilicic acid is chemically inert and acts as an absorbent of colloidal particles. Highly soluble in water, it affects the water holding capacity of soil, adding to its effect on soil formation and structure (Matichenkov *et al.*, 2000).

1.4.3 Silicon Supply

The direct source of Si in the soil solution above pH 8 as SiO_4, which is present at concentrations normally ranging from 0.1mM to 0.6mM. Plants growing in soil are therefore exposed to Si (Epstein, 1999a). However, these siliceous nutrients are almost insoluble, and addition of silicon fertilizers is proposed to supply plants with

adequate concentrations. Various compounds can be used as Si fertilizers, but the grade and quality may vary within compounds (Datnoff *et al.,* 1997).

According to Watteau and Villemin (2001), compost was the main source of Si before silicon fertilizers were introduced. With an average of approximately 5% SiO_2 contained in compost, the average amount of Si applied to fields decreased from 330kg.ha^{-1} in 1945 to 100kg.ha^{-1} in the 1990's with the decrease in compost use (Ma and Takahashi, 2002). The straw of monocotyledonous-plants, especially rice, contains up to 20% SiO_2. This source is, however, only available for a short period since the concentration of Si in the soil decreases. Si is however slowly released from compost, and if proper compost is made from the straw, long term benefits can be seen (Rogers-Gray and Shaw, 2004).

Potassium silicate, a slow-release fertilizer, first appeared in 1978, with fly ash produced from a coal power-plant used as silicate material (Ma and Takahashi, 2002). According to standards, the fertilizer should contain more than 20% K_2O of citrate-soluble potassium, 25% SiO_2 of 0.5M HCl-soluble silicate, 3% MgO of citrate-soluble magnesium, and less than 3% of non-reactive water-soluble potassium (Adatia and Besford, 1986). During 1986 a liquid potassium silicate, which is guaranteed by 6% of water-soluble potassium and 12% of water-soluble SiO_2, appeared and is produced by diluting potassium silicate and potassium carbonate in water (Ma and Takahashi, 2002).

Calcium silicate slags were used after the Second World War to fertilize mainly rice fields, especially in Japan, and in 1955 they were registered as a silicate fertilizer (Ma and Takahashi, 2002). Slag is made by melting ore containing Fe, Mn, Ni, and Cr with limestone in a furnace and then cooled by floating material on the surface (Elawad *et al*., 1982). As a fertilizer, slags must have more than 20% of 0.5N HCl soluble SiO_2, more than 35% alkali component and the toxic component must be under the permissible limit (Ma and Takahashi, 2002).

Anderson and Snyder (1992) reported that most calcium silicate slags contain trace amounts of P, generally in excess of 10g P.kg^{-1}, which amounts to a commercial application rate of 6.7Mg.ha^{-1}, resulting in P application rates of up to 67kg.ha^{-1}. This can become a limiting factor if calcium silicate slags are applied annually at commercial dosages (Elawad *et al*., 1982). This fertilizer was manufactured by Japan from 1950. Its production entails the melting of phosphate rock with serpentinite,

followed by grinding after cooling. This fertilizer contains 16-26% soluble SiO_2 (Ma and Takahashi, 2002).

The present commercial silicate fertilizers are not suitable for use in nursery beds because they contain alkali components that raise pH and weaken the resistance to diseases (Elawad *et al*., 1982). Silica gel, however, does not increase the pH and is therefore a good source of silicon to use for seedlings. Official silica gel fertilizers should contain more than 80% of 0.5N sodium hydroxide soluble SiO_2 (Ma and Takahashi, 2002).

Porous hydrate calcium silicate (tobamolite) is used for light wall material in construction and is produced from quarry lime, quartz and cement, which are reacted at 180°C at 10atm pressure (Adatia and Besford, 1986). This fertilizer should contain more than 15% of 0.5N HCl soluble SiO_2 and more than 15% base component (Ma and Takahashi, 2002).

1.4.4 Application Methods

1.4.4.1 Foliar Sprays

Silica deposition in plant surface cell layers, especially the epidermis, has a bearing on physical surface properties (Epstein, 1999a). During a study done by Menzies *et al*., (1992), they found that foliar applications of potassium silicate at 17 and 34mM Si are as effective as a 1.7mM root application. When Si is applied to foliage, concentrations need to exceed the amount applied to soil by a factor 10 (Bowen *et al*., 1995). Foliar sprays may however lead to whitish spots on Si-sprayed leaves and fruit, and could affect marketability of the product (Bowen *et al*., 1992).

1.4.4.2 Soil Application

Most silicon fertilisers are applied as a solid, similar to ordinary agricultural fertilisers, to the soil. The concentration applied depends on the fertiliser used (Bowen *et al.,* 1995). Application of Si to Si-deficient soils creates the possibility of reduced fertiliser rates to be applied in successive years after Si application, and with regards to disease prevention, to reduced fungicide applications (Seebold *et al*., 2004).

1.4.4.3 Combined Application of Si and Fungicides

Regardless of the silicon concentration applied, the percentage of Si in plant tissue is significantly higher than that of untreated control groups (Seebold *et al.,* 2004). Application of Si in the form of calcium silicate slag (elemental Si at 1Mt.ha^{-1}) was equivalent to seven applications of a fungicide over a three-month period for suppressing gray leaf spot caused by *Magnaporthe grisea* (T.T. Hebert) Barr in St. Augustine grass, which suggests that fungicides may be better managed with Si (Brecht *et al.,* 2004). The application of fungicides can in some cases even be eliminated if Si is applied. The residual activity of Si-residuals in soils suggests that annual applications may not be necessary (Seebold *et al.*, 2004).

1.4.4.4 Trunk Injections

Anderson *et al*. (2004) first reported the application of potassium silicate (200ppm) with trunk injections into avocado trees affected by *P. cinnamomi* root rot. With the application of silicon, a mean tree health improvement of 31.1% was seen, compared to a 3.6% decline in control trees. Injections also aided in reduction of postharvest fruit diseases.

1.4.4.5 Si Accumulators and their Distribution

Jones and Handreck (1967) were the first to divide plants into groups in relation to their silicon concentration. This grouping divided plants into accumulators (10-15% dry weight) (wetland grasses), intermediates (1-3% dry weight) including dry land grasses, and non-accumulators (< 1% dry weight) (dicotyledons). Ma and Takahashi (2002) proposed another grouping system. Group A (which includes monocotyledons, Pteridophytes and Bryophytes) contains more than 1.5% Si; group B (monocotyledons, dicotyledons, Gymnosperms and Pteridophytes) contains less than 0.25% Si; and group C has a Si/Ca ratio of 0.54 with a Si content of 0.86%. This suggests that plants in group A take up Si actively, but plants in group B reject the uptake of Si. Plants contained in group C may take Si up passively.

1.4.5 Silicon Uptake

1.4.5.1 Uptake Form of Silicon

Optimal uptake of Si from the soil solution is influenced by soil physical and chemical factors including soil pH, clay percentage and $CaCO_3$ content (Liang *et al*., 1994). The chemical form of the Si molecule depends on the pH of the growth solution, and therefore the uptake of Si, depends on the pH as well (Takahashi and Hino, 1978; Ma and Takahashi, 2002), although the aqueous chemistry of silicon is dominated by silicic acid at biological pH ranges (Fauteux *et al*., 2005). When a growth solution is below pH 8.0, Si is present as a non-dissociated silicic acid molecule [$(H_4SiO_4)_n$, n=2,3]. Si is therefore considered to be in the form of an uncharged molecule in usual culture solutions (Ma and Takahashi, 2002), ranging from 0.1 to 0.6mM, roughly two orders in magnitude higher than the concentrations of P in solution (Epstein, 1999a).

Yoshida *et al*. (1962) fractioned silicon contained in rice into three forms, these being colloidal silicic acid, silicate ion and silica gel; and reported silica gel to constitute 90% of total silicon concentration in plants. It had, during the time of study, not been clear how silicon form changes with growth progression, or how much silicon is in the cell sap, and which form is the most important for rice growth. This was confirmed when Lewin and Reimann (1969) reported the form of amorphous silica present in plants is that of a silica gel, constituting 90 to 95% of the total silicon in the rice plant, with the content of silicic acid ranging from 0.5 to 8% of the total silicon. Silicon contained in the xylem sap exists entirely in the form of monosilicic acid (Epstein, 1999a). It could therefore be concluded that plants take up Si in the form of a non-dissociated molecule, silicic acid, which is superior to the ionic form uptake (Ma and Takahashi, 2002).

Quantitatively, Si concentrations fall in the same range as those of inorganic macronutrients, although its variability is wide, corroborating the observation that the Si content of monocotyledons is by far larger than that of dicotyledons (Epstein, 1999b). Ma *et al.* (2003) has also reported the concentration of silicon in plants to be controlled genetically.

1.4.5.2 Kinetics of Si Uptake

Richmond and Sussman (2003) stated that silicon uptake may either take place passively with the uptake of water by plants, or as an active form of nutrient recruitment. Ma and Tamai (2002) investigated whether Si uptake is induced in plants and concluded that, although the uptake of Si increased linearly with time, no difference in Si uptake between plants previously exposed to Si or not exist, suggesting that the uptake of Si by plant roots is not inducible (Ma and Takahashi, 2002).

Kelly *et al*. (1998) reported that plants themselves alter the chemical form and availability of soil Si. As a rule, most of the silicon absorbed is channeled from the roots to the shoots, and that within the shoots, distribution is far from even. Such differential, localized distributions of Si have often been mentioned in both roots and shoots (Hodson and Evans, 1995). Ma *et al.* (2001) studied the effect of root form (root hairs vs. lateral roots) on silicon uptake and concluded that lateral roots contribute to silicon uptake in rice plants, whereas root hairs do not.

1.4.5.3 Effect of Transpiration on Si Uptake

Good evidence exists to assume the uptake of silicic acid in the transpiration stream through the xylem and subsequent distribution throughout the plant to be passive, resulting in constant silica accumulation in the aerial plant parts, particularly in epidermis cells, as water is lost through transpiration (Ma *et al*., 2001). The movement of silicic acid across the root into the transpiration stream can however not be explained by passive move diffusion and mass flow in water, and it appears that silicic acid enters xylem sap of plants, including beans and rice, against a concentration gradient (Raven, 2001). The influence of silicon uptake, either passive or otherwise, on transpiration can be physiologically important, as it has been reported that the transpiration rate of rice plants can be increased by as much as 30% compared to untreated plants (Ma and Takahashi, 2002). This implicates silicon in playing an important role in water economy management in plants (Lewin and Reimann, 1969). Transpiration does, however, play an important role in the translocation of Si in plants from the roots to the shoots. The concentration of Si in leaf blades is high as a result of a high transpiration rate (Ma and Takahashi, 2002). The rate of transpiration is

regulated by the amount of silica gel associated with cell wall cellulose of epidermis cells. Thus, a well-thickened layer of silica gel helps in decelerating water loss, while epidermal cell walls with less silica gel will allow water loss at an accelerated pace (Lewin and Reimann, 1969). Ma (1990) stated that silicon may promote photosynthesis by decreasing transpiration, improving light transmission and improving light-receiving plant form. These effects of silicon are, however, dependant on growth conditions such as humidity, temperature and light intensity, and it is therefore not surprising that the effect of silicon is sometimes easily observable, whilst in other instances it is hardly noticeable.

1.4.5.4 Effect of Nutrient Salts on Si Uptake

It was proposed that the soil pH value, clay content and $CaCO_3$ concentration significantly affect the availability of silicon to plant material (Liang *et al.,* 1994). Because rice roots take up Si in a non-charged form, the effect of nutrient ions on Si uptake in all plants is considered to be small. Excess nitrogen however causes a decrease in Si uptake, which suggests that some nutrient salts affect Si uptake. Meyer and Keeping, 2005b) Fu *et al*. (2001) investigated the effect of rare earth elements (REE) on silicon uptake and concluded that the abundance of silicon had no relationship with REE concentrations in both soil and plant samples.

1.4.5.5 The Effect of Pruning on Si Uptake

The amount of silica present in plant tissues can be increased by defoliation of the plant. Tissue silicification can be increased by defoliation and is affected by the availability of soluble silica in the nutrient medium (McNaughton *et al.,* 1985).

1.4.5.6 Participation of Metabolism in Si Uptake

Hydrogen sulfide (H_2S) and NaCN inhibit the uptake of silicon to a similar extent as K and P, which suggests that the uptake of Si in rice is an aerobic metabolism-dependent active process (Ma *et al*., 2001). Plants contain metabolic inhibitors that inhibit the uptake of Si, and therefore ATP may be required for the uptake of Si, while a metabolism partially related to P uptake is also involved in Si uptake. Light irradiation supplies sugars during photosynthesis as well as stimulates transpiration

through stomatal opening, which in turn stimulates Si uptake (Ma and Takahashi, 2002).

1.4.6 Deposition of Silicon

Monosilicic acid forms stable organic hydroxyl-containing complexes. Bio-silica has also been implicated in various bio-molecules including associations with proteins and carbohydrates (Bond and McAuliffe, 2003), and in complexes with sugars and sugar derivatives bonded to hypervalent silicon forms (Fauteux *et al*., 2005).

Silica is typically deposited in rice plants in the form of silica bodies, formed in the epidermis, silica and bulliform cells (Agarie *et al*., 1996). Silica and cork cells are found in rows over veins. These cells are found to occur in the epidermal layer above and below vascular bundles (Sangster *et al.,* 2001). Bulliform cells are only seen in the upper epidermal layer between vascular bundles, and silicon is polymerized inside these silicon-treated plant cells as $SiO_2.nH_2O$. These polymerized silicic acids in the cells bind with cellulose, forming a strong bonded silico-cellulase membrane, with silicified cell walls, becoming lignified as cells mature (Agarie *et al.,* 1996).

Clanning and Eleuterius (1992) reported on the deposition of Si in seeds of both mono- and dicotyledonous plants, and concluded that silicon distribution is related to certain epidermal structures including trichomes, ridges, raised areas and hairs, and is also included into the cell wall. They reported silica content of seeds to be as high as 50% of the ash. Wutscher (1989) reported silicon to accumulate in citrus leaves and feeder roots and not to be dependant on plant age. Lux *et al*. (2003) reported that bamboo (*Phyllostachys heterocycla* Mitf.) accumulated 7.6 and 2.4% silicon in their leaves and roots respectively and that this silicon was impregnated into the inner tangential endodermal cell walls.

1.4.7 *In Vitro* Effect of Silicon on Plant Pathogens

Recent literature indicated the direct suppressive effect of silicon on fungal growth *in vitro*. Biggs *et al*. (1997) reported a 65% growth reduction of the brown rot pathogen *Monilinia fructicola* (G. Wint) Honey of peach fruit on potato dextrose agar (PDA) amended with calcium silicate compared to control treatments.

In-vitro dose-responses towards soluble potassium silicate (20.7% SiO_2) were determined for *P. cinnamomi*, *Sclerotinia sclerotiorum* (Lib.) De Bary*, Pythium* F-

group, *Mucor pusillus, Drechslera* sp, *Fusarium oxysporum* Schltdl. W. Snyder & H.N. Hansen, *Fusarium solani* (Mart.) Appel Wollenw., *Alternaria solani* Sorauer, *Colletotrichum coccoides* (Wallr.) S. Hughes, *Verticillium tricorpus* I. Isaac, *Curvularia lunata* (Wakker) Boedijn and *Stemphylium herbarum* E. Simmons by Bekker *et al*. (2006). Inhibition of mycelial growth was dose-related with 100% inhibition at 80ml (pH 11.7) and 40ml (pH 11.5) soluble potassium silicate per litre of agar, for all fungi tested with the exception of *Drechslera* sp. and *F. oxysporum* at 40ml in one experiment. *S. sclerotiorum* and *P. cinnamomi* were completely inhibited at all soluble potassium silicate concentrations between 5 and 80ml.l^{-1} agar, while all the other fungi were only partially inhibited at potassium silicate concentrations of 5, 10 and 20ml.l^{-1} agar. Percentage inhibition was positively correlated with silicon concentrations. Soluble potassium silicate raised the pH of unameliorated agar from 5.6 to 10.3 and 11.7 at potassium silicate concentrations of 5 and 80ml.l^{-1} agar, respectively. Subsequent investigations by the same author into the effect of pH in the absence of potassium silicate showed that fungal growth was only partially inhibited at pH 10.3 and 11.7. Clearly, potassium silicate has an inhibitory effect on fungal growth *in vitro* and this is mostly fungicidal rather than attributed to a pH effect.

1.4.8 *In Vivo* Effect of Silicon on Plant Pathogens

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. To provide maximum protection, and therefore minimize disease development, silicon must be applied continuously (Bowen *et al*., 1992). Bélanger and Benyagoub (1997) however stated that soluble silicon appears to be an effective, but not exclusive part of an integrated disease control strategy. Numerous diseases have been effectively controlled by silicon application (Table 1).

Table 1.1: Diseases controlled by silicon application

Host plant	**Disease**	**Pathogen**	**Reference**
St. Augustine grass	Grey leaf spot	*Magnaporthe grisea* (T.T. Hebert) Barr	Brecht *et al*. (2004)
Tomato	Bacterial wilt	*Ralstonia solanacearum* (Smith)	Dannon and Wydra (2004)
Rice	Brown spot	*Pyricularia oryzae* Cavara	Seebold *et al*. (2004)
Rice	Neck rot	*Bipolaris oryzae* (Breda de Haan) Shoemaker	Seebold *et al*. (2004)
Cucumber	Powdery mildew	*Sphaerotheca fuliginea* (Schltdl. Fr.) Pollacci	Adatia and Besford (1986)
Peach	Brown rot	*Monilinia fructicola* (G. Wint) Honey	Biggs *et al*. (1997)
Avocado	Anthracnose	*Colletotrichum gleosporioides* (Penz.) Penz. & Sacc.	Anderson *et al*. (2005)

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 hindered. Similar findings have been reported for powdery mildew on cucumber (Chérif *et al*., 1992), muskmelon and zucchini squash (Menzies *et al*., 1992), wheat (Bélanger *et al*., 2003) and rice (Rodriguez *et al*., 2003).

Adatia and Besford (1986) reported a complete inhibition of *Sphaerotheca fuliginea* causing powdery mildew on cucumber plants amended with 100 $mg.l^{-1}$ SiO_2. This reduction due to silicon amendment has been shown to be coincident with the accumulation of silicon in leaves (Bowen *et al.,* 1992). Coupled to this they reported a reduction of haustoria formation, and an increase in phenolic production.

Korndörfer *et al*. (1999) reported a 64% decrease in rice grain discolouration caused by several different pathogens including species such as C*urvularia*, *Fusarium* and *Helminthosporium*, after silicon application, with reduced sporulating lesion number, lesion size, rate of lesion expansion, number of spores per lesion, and diseased leaf area, and an increased incubation period.

Seebold *et al.* (2000) reported leaf and neck blast on partially resistant and susceptible rice cultivars to be reduced by silicon amendment to disease levels found in resistant

cultivars without silicon. Seebold *et al*. (2004) thereafter concluded that silicon showed similar results and sometimes outperformed modern chemicals used to control leaf and neck blast in rice caused by *Bipolaris oryzae*, and reported that the application of silicon to Si-deficient soils may permit the use of reduced fungicide rates to manage disease severity.

Anderson *et al*. (2004) reported on disease incidence of avocado fruit harvested from trees injected with 1000ppm potassium silicate as a trunk injection. Fruit harvested two and three months after injection indicated significantly lower levels (50% of the total number of fruit) of anthracnose development, but fruit harvested only two weeks after injections showed no difference in disease severity compared to control fruit. This was confirmed by a two year study reporting a decrease in severity and incidence of anthracnose on fruit from trees treated with silicon (Anderson *et al*., 2005).

1.4.9 Mechanism of Disease Suppression by Silicon

Silicon has been implicated in the induction of numerous disease suppressive mechanisms. These mechanisms are not exclusive to certain plant species and do not function in isolation, but rather forms part of a conglomeration of mechanisms that induce disease suppression in plants. However, through ongoing research, the induction of host defence responses seems to be the primary mechanism of disease suppression (Fawe *et al*., 2001).

1.4.9.1 Mechanical Barriers

Resistance of a plant to pathogens may be attrabuted to specific accumulation and polymerisation of $Si(OH)_4$ in cell walls. Wagner (1940), studying the effect of silicon on powdery mildew development on wheat, was the first to speculate on the mode of action of silicon. He noted that the positive correlation between silicic acid concentration at the infection site and the degree of mildew resistance is indicative of an effect of silicon on disease suppression (Bowen *et al*., 1995). Chérif *et al.* (1994) and Datnoff *et al*. (1997) stated that this suppression is made possible by the increased silicification of the epidermal cells. They hypothesised that Si impedes the initial penetration of spore germ tubes, leading to reduced haustoria penetration in Si-treated plants. Datnoff *et al.* (1991) proposed the association of silicon with cell wall constituents, making cell walls less accessible to the enzymatic degradation by fungi.

Although it was thought that this mechanism by which Si provides protection is only effective against foliar fungal diseases, it has been proved that Si is equally suppressive towards root-infecting pathogens (Bowen *et al*., 1995).

This link between silicon deposition and pathogen resistance stems from the fact that Si accumulates at sites of infection (Fauteux *et al.,* 2005). Ma *et al.* (2002) suggested an active transport system for silicon, at least in rice plants, which is due to a higher transpiration rate at sites where the cuticle is damaged rather than active transport in a defence pathway. Rodriguez *et al*. (2005) promoted the hypothesis for an active participation of silicon in disease response, whilst the possibility of a physical barrier in the rice-*Magnaporthe grisea* (T.T. Hebert) Barr disease system may not be excluded. Although the hypothesis of cell wall reinforcement has been contested throughout recent years, numerous studies confirm this hypothesis (Kunoh and Ishizaki, 1975; Chérif *et al.,* 1992; Menzies *et al*., 1992; Bowen *et al*., 1992, 1995).

1.4.9.2 Induction of Plant Enzymes

Amendment of nutrient solutions with soluble potassium silicate reduces root decay and wilting of cucumber plants after inoculation with *Pythium ultimum* (Chérif *et al.,* 1992). It was thought that Si acts as a mechanical barrier by accumulating at the point of hyphal entry, but through Electron Dense X-ray (EDX) analysis, silicon accumulation was not detected in the root and hypocotyl tissue of inoculated cucumber plants. Fungal hyphae are able to penetrate silicon amended cucumber hypo-cotyledons and roots readily. However, 48h after infection significant differences in the rate and extent of colonisation of Si- and Si+ plants are detected (Chérif *et al.,* 1992).

Samuels *et al*. (1993), using the powdery mildew-cucumber pathogen system, showed that within a short period of time after Si application is ceased, all prophylactic effects recede. Thus, interruption of silicon application leads to resistance loss even though opal phytoliths accumulate and, according to the mechanical barrier hypothesis, pathogen development should be slowed.

Chérif *et al*. (1992, 1994) proposed that soluble Si activates defence mechanisms in cucumber against *Pythium*, as reflected by enhanced activity of peroxidases, chitinases and polyphenoloxidases, and increased accumulation of phenolic compounds. This was also reported to be true in wheat (Bélanger *et al.,* 2003).

Silicon is an effective inducer of resistance against bacteria in tobacco (Schneider and Ullrich, 1994). They reported increased levels of B-1,3-glucanase, polyphenoloxidase, lysozyme and phenylalanine ammonia lyase concentrations. Silicon amended cucumber plants showed concentrations of Ribulose-1,5-bisphosphate-carboxylase to be 31% higher on a leaf fresh weight basis, and 50% higher on a leaf area basis (Adatia and Besford, 1986). A significant decrease in the rate and extent of colonisation of *Pythium ultimum* 48h after inoculation was also found in silicon amended plants compared to control treatments, indicating defence responses observed in Si+ plants to differ in both the extent and speed of inoculation (Chérif *et al.,* 1994). Rodriguez *et al.* (2005) reported the differential accumulation of peroxidase and glucanase in silicon amended rice plants. Resistance is, however, due to an increase in the whole group of defence genes and not only to singular increases in certain enzymes (Schneider and Ullrich, 1994).

Increased peroxidase and Poly-Phenylene Oxide (PPO) activities are observed in Si+ roots within two days after inoculation with *P. ultimum*, whereas comparable activity is only detected 10 days after inoculation in Si- plants (Rémus-Borel *et al*., 2005). It may be of major importance to notice the relation between peroxidase and PPO activities and Si stimulated accumulation of polymerized phenolics. This may be an indication that the participation of the oxidative metabolism of phenolic compounds is more important than the presence of phenolic constituents (Chérif *et al.,* 1994). Rémus-Borel *et al*. (2005) confirmed this when they reported the production of phytoalexins in reaction to silicon application in response to powdery mildew infection of wheat plants.

Silicon appears to activate the natural host defence mechanisms, which results in enhanced plant defence to *P. ultimum* attack. Plant cells that received silicon react to *P. ultimum* invasion by a marked accumulation of densely stained amorphous material, while no discernible response is seen in infected cells from Si- plants (Chérif *et al.,* 1992). Necrotic hyphae occur in this amorphous material suggesting that this specific plant response is a potential barrier that restricts pathogen growth. It may also be deleterious to the fungus. The efficiency, with which this response prevents fungal attack, is likely to be contingent upon the magnitude and speed of the expression of antifungal compounds (Rodriguez *et al*., 2005).

The activation of a gene regulation system by Si should be considered (Wingate *et al*., 1988). The presence or absence of these genes does not determine resistance or

susceptibility, but the magnitude and speed with which the gene information is expressed. The mechanisms by which these systems are signalled and activated to initiate defence molecule production remain unknown (Chérif *et al.,* 1992)

1.4.9.3 Induction of Fungitoxic Metabolites

Conclusive evidence proves Si increases accumulation of fungitoxic metabolites in plants in a pattern typical of phytoalexins. The release of these fungitoxic metabolites as a result of silicon application, contributes to the enhanced resistance to pathogens (Fawe *et al*., 2001). Cucumber plants synthesize phytoalexins as a result of silicon acting as an elicitor, with a variable set of anti-fungal compounds being produced. For phytoalexins to form, numerous anti-fungal pathways in plant cells, that include acid hydrolyses, are induced (Fawe *et al*., 1998). This hydrolysis is responsible for the convergence of glycosidic substances to aglycones (Chérif *et al.,* 1994). Both preformed and neo-synthesized conjugates (as a result of hydrolysis) play an important role in the biosynthesis of phytoalexins, where the hydrolysis is of primary importance in anti-fungal activity. Cucumber plants possess a pool of conjugates that are raised or modified by Si treatment from which new anti-fungal metabolites can be released. Si prompts the accumulation of phenol-like material (such as the phytoalexin rhamnetin) that is deleterious to fungal haustoria (Fawe *et al*., 1998).

Anderson *et al*. (2004) stated that analysis of avocado fruit from trees injected with silicon for the control of anthracnose indicate higher levels of Mn, which possibly can contribute to disease suppression, as Mn is an important cofactor in synthesis of phenols and lignin necessary for plant defense. Manganese also inhibits the activity of pectolytic enzymes produced by fungi.

In the rice-*Magnaporthe grisea* disease system, higher levels of momilactone phytoalexins were found to be present in leaf extracts from plants amended with silicon compared to unamended plant leaf extracts (Rodriguez *et al*., 2004). The more efficient stimulation of the terpenoid pathways in silicon amended plants, coinciding with the increase in momilactone levels, appears to be a factor contributing to enhanced rice resistance to blast. This again, indicates silicon plays an active part in rice blast resistance, as opposed to merely enhancing formation of a physical barrier (Fawe *et al*., 2001).

Although silicon has 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*, where concentrations of phenolics in inoculated plants are double that of uninoculated plants six days after inoculation (Chérif *et al.,* 1994).

Silicon amplifies the chemical defence of plants, although plant physiological roles have not yet been determined or fully understood. Resistance induced by Si is similar to Systemic Acquired Resistance (SAR), but Si-resistance is lost when the Si-source is removed whereas SAR is a long-term effect. Polymerisation of Si, however, leads to its inactivation as an inducer of resistance (Fawe *et al.,* 1998).

1.4.10 Stress Related Role

Silicon appears to have an alleviating effect on not only biotic (Kvedaras *et al*., 2005; Meyer and Keeping, 2005a; Meyer and Keeping 2005b), but also abiotic stress (Bowen *et al*., 1995). This suggests the possibility that the effect of Si on plant growth and performance are only evident when plants are under some form of stress. Further research is required to determine whether a physiological role for Si exists in commercially important crops, especially in vegetable crops where these crops are grown in soil-less culture with a Si concentration usually less than 0.17nM in the form of SiO_2. Factors leading to stress of a physical nature include temperature, light, wind, water, drought, radiation, frost and ultraviolet radiation (Ma and Takahashi, 2002).

If excess water is lost during transpiration, stomata close and a decrease in photosynthetic rate occurs. Transpiration mainly occurs through the stomata and partly through the cuticle (Ma *et al*., 2001). 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. This can be a great advantage, especially in plants with thin cuticles (Ma and Takahashi, 2002). Gong *et al*. (2005) reported that silicon improves the water status of drought stressed wheat plants, with regard to water potential and water content in leaf tissue, compared to untreated plants. Biomass accumulation due to lower water stress was reported in sugarcane after silicon fertilization (Singels *et al*., 2000).

1.4.11 Beneficial Impact of Silicon on Other Nutrient Elements

The effect of silicon on plant growth and disease development in plants is related to the interaction of silicon with other essential and non-essential plant growth elements. Sistani *et al.* (1998) reported that rice plants respond to silicon as well as to N, P and K application, with silicon comprising a major part of rice crops residues. Application of silicate fertilizers increase levels of P, Si, Ca, and Cu, and reduce N, K, Mg, Fe, Mn and Zn levels in sugarcane leaves (Elawad *et al*., 1982). Silicate materials also increase pH, Si, P, Ca and Mg in the soil (Sistani *et al.,* 1998).

Nutrient content (including Si) of avocado fruit has been reported to influence fruit quality and ripening. Hoffman *et al*. (2002) reported significant positive correlations between fruit Ca and Mg concentrations, the (Ca + Mg)/ K ratio and the number of days for the fruit to reach ripeness. Negative correlations are observed between these nutrients and anthracnose and mesocarp discolouration. Fruit from high yielding trees are generally smaller, have a lower anthracnose incidence and ripen slower, and have higher flesh Ca concentrations. In barley plants experiencing salt stress, the addition of silicon leads to increased calcium concentrations in shoots and decreased sodium levels in shoots and roots (Liang, 1999). However, Ma (1990) reported that silicon decreases calcium uptake, but calcium has no effect on silicon uptake or on the silicon form in which it occurs in plants.

Wutscher (1989) reported a strong correlation between silicon levels and that of S, P, Fe, Mg, Mn, Cu, Zn and Mo, especially in tree bark, leaves and feeder roots of Valencia oranges (*Citrus sinensis* L.). Morikawa and Saigusa (2003) reported the mean N, K, Mg, Cu and Zn content to be lower in old leaves compared to young leaves, while P, Ca, Fe, Mn and Si content are higher in older compared to young leaves. Although silicon is not seen as essential for blueberry growth, silicon was the element that accumulated the most in leaves. Korndörfer *et al*. (1999) reported the alleviation of Fe toxicity symptoms by silicon application. It is known that Si reduces Fe and Mn toxicity, and it is thought that Si increases the ‘oxidising power’ of roots making Mn and Fe less soluble. Silicon may alleviate this toxicity not only because it reduces absorption, but also increases the internal tolerance level of the plant to excess of these elements in the tissue. Richmond and Sussman (2003) reported that complex formation of silicon with heavy metals (sometimes forming silica-heavy

metal precipitates) may be part of the toxicity amelioration mechanism. Maize plants treated with silicon released 15 times more phenolics than untreated plants, and these flavanoid-phenolics (i.e. quercetin and catechin) have strong Al-chelating abilities and may provide heavy metal tolerance in plants.

In the presence of Si, the negative effect of excess nitrogen on the plant (for example turgor-loss, reduced yields etc.) is reduced, with the positive effects resulting in higher yields (Ma and Takahashi, 2002).

Although silicon does not affect P availability for root uptake in soil solutions, it does increase the uptake of P in acid soils (Clements, 1965), improve availability of P in the plant through decreased Mn uptake, and assures good transport throughout the plant (Ma and Takahashi, 2002). Ma (1990) reported silicon to increase P concentration in photosynthetic active plant parts of rice crops, but that this did not occur when P nutrition was high. This may suggest that under low P stress, P utilization may be improved by silicon addition. Under conditions of high P availability, silicon reduces P uptake.

The presence of Si in plants can also cause amelioration of aluminium toxicity in diverse plants including sorghum, barley, and soybean, but not in rice, wheat (Hodson and Evans, 1995), cotton (Li *et al*., 1989; Hodson and Evans, 1995) and pea (Benhamou *et al*., 1996). This indicate species differ considerably in the amounts of Si and Al uptake and transport, suggesting very high Si and Al accumulation to be mutually exclusive (Hodson and Evans, 1995). Corrales *et al*. (1997) reported that pretreatment of Al sensitive maize cultivars with silicon results in Al amelioration and that this effect is due to a decrease in Al uptake and exclusion of Al from silicon pretreated root tips. Decreased Al uptake is however not due to a decrease in Al availability in the growth solution.

Silicon supply alleviates necrotic browning but does not inhibit chlorosis due to toxic Mn concentrations in the growing solution, nor alter the Mn uptake (Horiguchi and Morita, 1987). This alleviation of Mn toxicity was also reported by Shi *et al*. (2005) in cucumber plants amended with silicon. Iwasaki *et al*. (2002) reported pretreatment with silicon not to be effective but concluded that silicon alleviates Mn toxicity if applied concurrently with Mn stress conditions.

1.5 CONCLUSIONS

Phytophthora cinnamomi root rot has been the main limiting factor in successful avocado production in countries such as Australia, South Africa and the USA. Infection is mostly limited to the fine feeder roots, which become black and brittle and eventually die off. Feeder roots may be difficult to find under trees with advanced root rot symptoms and this feeder root dieback may impose severe water stress on the tree. Visible symptoms include wilted and chlorotic foliage and eventually defoliation and die-back of branches depending on root rot severity.

Numerous control measures have been implemented to control root rot, but a well managed program is necessary to ensure disease suppression. Biological control and selection of resistant host rootstocks are important measures taken to ensure reduction of Phytophthora root rot. Resistance is thought to be due to increased levels of lignin and phenolics after inoculation with *P. cinnamomi*.

Chemical control, however, 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 and are believed to control *Phytophthora* spp. by a combination of direct fungitoxic activity and stimulation of host defence. Darvas (1983) first reported the use of a trunk injection method obtaining “outstanding control” of *P. cinnamomi* by fosetyl-Al and this remains to date the most effective application method.

The possibility of *Phytophthora* resistance to fosetyl-Al and H_3PO_3 is a major threat to not only the avocado industry, but the horticultural industry as a whole. To this end, studies have been conducted to determine the effect of potassium silicate application on *P. cinnamomi* disease development in avocado plants, to ascertain if it is a viable alternative treatment to phosphonate fungicide. The beneficial effects of silicon on disease suppression have been indicated by numerous authors. Methods of suppression include increased mechanical barriers, plant enzymes and fungitoxic compound release.

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CHAPTER 2
IN VITRO ASSESSMENT OF THE ANTIFUNGAL ACTIVITY OF POTASSIUM SILICATE AGAINST SELECTED PLANT PATHOGENIC FUNGI

2.1 ABSTRACT

Silicon is a bioactive element only recently implicated as having fungicidal properties. The present study examines the use of liquid potassium silicate for activity against several phytopathogenic fungi. *In vitro* dose-responses towards soluble potassium silicate (20.7% silicon dioxide) were determined for *Alternaria solani, Colletotrichum gloeosporioides, Curvularia lunata, Drechslera* sp., *Fusarium oxysporum, Fusarium solani, Glomerella cingulata, Lasiodiplodia theobromae, Mucor pusillus, Natrassia* sp., *Pestalotiopsis maculans, Phomopsis perniciosa, Phytophthora capsicii, Phytophthora cinnamomi, Pythium* F-group, *Sclerotinia sclerotiorum, Sclerotium rolfsii, Stemphylium herbarum* and *Verticillium tricorpus*. Inhibition of mycelial growth was dose-related with 100% inhibition at 80ml (pH 11.7) and 40ml (pH 11.5) soluble potassium silicate per litre of agar, for all fungi tested in two replications with the exception of *Natrassia* sp., *G. cingulata, F. oxysporum* and *C. gloeosporioides* at 40ml in one replication. For all replications, *P. cinnamomi, P. perniciosa, P. maculans, L. theobromae, G. cingulata, Natrassia* sp., and *C. gloeosporioides* were only partially inhibited at 5, 10 and 20ml soluble potassium silicate per litre of agar, but percentage inhibition was positively correlated with dosage concentrations. Soluble potassium silicate raised the pH of the agar from 5.6 (unameliorated agar) to 10.3 and 11.7 at concentrations of 5 and 80ml potassium silicate per litre of agar, respectively. Increased pH, in the absence of potassium silicate, only partially inhibited mycelial growth. Clearly, potassium silicate had an inhibitory effect on fungal growth *in vitro* and this was mostly fungicidal rather than a consequence of raised pH or the nutritional value of potassium.

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2.2 INTRODUCTION

Numerous studies have shown increased resistance to plant fungal diseases in response to silicon applications, including increased resistance to powdery mildew (*Uncinula necator* (Schwein) Burrill) in grapes (Bowen *et al*., 1992); powdery mildew (*Sphaerotheca fuliginea* (Schltdl. Fr.) Pollacci) in cucumbers (Adatia and Besford, 1986; Belanger *et al.*, 1995); powdery mildew (*Erysiphe cichoracearum* D.C.) in muskmelons (Menzies *et al*., 1992); grey leaf spot (*Magnaporthe grisea* (T.T. Hebert) Barr) in St. Augustinegrass (*Stenotaphrum secundatum* Kuntze.) (Brecht *et al*., 2004), brown spot (*Pyricularia oryzae* Cavara) and neck rot [*Bipolaris oryzae* (Breda de Haan) Shoemaker] in rice crops (Seebold *et al*., 2004).

Wainwright (1993) suggested nutrient-free silica gel supports fungal growth, with the gel itself acting as a nutrient source and stimulating spore formation. Visible mycelial growth was reported by Wainwright *et al*. (1997) to be present on silicic acid amended media. They however ascribed this growth to silicon acting only as a physical contact surface for spores, rather than acting as a nutrient source.

This may have been the case, as recent literature reported on the inhibitory effect of silicon on fungal growth. Biggs *et al*. (1997) reported a 65% growth reduction of *Monilinia fructicola* (G.Wint.) Honey, the causal fungus of brown rot of peach fruit, on Potato Dextrose Agar (PDA) amended with calcium silicate compared to control treatments. Menzies *et al*. (1992) conducted a study to determine the *in vitro* effect of silicon on conidial germination and germ tube growth. They concluded that potassium silicate had no effect on either factors tested. The effect of potassium silicate on mycelial growth was however not mentioned. Wainwright *et al*. (1994) reported that, although fungal mycelia formed when inoculum particles were placed on nutrient free silica gel, no mycelia could be found on the gel surface and growth was therefore most probably supported by nutrients from the inoculum.

The aim of the current *in vitro* study was therefore to assess whether potassium silicate has any fungicidal effect on fungal growth, and if inhibition is found, at what concentration is *Phytophthora cinnamomi* effectively inhibited.

2.3 MATERIALS AND METHODS

2.3.1 Fungal Isolates

Fungal isolates maintained on potato dextrose agar (PDA) were obtained from the Mycological Herbarium of the Plant Protection Research Institute, Agricultural Research Council (ARC), Pretoria, South Africa; Westfalia Technological Services (WTS), Tzaneen, South Africa; and the University of Pretoria, South Africa, culture collections. Fungi were selected on the basis of availability of cultures as well as the importance as pathogens (Table 2.1).

2.3.2 Siliceous Material

Potassium silicate was obtained from Ineos Silicas (Pty) Ltd. (Mean weight ratio SiO_2: K_2O = 2.57; Mean K_2O = 8.0%; Mean SiO_2 = 20.7 %; Mean total solids = 28.75%).

2.3.3 Agar Preparation and Assay

Sterilization of soluble potassium silicate by means of autoclaving resulted in a solidified silicon mass. It was therefore passed through a 0.45µm millipore filter to remove fungal spores and other possible contaminants, and added to autoclaved potato dextrose agar (PDA) prior to solidification to obtain final concentrations of 5, 10, 20, 40 and 80ml soluble potassium silicate per litre of PDA, with non-ameliorated PDA (i.e. with no silicon) as control.

Concentrated soluble potassium silicate has a pH of 12.7, which perceptibly increases the pH of a PDA media. Unamended PDA has a pH of 5.6 but upon addition of 5, 10, 20, 40 and 80ml soluble potassium silicate per litre of agar, the pH of the PDA was raised to 10.3, 10.7, 11.2, 11.5 and 11.7 respectively (Bekker *et al*., 2006). To assess the effect of pH alone on mycelial growth, PDA control plates were adjusted to pH 10.3, 10.7, 11.2, 11.5 and 11.7 using potassium hydroxide. This assessment of the effect of pH was however limited to certain isolates (Table 2.2).

Agar was aseptically dispensed into 9cm diameter plastic Petri dishes. A 5mm diameter mycelial disc taken from a seven-day-old fungal culture on PDA was transferred to the centre of a Petri dish containing PDA either amended with soluble potassium silicate or potassium hydroxide, or unamended control plates. This was performed for each fungus. Ten replicates were included for each treatment. Plates

were incubated at 25˚C in the dark, with colony diameters measured every second day for eight days.

2.3.4 Statistical Analysis

Percentage inhibition was calculated according to the formula (Biggs *et al*., 1997):

$$\textit{Percentage inhibition} = \frac{(C-T) \times 100}{C}$$

Where C = colony diameter (mm) of the control
T = colony diameter (mm) of the test plate

This experiment was repeated for all fungi and results presented are pooled data. Data were subjected to analysis of variance (ANOVA). Mean differences were separated according to Duncan's multiple range test ($P < 0.05$).

2.4 RESULTS AND DISCUSSION

Soluble potassium silicate (20.7% silicon dioxide) inhibited all mycelial growth of *P. cinnamomi* (Figure 2.1), *P. capsicii, S. rolfsii* and *S. sclerotiorum* at all concentration tested from 5 to 80ml.l^{-1} potassium silicate (Table 2.2). Soluble potassium silicate however completely suppressed mycelial growth of all fungi tested at concentrations of 40 and 80ml.l^{-1} potassium silicate, except for *Drechslera* sp. (mean percentage inhibition of 98.35). For all fungi tested, treatments within each fungal group differed with highly significant differences in colony diameter already prevalent within two days after inoculation as can be seen in the data collected for *P. cinnamomi* (Table 2.3). Suppression of mycelial growth at concentrations of 5 - 20ml.l^{-1} potassium silicate varied in the degree of suppression at these low concentrations and was not consistent between replications. This implies a difference in sensitivity of different fungi to potassium silicate inhibition, and effective inhibition concentrations are to be determined for each fungus. Although the concentration gradient in this study resulted in inhibition variability between different fungi tested, the suppressive effect that soluble potassium silicate has on fungal mycelial growth cannot be debated.

While inhibition of mycelial growth on potassium hydroxide amended PDA ranged from 0 – 100%, in some cases mycelia were induced to grow faster and in the case of

Alternaria solani growth was 13.8% faster at pH 11.2 (Table 2.2). Nevertheless, the effect of pH on mycelial growth is inconsistent. Although these results are variable, the effect of added soluble potassium silicate is apparent. The potassium silicate has a direct impact on mycelial growth and the observed inhibition is not solely the result of the potassium silicate changing the pH of the medium. In all fungi tested, mycelial growth continued at high pH in the absence of potassium silicate, although at a slower rate as can be seen in *P. perniciosa* (Figure 2.2), and this implies that soluble potassium silicate has a two-fold effect on fungal growth.

From results obtained in the current study, it can be deducted that, if silicon is applied to plants, it may form an impenetrable barrier to pathogens on the plant surface, preventing colonisation. Ma *et al*. (2001) reported that the applications of silicon to leaves could possibly prevent mildew colonisation and consequent infection, and that this approach may be useful in crops with a passive or rejective silicon uptake mode. Although the current study does not deny the activation of plant defence mechanisms including induced systemic resistance (ISR) by silicon (Fawe *et al*., 1998, 2001), it is proposed that silicon may act as the first protecting barrier in silicon treated plants, and may inhibit pathogen colonisation and consequential infection by inhibiting fungal growth on the plant surface.

Low potassium silicate concentrations resulted in increased fungal growth of *F. oxysporum* (-8.2% inhibition) and *Verticillium tricorpus* (-10.13% inhibition) at 5ml.l^{-1} potassium silicate, and *F. solani* (-8.61%; -11.27% inhibition) at 5 and 10ml.l^{-1} potassium silicate respectively (Table 2.2). This phenomenon has been observed in numerous fungi by Wainwright *et al.* (1997). Although highly improbable, the silicon could also act as additional nutrient supplementation and induce faster mycelial growth. It is however interesting to note that wherever low soluble silicon concentrations induced faster mycelial growth, all pH control groups of the same fungus showed similar results. It is therefore possible that faster mycelial growth in certain fungi is due to an affinity of those fungi for higher pH growing conditions. The precise reason for this is however yet to be determined. This has implications if silicon is to be used as a fungicide and it should therefore be ensured that silicon addition does not increase (water soluble) concentrations in soil to a level conducive to the growth of these fungi.

Seebold *et al*. (1995) and Seebold (1998) reported silicon tested as a fungicide under field conditions resulted in a 40% suppression of rice neck blast (*Magnaporthe grisea*

(T.T. Hebert) Barr). Complete suppression of a particular fungus in the current study however occurred at different concentrations and is variable. The suppression level of each fungus is therefore to be determined *in vitro* before *in vivo* investigations are initiated.

2.5 CONCLUSION

Soluble potassium silicate completely suppresses mycelial growth of all fungi tested at concentrations of 40 and 80ml.l^{-1} potassium silicate. Suppression of mycelial growth at concentrations of 5 - 20ml.l^{-1} potassium silicate vary in the degree of suppression at these low concentrations and is not consistent between replications. This implies a difference in sensitivity of different fungi to potassium silicate inhibition, and effective inhibition concentrations are to be determined for every fungus. In all fungi tested, mycelial growth continued at high pH in the absence of potassium silicate, although at a slower rate which implies that soluble potassium silicate has a two-fold effect on fungal growth, of which the direct effect on fungal growth overrides the effect of pH. The inclusion of potassium hydroxide as a control treatment eliminated any potential role potassium may play in enhancing or suppressing mycelial fungal growth. It is proposed that silicon may act as the first protecting barrier in silicon treated plants, and may inhibit pathogen colonisation and consequential infection by inhibiting fungal growth on the plant surface.

Low potassium silicate concentrations resulted in increased fungal growth in *F solani*, *F. oxysporum* and *V. tricorpus* isolates. This can be due to the presence of nutritional elements present overriding the inhibitory effect of silicon. Although highly improbable, the silicon could also act as additional nutrient supplementation and induce faster mycelial growth. It is however interesting to note that wherever low soluble silicon concentrations induced faster mycelial growth, all pH control groups of the same fungus showed similar results. Complete suppression of a particular fungus in the current study however occurred at different concentrations and is variable. The suppression level of each fungus is therefore to be determined *in vitro* before *in vivo* investigations are initiated. The results of this study clearly indicate that soluble potassium silicate (20.7% silicon dioxide) has fungicidal activity. Effective application of these results in a field trial is however paramount to determine the commercial value of water soluble potassium silicate as a fungicide.

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Table 2.1: Fungal pathogens tested for their ability to grow in the presence of soluble potassium silicate (20.7% silicon dioxide).

Fungus	**Isolate nr.**	**Host plant**	**Disease name**
Alternaria solani Sorauer	UPGH100	Tomato	Early Blight
Colletotrichum coccoides (Wallr.) S. Hughes	-	Tomato	Anthracnose
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.	PPRI3848	Avocado	Anthracnose, Stem end rot
Curvularia lunata (Wakker) Boedijn	UPGH105	Lettuce	Leaf spot
Dreschlera sp.	UPGH106	Tomato	Leaf spot
Fusarium oxysporum Schltdl. W. Snyder & H.N. Hansen	UPGH110	Banana	Wilt
Fusarium solani (Mart.) Appel Wollenw.	UPGH111	Cucumber	Wilt
Glomerella cingulata (Stoneman) Spauld. & H. Schrenk	PPRI6360	Avocado	Anthracnose
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	PPRI6630	Avocado	Stem end rot
Mucor pusillus	-	Tomato	Postharvest rot
Natrassia sp.	PPRI6718	Avocado	
Pestalotiopsis versicolor (Spreg.) Steyaert	PPRI5564	Avocado	Stem end rot
Phomopsis perseae Zerova	PPRI6005	Avocado	Stem end rot
Phytophthora capsicii Leonian	UPGH118	Tomato	Root Rot
Phytophthora cinnamomi Rands	-	Avocado	Root Rot
Pythium F-group	UPGH009	Lettuce	Root Rot
Sclerotinia sclerotiorum (Lib.) De Bary	UPGH122	Lettuce	Rot
Sclerotium rolfsii Sacc.	UPGH123	Tomato	Wilt
Stemphylium herbarum E. Simmons	UPGH124	Lettuce	Leaf spot
Verticillium tricorpus I. Isaac	-	Lettuce	Wilt

Table 2.2: Mean percentage inhibition of different fungi at different potassium silicate (20.7% silicon dioxide) or potassium hydroxide concentrations on ameliorated potato dextrose agar.

Pathogen	Percentage inhibition										
	5ml Si (pH 10.3)	**10ml Si (pH 10.7)**	**20ml Si (pH 11.2)**	**40ml Si (pH 11.5)**	**80ml Si (pH 11.7)**	**pH 10.3**	**pH 10.7**	**pH 11.2**	**pH 11.5**	**pH 11.7**	**Pr.F.**
Alternaria solani	22.9	100.0	100.0	100.0	100.0	33.5	11.8	-13.8	-13.0	24.8	< 0.001
Colletotrichum coccoides	70.43	77.88	95.16	100.0	100.0						< 0.001
Colletotrichum gloeosporioides	15.7	76.4	100.0	100.0	100.0	20.4	17.1	16.2	26.5	57.8	< 0.001
Curvularia lunata	3.17	41.8	99.03	100.0	100.0						< 0.001
Drechslera sp.	13.25	21.52	62.9	98.35	100.0						< 0.001
Fusarium oxysporum	-8.2	23.7	96.2	100.0	100.0	-8.2	-8.2	-8.2	-8.2	-2.3	< 0.001
Fusarium solani	-8.61	-11.27	72.17	100.0	100.0	-0.07				30.1	< 0.001
Glomerella cingulata	9.3	79.7	100.0	100.0	100.0	0.0	0.0	9.3	14.0	32.0	< 0.001
Lasidiodiploidia theobromae	11.3	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	43.0	< 0.001
Mucor pusillus	50	92.86	100.0	100.0	100.0						< 0.001
Natrassia sp.	46.4	100.0	100.0	100.0	100.0	8.4	22.5	0.0	19.0	34.0	< 0.001
Pestaliopsis maculans	23.6	100.0	100.0	100.0	100.0	18.3	7.7	14.3	44.0	77.0	< 0.001
Phomopsis perniciosa	41.8	100.0	100.0	100.0	100.0	4.6	13.6	18.8	34.9	64.7	< 0.001
Phytophthora capsicii	100.0	100.0	100.0	100.0	100.0	22.0	31.8	19.4	47.7	49.9	< 0.001
Phytophthora cinnamomi	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	< 0.001
Pythium F-group	32.9	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	96.7	< 0.001
Sclerotinia sclerotiorum	100.0	100.0	100.0	100.0	100.0						< 0.001
Sclerotium rolfsii	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	< 0.001
Stemphylium herbarum	5.14	14.89	89.24	100.0	100.0	-0.04				1.5	< 0.001
Verticillium tricorpus	-10.13	25.22	54.78	100.0	100.0	-0.05				11.2	< 0.001

Table 2.3: Mycelial growth of *Phytophthora cinnamomi* incubated for eight days at 25˚C on PDA. Agar was either amended with soluble potassium silicate (20.7% silicon dioxide) to create a concentration range, or potassium hydroxide to increase agar pH, mimicking the pH effect of soluble silicon (20.7% silicon dioxide).

	Mean colony diameter (mm)[x]			
Treatment	Day 2	Day 4	Day 6	Day 8
0ml/l [y]	23.9 a[z]	48.2 a	61.8 a	72.3 a
5ml/l	5.3 c	10.8 d	15.7 e	16.1 e
10ml/l	5.0 c	5.0 e	5.0 f	5.0 f
20ml/l	5.0 c	5.0 e	5.0 f	5.0 f
40ml/l	5.0 c	5.0 e	5.0 f	5.0 f
80ml/l	5.0 c	5.0 e	5.0 f	5.0 f
pH 10.3	17.5 b	28.1 b	45.9 b	55.6 b
pH 10.7	12.4 b	25.2 b	42.1 b	50.3 c
pH 11.3	15.5 b	30.7 b	46.1 b	56.9 b
pH 11.5	7.9 c	19.9 c	31.1 c	39.9 d
pH 11.7	7.9 c	18.68 c	25.6 d	38.2 d

[x] Means of 10 plates/ treatment. Measurements include initial 5mm diam. mycelial disc

[y] Concentration of the active ingredient per litre of PDA growth medium

[z] In each column, values followed by the same letter do not differ significantly (F.Pr < 0.01)

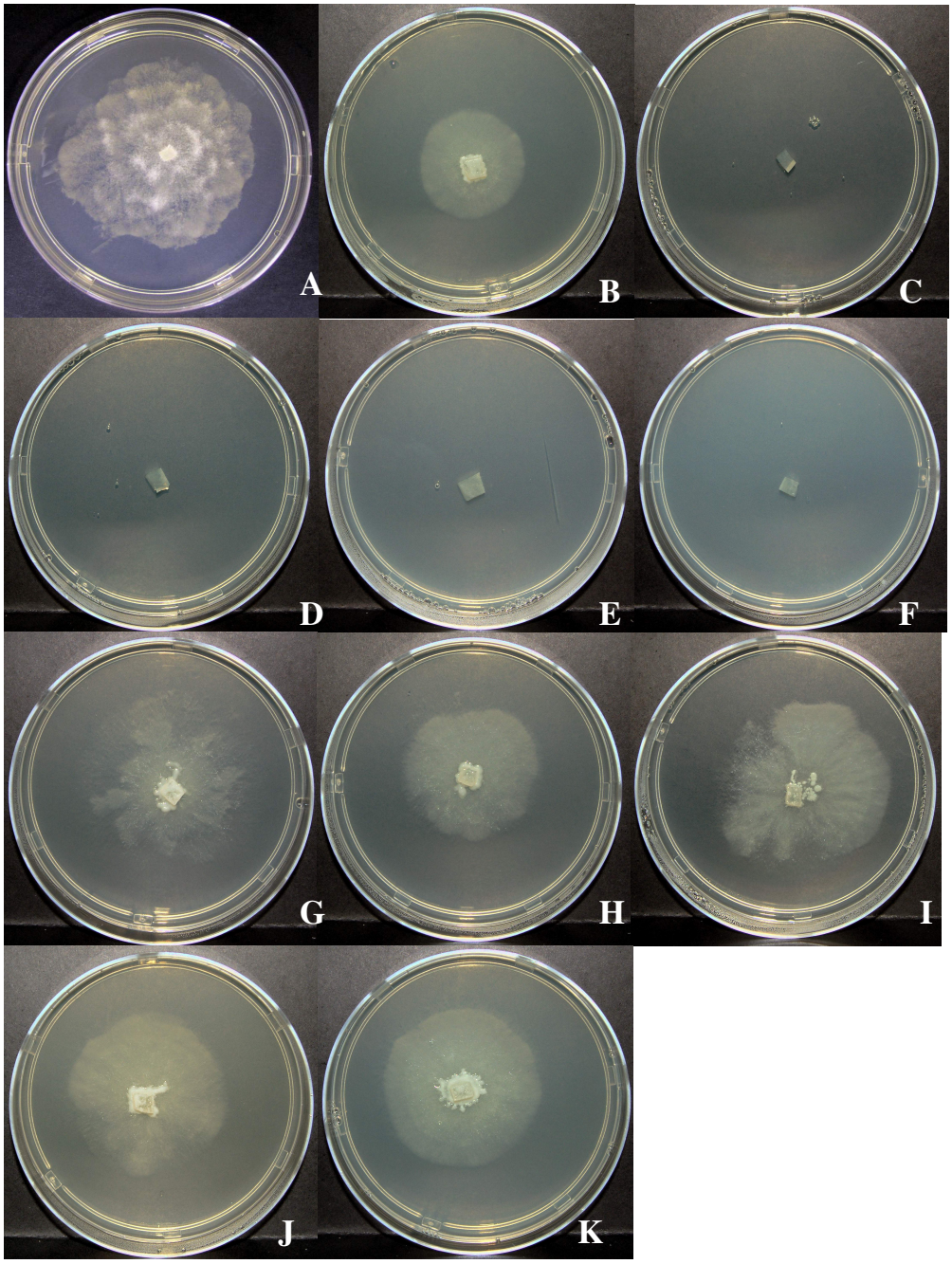

Figure 2.1: Mycelial growth of *Phytophthora cinnamomi* in response to 0ml (pH 5.6)(A), 5ml (pH 10.3) (B), 10ml (pH 10.7) (C), 20ml (pH 11.2) (D), 40ml (pH 11.5) (E), and 80ml (pH 11.7) (F) soluble potassium silicate (20.7% silicon dioxide) per litre of potato dextrose agar (PDA) compared to a potassium hydroxide control group including pH 10.3 (G), pH 10.7 (H), pH 11.2 (I), pH 11.5 (J), and pH 11.7 (K).

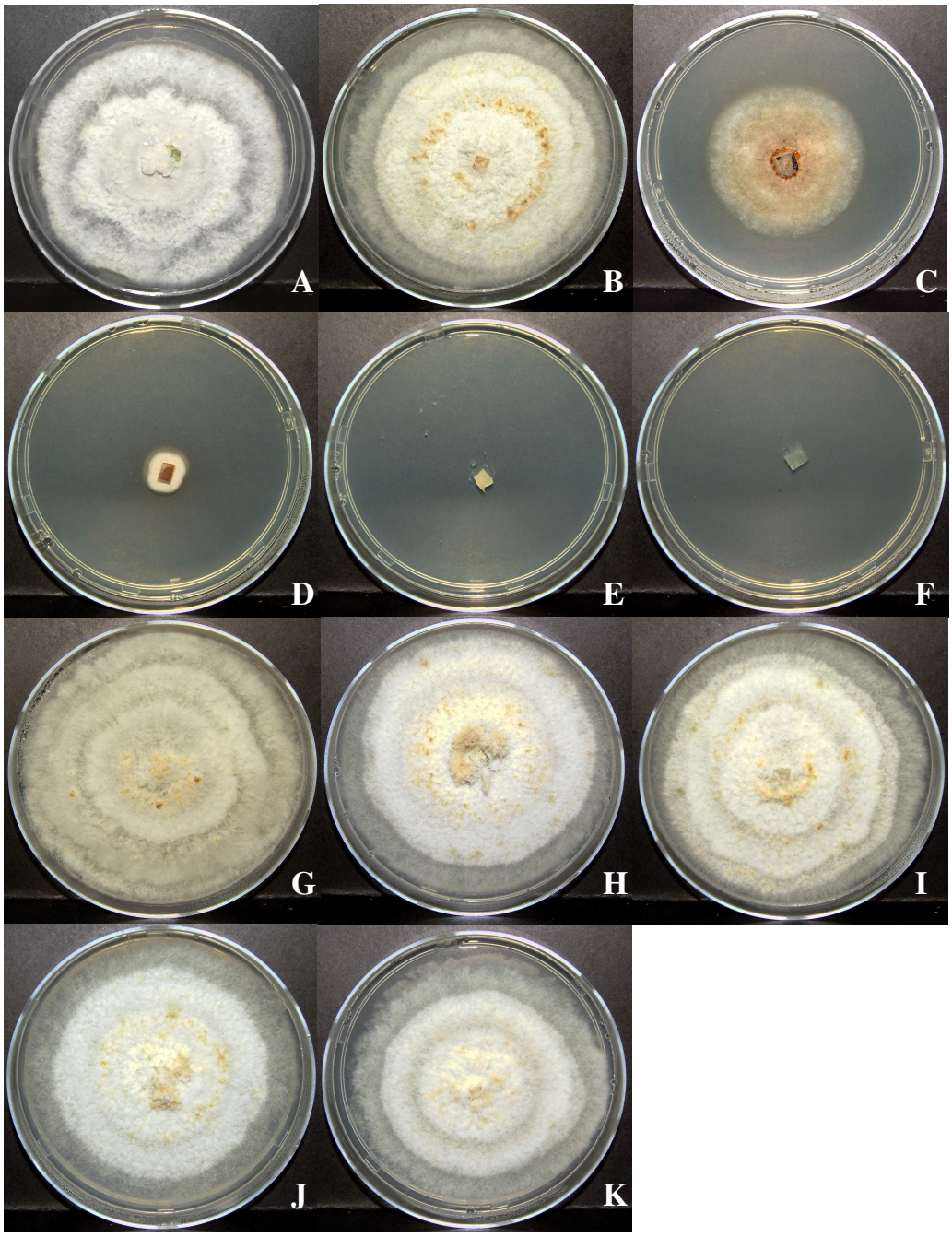

Figure 2.2: Mycelial growth of *Phomopsis perniciosa* in response to 0ml (pH 5.6)(A), 5ml (pH 10.3) (B), 10ml (pH 10.7) (C), 20ml (pH 11.2) (D), 40ml (pH 11.5) (E), and 80ml (pH 11.7) (F) soluble potassium silicate (20.7% silicon dioxide) per litre of potato dextrose agar (PDA) compared to a potassium hydroxide control group including pH 10.3 (G), pH 10.7 (H), pH 11.2 (I), pH 11.5 (J), and pH 11.7 (K).

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.

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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).

CHAPTER 4

EFFICACY OF WATER SOLUBLE POTASSIUM SILICATE AGAINST PHYTOPHTHORA ROOT ROT OF AVOCADO UNDER FIELD CONDITIONS

4.1 ABSTRACT

Phytophthora root rot is the most important disease of avocados worldwide. *P. cinnamomi* causes rot of the feeder roots and depending on root rot severity, may lead to tree death. Although cultural practices, biological control and resistant cultivars play an important role in suppression of the disease, the avocado industry relies almost solely on phosphonate fungicides for control of root rot. The possibility of development of resistance against this group of fungicides is a concern and to this end silicon was investigated as a possible alternative treatment. An orchard of thirteen year old 'Hass' avocado trees on 'Duke7' seedling rootstocks was selected. This orchard was naturally infested with *P. cinnamomi*. Potassium silicate was applied as either a soil drench or a trunk injection. Three silicon (Si x 3) soil drench applications resulted in significantly higher root densities compared to the control and potassium phosphonate (Avoguard®) treatments. Significant differences in root density were obtained during March 2005 between Si x 3 (5.54%) and Si x 2 (4.45%), compared to the potassium phosphonate treatment (2.16%) and untreated control (2.35%). These differences were negated during drier periods (May 2005) with no significant differences occurring between treatments. However, from November 2005 to July 2006, Si x 3 soil drench treatments resulted in significantly higher root densities compared to the untreated control and potassium phosphonate treatments. These results correlated with tree canopy ratings. All potassium silicate soil drench treatments resulted in lower disease ratings (canopy condition) over the 18 month period of data collection, with significant differences obtained at all data collection dates, except July 2006, when potassium silicate soil drench treatments (viz. Si x 1 = 2.55, Si x 2 = 2.4 and Si x 3 = 2.55) resulted in similar disease ratings as those observed in the control (3.15) and potassium phosphonate treatments (2.95). This indicates that potassium silicate soil drench treatments reduced drought stress, apart from reducing disease stress. The effect of a potassium silicate stem injections did not result in differences in tree root densities or canopy ratings. Silicon x 3 also significantly increased total yield per tree as well as the number of fruit per tree in comparison to the untreated control. No clear effect of silicon on post harvest diseases was observed.

4.2 INTRODUCTION

Avocados (*Persea americana* Mill.) are widely distributed throughout South Africa, with the most important cultivars being 'Fuerte' and 'Hass' (Knight, 2002). True to the phenological model, avocado roots display rhythmic growth (termed flushes), which alternate with quiescent periods (Wolstenholme, 1981). Consequently, the balance between root and shoot mass must always be maintained. Wolstenholme (1987) described the avocado tree root system as relatively inefficient, although feeder roots may reach as deep as 1m (Whiley, 1994). The majority of these white, unsuberized feeder roots are, however, found in the upper 0.6m of soil (Pegg *et al*., 2002). Tree performance is ultimately reflected in yield and quality, and these factors are governed by the condition of the root system, and the severity of pathogen attack on avocado roots.

Phytophthora root rot, caused by the fungus *Phytophthora cinnamomi* Rands, is the most important and destructive disease of avocados worldwide (Pegg *et al*., 2002). Phytophthora root rot has been the main factor limiting successful economic avocado production in countries such as Australia, South Africa and the USA (Coffey, 1987). It attacks trees of all ages, and may kill both nursery and large bearing trees. *Phytophthora cinnamomi* causes rot of feeder roots (Anon, 2004), although invasion of larger roots has also been reported (Pegg *et al*., 2002; Anon, 2004). A moderate tolerance is often observed in avocado trees which do not show degradation of canopy condition (Ploetz and Parrado, 1988). However, symptoms normally manifest in the canopy, resulting in foliage becoming wilted and chlorotic, leaves abscising and branches rapidly dying back. Occurrence of these symptoms depends on root rot severity. In infected trees new leaf growth is minimal, and if leaves do form, they are small and pale green. Fruit set is usually low in root rot affected trees, and fruits are small. Because roots are unable to control salt uptake, chloride accumulates in leaves and may reach toxic levels, resulting in scorching of leaf margins and tips (Whiley *et al*., 1987). The effect of Phytophthora root rot on photosynthate accumulation and storage is of major importance, as infection leads to lower water potential, reduced stomatal openings, and reduced water and nutrient uptake (Sterne *et al.,* 1977, 1978; Whiley *et al.,* 1986).

Prevention of Phytophthora root rot is difficult, and control measures are mostly limited to cultural practices, including the selection of virgin sites and clean plant material (Ohr and Zentmyer, 1991). The use of biological methods to control *P. cinnamomi* has been investigated by numerous authors (Pegg, 1977; Casale, 1990; Duvenhage and Kotze, 1993), and McLeod *et al.* (1995) reported a reduction in *P. cinnamomi* populations of more than 50% with application of *Trichoderma* isolates. To date, host resistance is the best

preventative method for reducing Phytophthora root rot (Coffey 1987). Some rootstocks express tolerance to root rot through the rapid regeneration of active feeder roots while in others the progress of infection in the root is inhibited (Phillips *et al.,* 1987). Avocado rootstocks bred for resistance include Dusa™ and Duke 7 (Kremer-Köhne and Duvenhage, 2000).

Chemical control however 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 *et al.,* 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 *et al.,* 2001).

Duvenhage (1994) reported 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.

In an attempt to find a viable alternative treatment for Phytophthora root rot of avocado, studies have been conducted to determine the effect of potassium silicate application on *P. cinnamomi* root rot development in both avocado nursery trees and trees in the field. The suppressive effects of silicon on plant diseases have previously been reported (Epstein, 1999; Ma and Takahashi, 2002). Methods of disease suppression by silicon include increased mechanical barriers (Datnoff *et al.,* 1997) and the production of plant enzymes (Samuels *et al.,* 1993) and fungitoxic compounds (Fawe *et al.,* 1998).

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

4.3 MATERIALS AND METHODS

4.3.1 Chemicals

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

4.3.2 Experimental Layout

An avocado orchard at an altitude of 847m in the Tzaneen area, South Africa (latitude 23° 43’ 60S; longitude 30°10’0E), was selected. Trees consisted of thirteen year old ‘Hass’ on

'Duke7' rootstocks planted at a density of 204 trees.ha^{-1} (7 x 7m spacing). Trees were on a southern facing slope. The presence of *Phytophthora cinnamomi* in the soil was confirmed by means of the citrus leaf baiting technique (Matheron and Tatejka, 1991). Virulent *P. cinnamomi* fungal isolates were obtained from avocado roots plated out on PARPH medium (Jeffers and Martin, 1986) and tested for pathogenicity before the trial was started in November 2004.

Temperature was measured every 30min from January 2005 to July 2006 using a HOBO® H8 data logger (Onset Computer Corporations, Bourne, MA, USA). The data logger was place inside a tree canopy that formed part of the experimental data group, 1.5m above soil level. Rainfall data was obtained from a rain gauge situated in the orchard. Mean bimonthly temperatures and rainfall are presented in Figure 4.1.

The soil drench trial (Experiment 1) consisted of 50 plants with 10 plants per treatment in a completely randomized block design (Appendix B). The trial where potassium silicate was applied as a trunk injection (Experiment 2) consisted of 20 plants with 5 plants per treatment organised in a completely randomised block design (Appendix B).

4.3.3 Standard Management Practices in the Orchard

Soil moisture content was determined by means of tensiometers at 0.3 and 0.6m below the soil surface and water was applied with drip irrigation when tensiometers readings dropped below -40kPa. Chemical fungicides as well as fertilisers were applied at critical periods (Appendix C) during the season according to nutritional requirements, as indicated by soil and leaf analyses. Weeds were managed by regular mechanical slashing between rows.

4.3.4 Treatments

4.3.4.1 Experiment 1

Silicon treatments consisted of trees drenched with a 20l solution of 20ml.l^{-1} soluble potassium silicate (20.7% silicon dioxide) (Bekker *et al.,* 2006) per tree either once, twice or three times in a growing season. Trees injected with potassium phosphonate (Avoguard®) were incorporated as a standard fungicide treatment. Untreated trees served as a control.

4.3.4.2 Experiment 2

Silicon treatments consisted of trees injected with either 20ml of 0.74ml.l^{-1} (200ppm; pH 10.35) or 20ml.l^{-1} (5405ppm; pH 11.46) potassium silicate (20.7% silicon dioxide), or with 20ml of a KOH solution (pH 10.35). These treatments were timed to correspond with the potassium phosphonate (Avoguard®) injections (Appendix C).

4.3.5 Root and Leaf Sample and Photographic Data Collection

For Experiment 1 data was collected from January 2005 to July 2006, and Experiment 2 from March 2005 to July 2006. Digital photographs (described hereafter) and root and leaf samples were taken every second month on the northern side of the tree, and fruit samples were taken at harvest. Trees were harvested in July 2005 and 2006, and fruit count size and total tree yield were determined for each tree.

4.3.6 Assessment of Tree Canopy Condition

The canopy condition was rated according to a Ciba Geigy (Darvas *et al.,* 1984; Bezuidenhout *et al.,* 1987) avocado tree rating scale from 0 to 10 where 0 = healthy looking tree and 10 = dead tree (Appendix D). Ratings were done every second month independently by two parties, as well as from digital photographs taken in the field.

4.3.7 Root Density Assessment

Ten sheets of newspaper were placed on top of one another, within the drip line of each tree, on the soil surface to cover a 0.5m^2 area, and covered with leaf mulch. Newspaper acted as a barrier to ensure avocado feeder roots do not grow into the mulch, but grow in a two-dimensional fashion on top of the soil surface. After two months, the mulch was carefully raked away, and the newspaper was removed. A digital photograph of the exposed feeder roots was taken at a set height of 75cm above the soil surface with a Konika Minolta Dimage Z5 camera (5 megapixel, 35-420mm lens). The newspaper was replaced every second month with new sheets and covered with mulch.

Photographs were analysed using the computer software ImageJ 1.33u (Wayne Rasband, National Institutes of Health, USA). 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), whereafter 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 were deleted from the picture (Figure 4.2). The picture was then computer analysed, an area fraction determined and recorded as a percentage root density.

4.3.8 Yield Data

In both experiments avocado fruit were harvested, packed into lug boxes, labelled and transported to the packhouse. Fruit size distribution was determined gravimetrically for individual trees using the international fruit count system. The count number equals the amount of fruit of a certain size that will fit into a 4kg carton (count 10 = 366 to 450g; count 12 = 306 to 465g; count 14 = 266 to 305g; count 16 = 236 to 265g; count 18 = 211 to 235g; count 20 = 191 to 210g; count 22 = 171 to 190g; and count 24 and smaller = < 170g. Yield data for Experiment 2 was not collected during July 2005.

4.3.9 Post-Harvest Disease Rating

The influence of silicon application during the growth season on the incidence of post-harvest diseases on fruit was monitored for two years. As part of the standard spray program in the orchard, fruit received two applications of copper oxychloride (Demildex®) during the 2004/2005 season and one application during the following season (Appendix C). Subsamples of two 4kg cartons of counts 16 (236 - 265g), 18 (211 - 235g) or 20 (191 - 210g) 'Hass' fruit from each tree were taken from the packhouse. Fruit was stored at 5.5°C for 28 days to simulate export conditions. Thereafter fruit was removed from cold storage and stored at 20°C in a temperature controlled room and allowed to ripen.

When fruit reached a firmness of 55 – 65pa, measured with a densimeter, it was cut open and rated according to the method described by Bezuidenhout and Kuschke (1982). Fruit were evaluated externally and internally for post-harvest diseases (anthracnose, stem end rot) and physiological disorders (pulp spot, grey pulp, bruising, vascular browning, cold damage, and lenticel damage). A rating scale of 0 – 3 was used where 0 = healthy fruit and 3 = 100% diseased.

Representative lesions of the different type of post-harvest diseases were selected for pathogen isolations. Fruit was surface sterilized by dipping it into 96% ethanol and left to dry on a work bench. This was repeated twice. Isolations were made by cutting small pieces of fruit pulp from the discoloured tissue on the fringes of lesions. Five pieces were taken from each lesion and plated onto PDA supplemented with 0.01% chloramphenicol. Plates were

incubated at room temperature until sporulation was visible. Representative colonies which developed from the avocado tissue were pure-cultured for identification. Cultures were identified microscopically.

4.3.10 Nutrient Analysis

Leaf and soil samples were taken during July for both 2005 and 2006. Analyses of avocado tissue and soil from the avocado orchard were done by Central Agricultural Laboratories (CAL), Pelindaba, South Africa. Four replicates of the plant material were analysed per treatment. Soil samples were pooled and analysed as a singular sample, and therefore no statistical analysis were done on soil samples.

4.4 RESULTS AND DISCUSSION

4.4.1 Root Health and Canopy Condition

Application of potassium silicate (20.7% silicon dioxide) as a soil drench to control Phytophthora root rot, affected root density positively (Figure 4.3). Higher root densities were recorded throughout the trial period in trees treated with potassium silicate application compared to that of potassium phosphonate (Avoguard®) injections. Significant differences were obtained during March 2005 between Si x 3 (5.54%) and Si x 2 (4.45%) compared to the potassium phosphonate (2.16%) and untreated control treatments (2.35) (Figure 4.4). These differences were negated during drier periods resulting in no significant differences between treatments (May 2005). However, from November 2005 to July 2006, Si x 3 resulted in significantly higher root densities compared to both the untreated control and potassium phosphonate treatments. One (Si x 1) silicon application per season resulted in significantly higher root densities compared to the control treatment except for March 2005 (2.3 vs. 2.35), May 2005 (2.52 vs. 1.39) and March 2006 (7.32 vs. 6.37). Two (Si x 2) silicon applications per season resulted in significantly higher root densities compared to the control during March 2005 (4.45) and for the period of January to July 2006. Differences in root density between treatments correlated with the availability of soil moisture, i.e. rainfall received throughout the season, although seasonal growth flushes and timing of silicon application also played a role. Soil water dissolves the applied potassium silicate. Adequate rainfall therefore ensures optimal quantities of silicon to be available for plant uptake. 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 *et al*., 1992). To provide maximum protection, and therefore minimize disease development, Bowen *et al.* (1992) suggested silicon to be applied continuously. Results from the current study concur with this, as three applications of silicon resulted in the best disease suppression and stimulation of new root growth. These results (root density) (Figures 4.3 & 4.4), were confirmed by tree canopy ratings (Figure 4.6) as trees that received silicon frequently, showed better canopy conditions compared to the control treatments.

The effect of potassium silicate as a stem injection to control *Phytophthora cinnamomi* severity was not significant in terms of differences in tree feeder root densities (Figure 4.5). Root densities of both potassium silicate injected trees and trees receiving potassium silicate as a soil drench increased under conditions of optimal rainfall. No significant trend could, however, be observed while the trial was conducted. Potassium phosphonate injected trees (12.4%) had significantly higher root densities compared to that of potassium silicate (8.16%) only during July 2006. Potassium hydroxide injections did not induce higher root densities during the summer months, but resulted in higher root densities compared to potassium silicate injected trees during May (KOH = 9.95% vs. 20ml.l^{-1} Si = 7.95%) and July 2006 (KOH = 10.86% vs. 0.74ml.l^{-1} Si = 11.3%). According to Kaiser (1993), a root flush occurs in avocado trees from autumn to early spring. The applied potassium in the form of potassium hydroxide may be translocated to the roots where it is incorporated into newly formed root tissue, explaining the higher root densities. The potassium applied as potassium silicate will not be freely transported to the root system as silicon in not easily translocated, and will therefore not have a similar effect.

Phenological cycling, rather than rainfall, was the determining factor in canopy condition. However, 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. During dry conditions, canopy condition deteriorated dramatically. This was nullified when rainfall resumed during Dec 2005 (Figure 4.6). All potassium silicate soil drench treatments resulted in lower canopy ratings over the 18 month period of data collection compared to the control. Significant differences were obtained at all data collection dates, except March and July 2006, when potassium silicate soil drench treatments had similar canopy ratings than those observed in the control (3.15 and 3.15) and potassium phosphonate treatments (2.90 and 2.95). This indicates that potassium silicate soil drench treatments reduced drought stress, concomitantly with reducing disease stress.

When potassium silicate was applied as a stem injection to avocado trees infected with *P. cinnamomi* and compared with KOH and potassium phosphonate (Avoguard®) injections,

potassium hydroxide resulted in the lowest disease rating over the period of data collection (Figure 4.7) except for March 2005. Results of potassium silicate injections did not show any clear trends. Anderson *et al*. (2004) injected avocado trees with a disease rating of 5.5 with a 200ppm (0.74ml potassium silicate) solution. They reported stimulation of epicormic buds, with "an eventual significant increase in canopy density", and a 31% mean tree health improvement. In the current study, no epicormic bud bursts were observed, and no simultaneous increase in canopy density was detected. No mention is made as to when epicormic bud burst was observed in relation to phenological cycling. and thus it could possibly be that the cycling observed by Anderson *et al*. (2004) was as a result of normal tree phenology.

If excess water is lost during transpiration, stomata close 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. This can be a great advantage in plants with thin cuticles (Ma and Takahashi, 2002). Gong *et al*. (2005) reported that silicon improved the water status of drought stressed wheat plants with regard to leaf water potential and water content, compared to untreated plants. This also seems to be the case in silicon treated avocado plants. Whiley *et al*. (1986) reported fosetyl-Al foliar sprays or metalaxyl soil applications resulted in higher xylem water potentials and treated plants showed fasted and more complete recovery from water stress due to Phytophthora root rot compared to uninfected trees. A similar situation may be occurring in silicon-treated avocado trees. However, in our study, the overriding influence of silicon seems to be its effect on disease suppression, and therefore canopy condition as an indicator of disease severity. Chérif *et al.* (1994) reported that although silicon had no effect on phenolic concentrations of plants in the absence of pathogen infection, significant differences can, however, be seen in inoculated plants compared to uninoculated control cucumber plants. Concentrations of phenolic compounds in inoculated plants were reported to be double that of uninoculated plants six days after inoculation. The differences seen in avocado canopy condition in our study can therefore possibly be attributed to disease suppression by silicon, and not other external factors influencing tree health.

4.4.2 Post-harvest Disease Rating

No significant differences were seen over a two-year period with regards to black cold damage between treatments. Although this was true for brown cold during 2006, significant differences were observed during 2005 (Table 4.1). Cold damage is a physiological disorder resulting from fruit being subjected to too low temperatures during storage. Woolf *et al*. (2003) reported that external cold damage occurs at storage temperatures below 3 °C. These temperatures cause dark, irregular, but clearly outlined patches on the fruit skin to appear after a few days. Severity is directly proportional to the degree of low temperatures experienced, and the length of time the fruit was subjected to these low temperatures (Swarts, 1984). In the current study, differences between treatments were most likely due to bad circulation in the cold storage room, and not to treatment factors implemented in the orchard. Stomata prominent in young avocado fruit regenerate due to lenticel formation, producing white or grey specks on the fruit rind surface. These become corky and rough, with the epidermis rupturing, causing lenticel damage (Scora *et al.,* 2002). During 2005, treatments receiving the least silicon resulted in the lowest lenticel damage rating with Si x 1 (0.507), Si x 2 (0.714) and control (0.721) differing significantly from the Si x 3 (1.021) soil drench, and the 0.74ml.l^{-1} (0.984) and 20ml.l^{-1} (1.021) potassium silicate injection treatments. During 2006 however, the 20ml (0.138) injection treatment resulted in the lowest rating of lenticell damage compared to all other treatments. Although significant differences were observed, no clear trends could be seen over the two-year period between treatments.

Anthracnose symptoms may develop either before or after harvest, although symptoms appearing after harvest only commence when fruit are ripened. Lesions initially appear as small, light brown circular lesions. As lesions enlarge they, however, become slightly sunken in the centre and change colour to dark brown or black. Symptoms are difficult to see on ripe 'Hass' fruit due to its' dark skin colour (Pegg *et al*., 2002) as a result of increased anthocyanin and decreased chlorophyll a and b levels in the fruit skin (Cox *et al*., 2004). The following fungi were isolated from lesions of 'Hass' avocado fruit in the current study: *Mucor pucillus*, *Botrytis cinerea*, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., and *Colletotrichum gloeosporioides* Penzig [telomorph *Glomerella cingulata* (Stonem.) Spauld & Schreck]. During the 2004/2005 season, significant differences in anthracnose ratings were observed between all treatments compared to the control treatment. Fruit from trees injected with 0.74ml.l^{-1} potassium silicate showed the lowest rating of anthracnose with an average rating of 0.143 per box of fruit. This was followed by fruit from potassium phosphonate (Avoguard®) treated trees (0.293), fruit from trees receiving three silicon applications (Si x 3;

0.279) and fruit from trees injected with 20ml.l^{-1} potassium silicate (0.3). No differences were recorded during the 2005/2006 season with regards to anthracnose rating. Anderson *et al.* (2004) injected four year old 'Hass' trees on clonal Velvic rootstocks using 1000ppm potassium silicate (equal to 37ml of a 20.7% silicon dioxide solution). Fruit from injected trees were harvested on three consecutive days, one month apart. Fruit harvested two weeks after injection did not differ significantly from fruit harvested from uninjected trees. However, fruit harvested six and 10 weeks after injection had significantly lower anthracnose ratings compared to uninjected trees. Their findings confirm results of the current study, indicating silicon injection may be a possible preventative measure to control anthracnose incidence and severity in avocado fruit. Anderson *et al.* (2005), however, stated that if silicon was mixed with phosphorous acid (80:20 v/v; pH 6.3), no control of anthracnose occurred. They propose that because silicon solubility was lower at a lower pH, silicon was unavailable to plants at such a low pH.

Stem end rot starts from the pedicel end of fruit and advances internally, causing rot of fruit flesh (Darvas, 1982). Externally, infection lesions turn brown to black coinciding with infectious advancement internally. Internal symptoms include flesh rot, leading to mycelial filled cavities, and are often associated with vascular discolouration (Darvas, 1982). The following fungi were isolated from lesions on the stem end of 'Hass' avocado fruit in the current study: *Phomopsis perseae* Zerova, *Rhizopus stolonifer* (Ehrenb. Ex Fr.) Vuill., *Botrytis cinerea*, *Lasiodiplodia theobromae*, *Alternaria alternata* (Fr:Fr.) Kiesl. and *Colletotrichum gloeosporioides* (telomorph *Glomerella cingulata*). During the 2004/2005 season, Si x 2 application resulted in the lowest average stem end rot rating of 0.4 per box of fruit (Table 4.1). Potassium phosphonate (Avoguard®) (0.7) and Si x 1 (0.757) applications had the highest rating of stem end rot. Surprisingly, Si x 3 (0.679) did not differ significantly from either the potassium phosphonate or Si x 1 treatments. During the 2005/2006 season, the Si x 1 (0.095) and the control (0.06) treatments had significantly higher ratings of stem end rot compared to all other treatments. Anderson *et al.* (2005) reported that injecting trees with silicon had no significant effect on stem end rot incidence and severity thereof.

Fruit harvested in 2005 from trees injected with potassium silicate (viz. ratings of 0.74ml.l^{-1} = 0.135 and 20ml.l^{-1} = 0.1) had significantly lower levels of bruising compared to other treatments (Table 4.1). No trend could be seen between soil drench applications of silicon and the control and potassium phosphonate treatments. No difference was seen between treatments during the 2005/2006 season, and no conclusive deductions can be made at this stage.

Darvas (1982) stated that stem end rot can frequently be associated with the browning of vascular tracts in infected fruit. In the current study, ratings of vascular browning in fruit harvested during 2005 showed a correlation with stem end rot ratings in the same fruit. Si x 2 (0.086) and Si x 3 (0.143) had lower ratings of vascular browning compared to potassium phosphonate (0.357), Si x 1 (0.4) and control (0.379) treatments (Table 4.1). During 2006, fruit from trees injected with 0.74ml.l^{-1} (0.1035) had significantly higher rating of vascular browning compared to silicon at 20ml.l^{-1} (0.0615) and potassium phosphonate (0.0575) treatments.

There was very low incidence of pulp spot over the two seasons and as a result there were no significant differences between treatments (Table 4.1).

There was no incidence of grey pulp in 2005, and even though the incidence was very low in 2006 there were some significant differences between treatments (Table 4.1). Fruit from trees receiving potassium silicate soil drenches showed a higher rating of grey pulp, with Si x 3 (0.0947) and Si x 2 (0.065) differing significantly from potassium phosphonate (0.01) and control (0.04) treatments.

4.4.3 Yield and Fruit Size

Total yield per tree of only Si x 2 (39kg.tree^{-1}) differed significantly from the control treatment (64kg.tree^{-1}) during 2005 (Table 4.2). During 2006, Si x 3 (158kg.tree^{-1}) was significantly different compared to all treatments with regards to the fruit yield per tree, followed by Si x 1 (111kg.tree^{-1}) and Si x 2 (104kg.tree^{-1}) differing significantly from potassium phosphonate (Avoguard®) (74kg.tree^{-1}) and the control treatment (16kg.tree^{-1}). There is, notwithstanding differences between treatments, a significant difference between total yields of 2005 and 2006. This is indicative of the occurrence of bi-annual (alternate) bearing prevalent in avocado orchards. Whiley (1994) reported that flower or fruit pruning to be an effective method to control alternate bearing. He stated that during a heavy crop set, this pruning may be effective to increase fruit size, but during a light bearing year, little differences could be seen in tree yield or fruit size. However, in the present trial no pruning occurred, resulting in a heavy crop set during 2006. The reason why Si x 1 (135kg.tree^{-1}) and Si x 2 (146.9kg.tree^{-1}) had lower yields compared to the control treatments (166kg.tree^{-1}) and potassium phosphonate (Avoguard®) (176kg.tree^{-1}) during 2006 are unclear. It is possible that the third silicon application was applied at a critical time in fruit development or tree

phenological cycle, and that this could have induced bigger-sized fruit, or reduced fruit drop during the second phenological fruit drop.

During 2005 the number of fruits from Si x 1 (222.6 fruits.tree^{-1}) and Si x 2 (189.7 fruits.tree^{-1}) treated trees were significantly lower compared to that of potassium phosphonate (Avoguard®) (294 fruits.tree^{-1}) treated trees and the control (348.1 fruits.tree^{-1}). During 2006, Si x 3 (780.5fruits.tree^{-1}) treated trees resulted in a significantly higher fruit number per tree compared to all other treatments, except for potassium phosphonate (Avoguard®) treated trees. Again, Si x 1 (648.3 fruits.tree^{-1}) and Si x 2 (700 fruits.tree^{-1}) had fewer fruit compared to the potassium phosphonate (Avoguard®) (840.8fruits.tree^{-1}) and control (780.5fruits.tree^{-1}) treatments.

Results from both total yield per tree and the number of fruit per tree indicate that Si x 3 is effective in, if not increasing yield and fruit number, sustaining tree health to a productive level. It should, however, be determined whether the amount of silicon applied, or the timing at which the third application was employed with regard to the tree phenological model, is the determining factor in increasing yield and number of fruit per tree.

No significant differences were seen between treatments over the two harvesting seasons with regards to fruit size in the 10 to 24 count size distribution. However, during 2005, the control treatment (28.52kg.tree^{-1}) showed higher yields in the fruit count increment smaller than 24. Hofman *et al.* (2002) reported fruit from 'Hass' trees with high fruit yields to be generally smaller, and to have a lower rating of anthracnose. This was reiterated in the current study within the fruits smaller that count 24. However, no differences were seen in lower fruit counts, and higher yields were only due to an increase in counts smaller than 24. Fruit size, especially in 'Hass' fruit, remains a problem. Marketing has moved towards 'ripe and ready' fruit, resulting in a niche market for smaller fruit. Producers, however, still aim to obtain maximum yields per unit area, and therefore larger fruit sizes to maximize their profit (Geldenhuis, Pers. com, Tzaneen). Although silicon increases the number of small fruit in 'Hass', especially with three applications timed correctly, this creates scope for other market explorations, or greater freedom during flower or fruit pruning.

In the silicon injection trial no differences were seen in terms of yield, the number of fruit or fruit count size (Table 4.3). This could be due to too low silicon concentrations in the injection solutions. Although Anderson *et al.* (2004) applied a 200ppm solution; they increased their solution concentration to 1000 – 2000ppm (Anderson *et al.,* 2005) during the consecutive experiment. Although their aim was to study the effect of silicon on anthracnose

incidence and severity, higher concentrations have a higher pH, rendering silicon more soluble, mobile, and therefore more efficient in plant tissue.

4.4.4 Nutrient Analysis

Nitrogen levels in all avocado leaf tissue were classified as deficient during 2005 according to standards set by Embleton and Jones (1964), Lahav and Kadman (1980) and Whiley *et al.* (1996a) (Table 4.4), except that of 0.74 ml Si (1.95%) and Si x 3 (1.58%) which were on the border of 1.6%, which is defined as being deficient. There were nonetheless no significant differences between treatments. This deficiency was nullified during the 2006 season by effective fertilizer applications (Appendix B), when all treatments, except Si x 3, were above the minimum level of deficiency. Phosphorous levels in leaf tissue of all treatments were below the deficiency level, indicating possible phosphorous stress. This is of interest as potassium phosphonate (Avoguard®) injections into tree stems leads to rapid translocation of this phosphorous product to photosynthetically active plant material, i.e. leaves. Schutte *et al.* (1988), however, reported that phosphite concentrations in avocado leaves peak three days after injections, and thereafter decrease steadily. The degree to which this decrease occurs is, however, not known. Si x 3 (0.13%) and 0.74ml.l^{-1} (0.12%) Si led to significantly higher phosphorous levels in leaf tissue during 2005 compared to all other treatments (0.1%). This effect of silicon was, however, not carried over to 2006, when no significant differences were observed between treatments.

Numerous authors (Boshoff *et al.*, 1996; Schoeman and Manicom, 2002) have reported on the beneficial effects of copper sprays on post-harvest disease incidence, *Colletotrichum gloeosporioides* in particular. Copper (Demildex) was therefore included into the spray program to inhibit post-harvest disease development. However, this leads to a build-up of copper in, not only soils, but avocado tissue, possibly leading to toxic levels in plants.

Significant differences between treatments were seen during 2005 with regards to boron concentrations in avocado leaf tissue. Si x 3 (39.25mg.kg^{-1} boron) was significantly different from all other treatments. Potassium phosphonate (Avoguard®), (34.75 mg.kg^{-1}), 0.74ml.l^{-1} Si (36 mg.kg^{-1}) and 20ml.l^{-1} Si (36 mg.kg^{-1}) were statistically similar, but still differed significantly from the control (29.75 mg.kg^{-1}). Although all treatments were within the recommended concentration, it does appear that silicon application increases the boron uptake. Although no significant differences were obtained with regards to boron concentration in avocado leaves during 2006, the same trend was observed. Whiley *et al.*

(1996b) reported that boron application may increase fruit set and quality. If silicon application increase boron uptake, this may result in additional benefits of silicon to the avocado plant.

Contrary to the expected outcome, silicon concentrations were not the highest in silicon treated avocado tissue. During 2005, Si x 3 (0.10%) had the lowest silicon concentration, and was statically different to both the potassium phosphonate (Avoguard®) (0.18%) and control (0.23%) treatments. During 2006 however, no significant differences were observed between the Si x 3 (0.30%), potassium phosphonate (Avoguard®) (0.15%) or the control (0.24%) treatments. These levels were however statistically different from the silicon injected treatments.

During 2005 potassium phosphonate (Avoguard®) (1.4%; 0.27%) and Si x 3 (1.4%; 0.3%) had significantly higher nitrogen and phosphorous concentrations in root tissue compared to the control treatment (1.1%; 0.13%)(Table 4.5). Schutte *et al*. (1988) reported the phosphite concentration in avocado roots to peak 21 days after potassium phosphonate (Avoguard®) injections, where after it decreases steadily. This may therefore explain the higher levels in root tissue treated with potassium phosphonate (Avoguard®). Silicon application may aid in phosphorous uptake by plant roots. There were, however, no significant differences between treatments during 2006 with regard to nitrogen or phosphorous concentrations in avocado root tissue.

No differences were obtained for copper concentrations between treatments over the two year period. Potassium phosphonate (Avoguard®) (188mg.kg^{-1}) and Si x 3 (155 mg.kg^{-1}) had significantly higher sodium concentrations in avocado root tissue compared to the control treatment (86mg.kg^{-1}). This effect was however not carried over to 2006. Roots from Si x 3 (2005 = 9110 mg.kg^{-1}; 2006 = 9090 mg.kg^{-1}) had significantly higher iron concentrations compared to all other treatments.

Roots from potassium phosphonate (Avoguard®) treated trees had significantly higher boron levels (108mg.kg^{-1}) compared to both that of the control (90mg.kg^{-1}) and Si x 3 treatments. This effect was again nullified during 2006. There was, however, no significant difference between treatments with regard to root zinc concentrations during 2006. During 2005, potassium phosphonate (Avoguard®) (3.35%) and Si x 3 (3.6%) had significantly higher silicon levels in the root tissue compared to the control (2.45%). This was the case for 2006 as well, where Si x 3 (4.75%) differed significantly from the potassium phosphonate (Avoguard®) (3.18%) and control (3.75%). This indicates that silicon is absorbed by avocado roots, but not effectively translocated in the plant to leaf tissue.

Due to the fact that no statistical analysis was done on soil samples (Table 4.6), only trends will be discussed. The pH of the potassium silicate used is 12.7 (Bekker *et al.,* 2006). This seems to have an effect on soil pH, as Si x 3 treated soil increased the pH from pH 4.73 during November 2004 to pH 5.28 during 2006. As expected, the silicon concentration of the soil receiving three treatments per year increased from 8.19% during 2004 to 18.2% during 2006.

Silicon appears to have an alleviating effect on not only biotic, but also abiotic stress (Bowen *et al*., 1995). This suggests the possibility that the effect of Si on plant growth and performance are only evident when plants are under some form of stress. The effect of silicon on plant growth and disease development in plants is related to the interaction of silicon with other essential and non-essential plant growth elements. Application of silicate fertilizers increased levels of P, Si, Ca, and Cu, and reduce N, K, Mg, Fe, Mn and Zn levels in sugarcane leaves (Elawad *et al*., 1982). Silicate materials also increased pH, Si, P, Ca and Mg in the soil (Sistani *et al.,* 1998).

Wutscher (1989) reported a strong correlation between silicon levels and that of S, P, Fe, Mg, Mn, Cu, Zn and Mo, especially in tree bark, leaves and feeder roots of Valencia oranges (*Citrus sinensis* L.). Korndörfer *et al*. (1999) reported the alleviation of Fe toxicity symptoms by silicon application. It is known that Si reduces Fe and Mn toxicity, and it is thought that Si increases the 'oxidising power' of roots making Mn and Fe less soluble (Ma, 1990). Silicon may alleviate this toxicity not only because it reduces absorption, but also increases the internal tolerance level of the plant to an excess of these elements in the tissue.

Toxicity of these elements depends on the availability of it to the plant for uptake, and this availability is determined primarily by soil pH. Increase in soil pH, as found in the current study, deems these metals insoluble, and therefore limits the uptake thereof (Ma, 1990).

4.5 CONCLUSION

The application of potassium silicate to *P. cinnamomi* infected trees resulted in higher feeder root densities than the control method currently implemented to inhibit the effect of *Phytophthora* infection on avocado trees. Differences in root density between treatments were however affected by the availability of soil moisture, although seasonal growth flushes and timing of silicon application also played a role. This was reiterated in tree canopy ratings, as trees that received silicon frequently had better canopy conditions compared to the control treatments. Results indicate that three silicon applications were the most effective to suppress

the disease and stimulate new root growth. Silicon application should however be timed according to the phenological model with the first application during the period of flowering and fruit set (September); the second to occur before the fruit drop (November); and the third application to be applied before the root flush during February to March (Kaiser, 1993).

Potassium silicate stem injections to inhibit *P. cinnamomi* disease severity were not effective in increasing feeder root densities. Potassium silicate injections did not show any significant trends throughout the trial period, and it is proposed that potassium silicate stem injections are not a viable method to inhibit Phytophthora root rot of avocado trees.

The application of potassium silicate to avocado trees to suppress the infection and spread of Phytophthora root rot seems to be most effective when applied as a soil drench. The possibility of physical barrier formation in roots will be limited as silicon is not actively transported in avocado tissue, and the expression of phenolic and other fungitoxic compounds were confined to plant parts receiving silicon.

Anthracnose severity during the 2004/2005 season was lower in fruit from trees treated with silicon. No significant differences were seen during the 2005/2006 season with regards to anthracnose incidence between treatments. Although some level of inhibition of stem end rot was observed in fruit from trees receiving silicon as a soil drench, results were not consistent, and fruit from silicon injected trees did not differ significantly from the control.

The application of potassium silicate to trees as a soil drench led to higher yields compared to the control treatment. It is possible that increased tree health due to a lower root rot disease severity led to a lower flower/fruit drop, resulting in higher yields compared to the control treatment. Results from both total yield per tree and the number of fruit per tree indicate that Si x 3 is effective in, if not increasing yield and fruit number, sustaining tree health to a productive level.

Three silicon applications resulted in higher boron concentrations in leaves compared to all other treatments and it appears that silicon application increases the boron uptake of avocado plants. Silicon application to avocado trees as a soil drench does not increase silicon translocation to avocado leaves. This indicates that silicon is absorbed by avocado roots, but not effectively translocated in the plant to leaf tissue.

Potassium silicate application to avocado trees as a soil drench leads to an increase in soil pH. This is an especially important additional benefit of silicon application as it is known that most avocado producing areas of South Africa have acidic pHs partly due to the high rainfall and low CEC (cation exchange capacity) of the soil in which avocados are cultivated.

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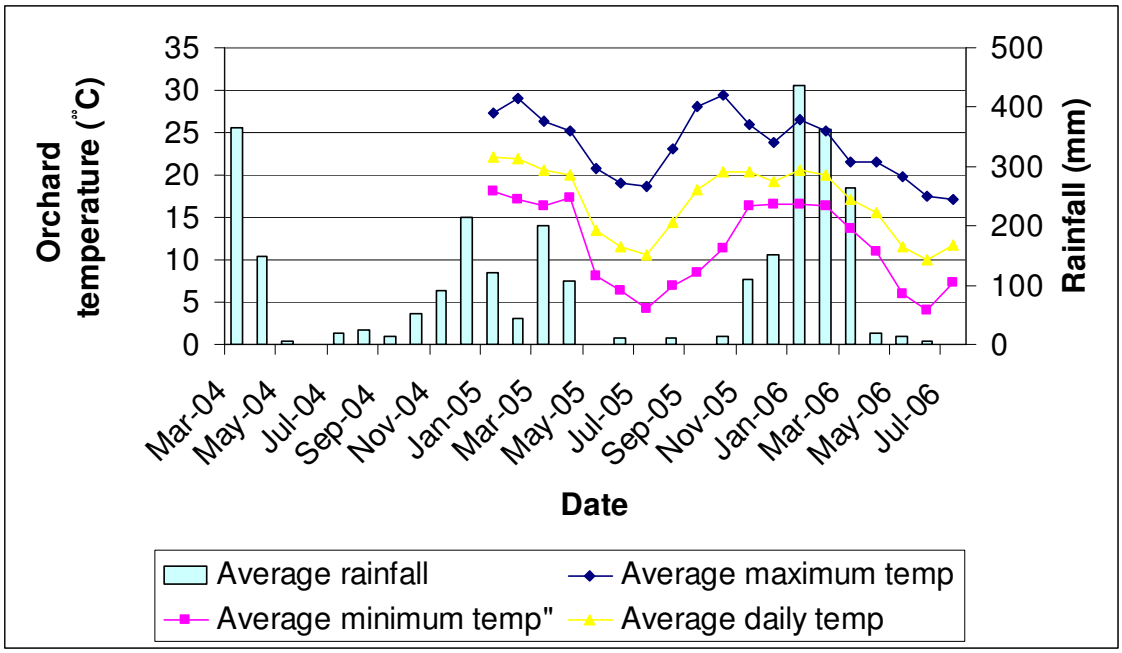


Figure 4.1: Mean bimonthly rainfall data for February 2004 to July 2006, and average maximum, minimum and mean temperatures for January 2005 to July 2006, measured in the orchard in the Tzaneen area, South Africa (latitude 23° 43’ 60S; longitude 30°10’0E).

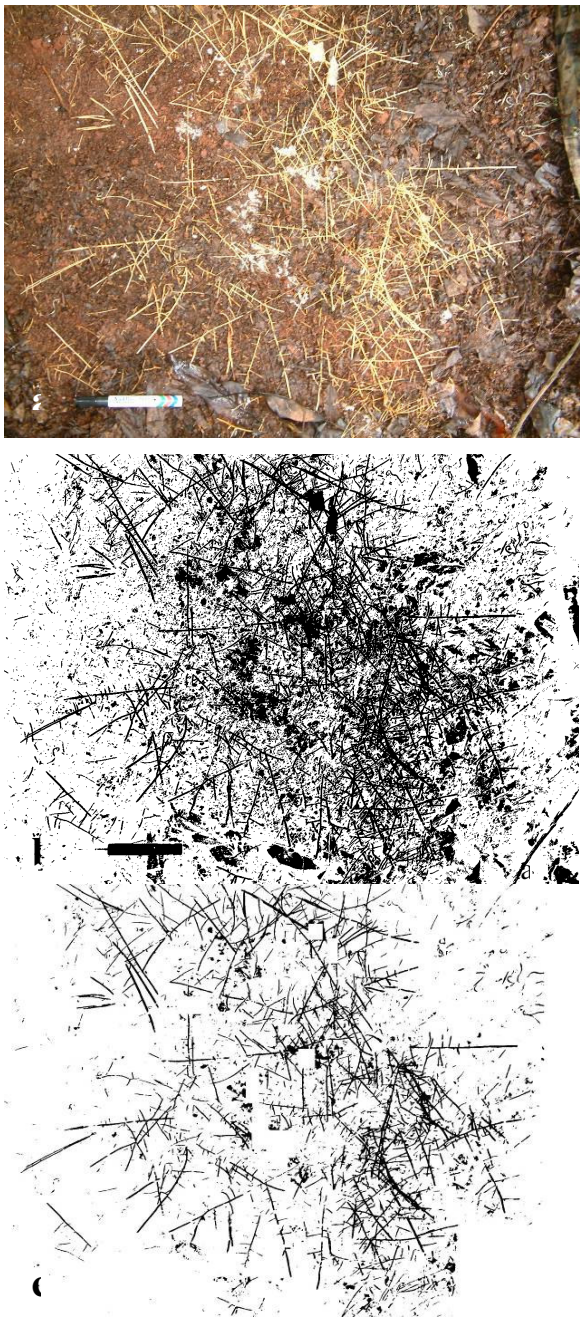


Figure 4.2: Representative photograph preparation for avocado root density determination by means of digital images analysed using ImageJ 1.33u software.

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

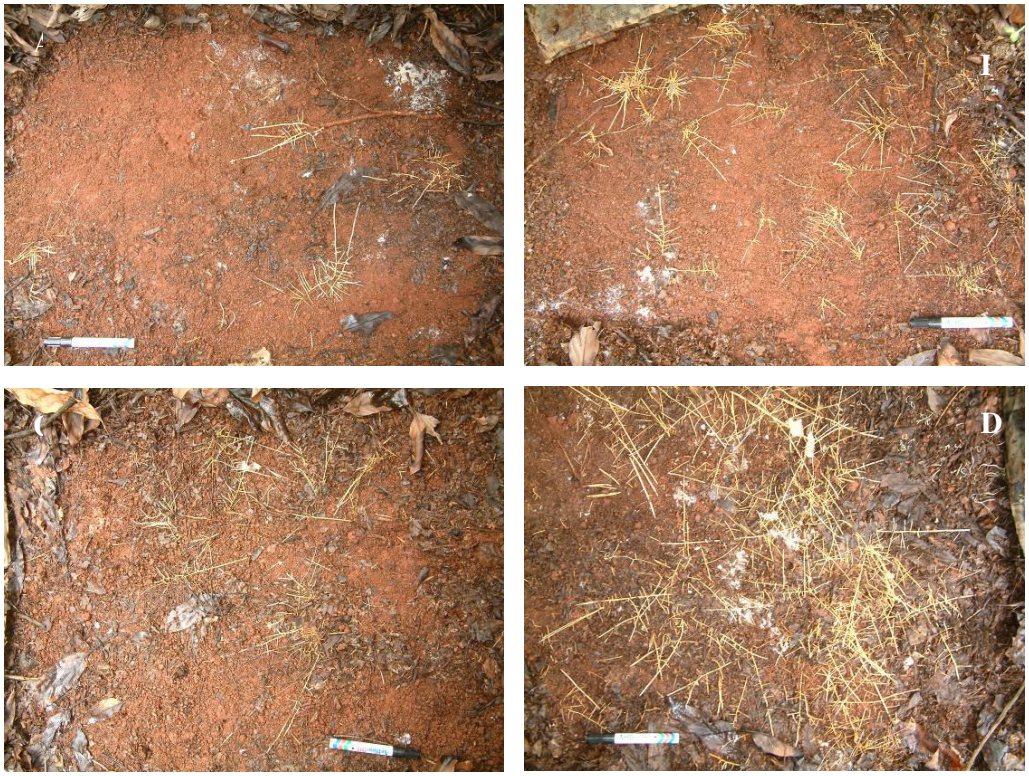
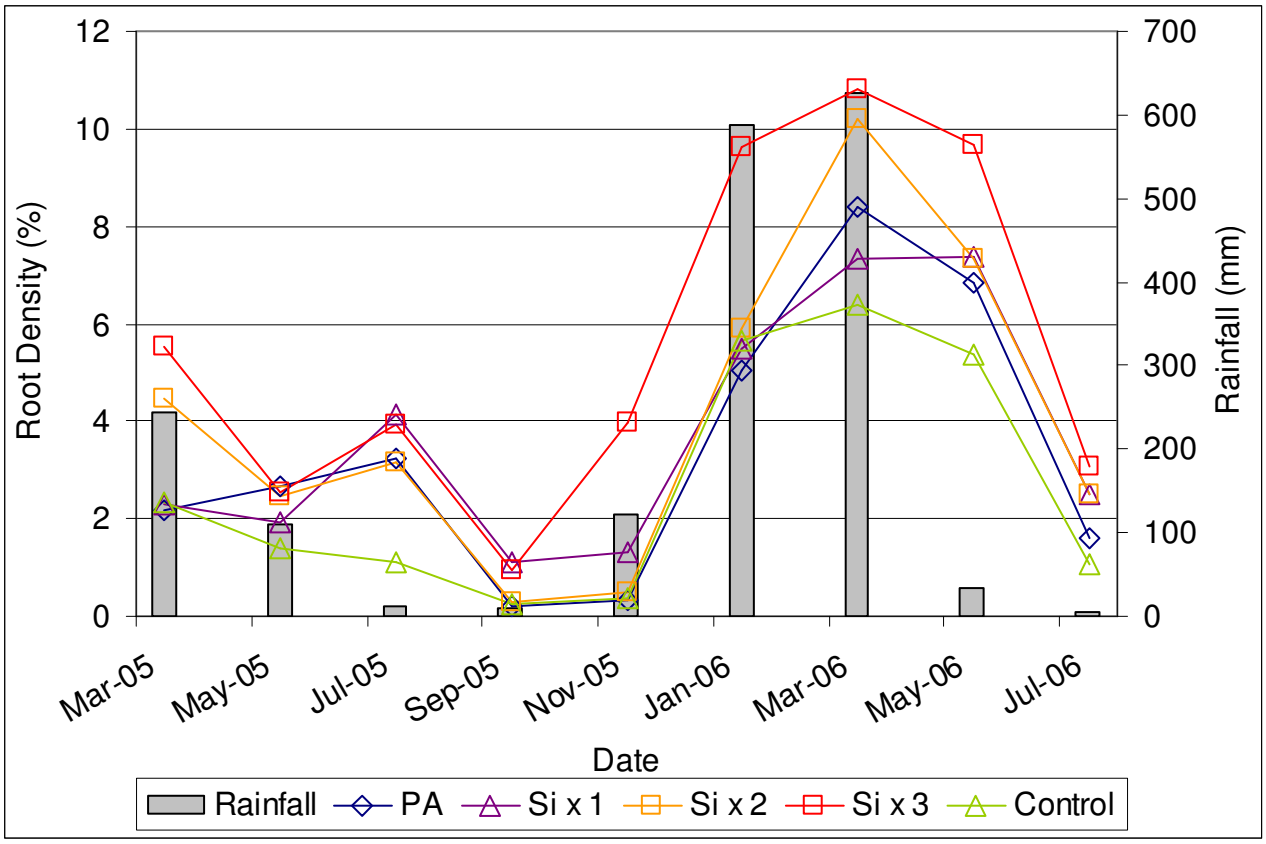
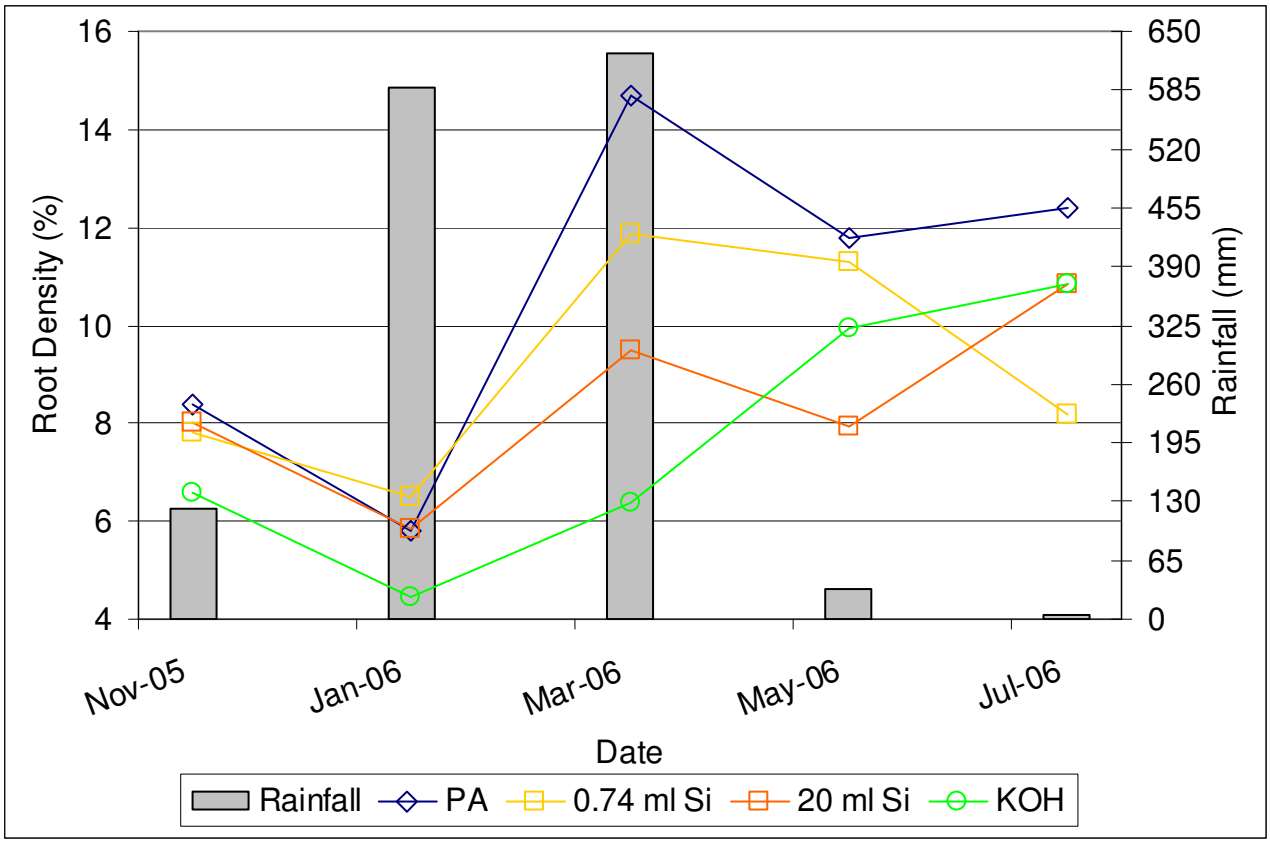


Figure 4.3: Digital images of avocado tree root densities after *P. cinnamomi* infected trees were subjected to the following treatments: A - Control, B - Si x 1 soil drench; C - Potassium phosphonate (Avoguard®) stem injection and D - Si x 3 soil drench.



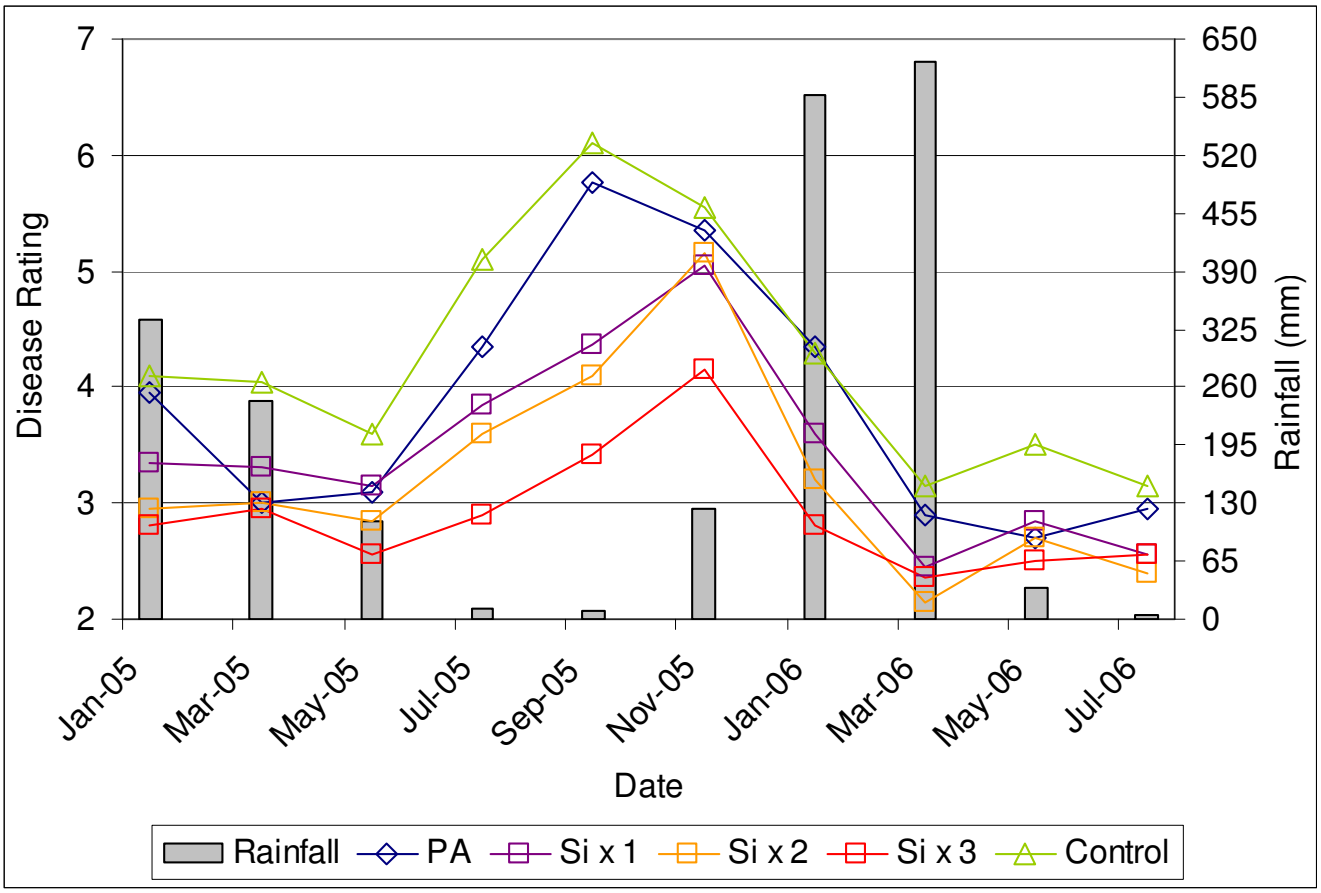
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	2.16a	2.65a	3.22b	0.20a	0.31a	5.04a	8.38b	6.85ab	1.60a
Si x 1	2.30a	1.93a	4.12b	1.09b	1.30b	5.49b	7.32ab	7.39b	2.48b
Si x 2	4.45b	2.46a	3.16ab	0.28a	0.48a	5.90b	10.18c	7.33b	2.49b
Si x 3	5.54b	2.52a	3.93b	0.93ab	3.98c	9.62c	10.82c	9.65c	3.06b
Control	2.35a	1.39a	1.12a	0.26a	0.38a	5.66a	6.37a	5.38a	1.06a
Rainfall (mm)	244	109	11	10	123	588	625	34	5

Figure 4.4: Avocado tree root density recorded over a period of 18 months to determine whether potassium silicate application as a soil drench to diseased avocado trees, could suppress *Phytophthora cinnamomi* disease severity and improve root density. Treatments consisted of either one (Si x 1), two (Si x 2) or tree (Si x 3) potassium silicate soil drench applications per year; trees injected with potassium phosphonate (Avoguard®) (PA) and trees receiving no treatment (control). Values in each column followed by different symbols indicate significant differences at a 95% level of significance.



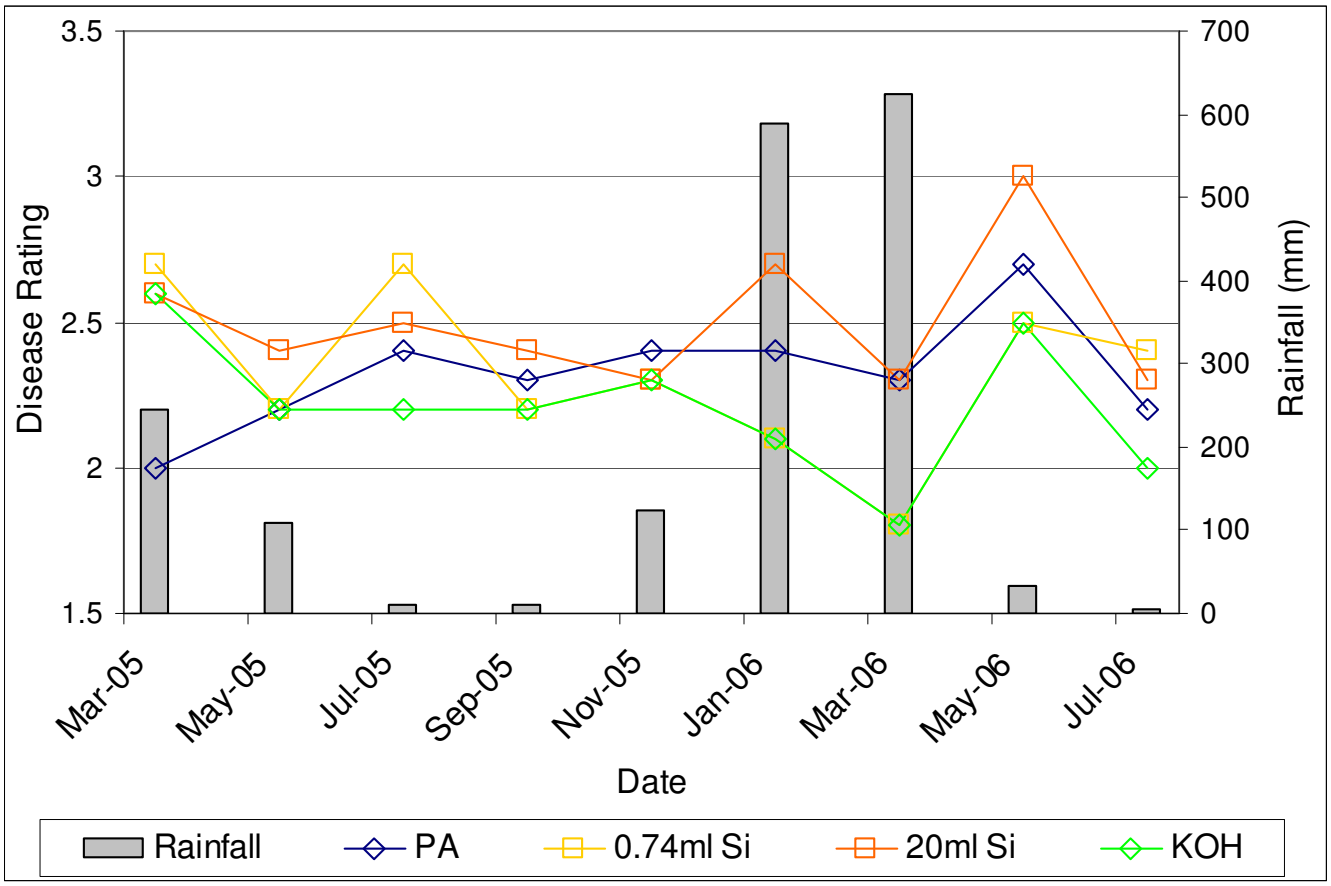
	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	8.38b	5.80b	14.68c	11.80c	12.40c
0.74 ml.l^{-1} Si	7.82ab	6.48c	11.88bc	11.30bc	8.16a
20 ml.l^{-1} Si	8.00b	5.86b	9.50b	7.95a	10.86b
KOH	6.56a	4.46a	6.36a	9.95b	10.86b
Rainfall (mm)	123	588	625	34	5

Figure 4.5: Avocado tree root density over a period of 10 months to determine whether potassium silicate applied as a stem injection to diseased avocado trees, could suppress *Phytophthora cinnamomi* disease severity and improve root density. Treatments consisted of biannual injections of either 0.74ml.l^{-1} or 20ml.l^{-1} potassium silicate solutions (20.7% silicon dioxide); a KOH solution at pH 10.35 or potassium phosphonate (Avoguard®) (PA). Values in each column followed by different symbols indicate significant differences at a 95% level of significance.



	Jan-05	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	3.90b	3.00a	3.10ab	4.35c	4.35b	5.35b	4.35c	2.90b	2.70a	2.95ab
Si x 1	3.35ab	3.30a	3.15ab	3.85bc	3.85b	5.05b	3.60b	2.45ab	2.85ab	2.55ab
Si x 2	2.95a	3.00a	2.85ab	3.60b	3.60ab	5.15b	3.20ab	2.15a	2.70a	2.40a
Si x 3	2.80a	2.95a	2.55a	2.90a	3.00a	4.15a	2.80a	2.35ab	2.50a	2.55ab
Control	4.10b	4.05b	3.50b	5.10d	5.10c	5.55b	4.30c	3.15b	3.50b	3.15b
Rainfall (mm)	355	244	109	11	10	123	588	625	34	5

Figure 4.6: Avocado canopy condition according to the Ciba Geigy disease rating scale, recorded over a period of 18 months to determine whether potassium silicate application as a soil drench to diseased avocado trees, could suppress *Phytophthora cinnamomi* disease severity. Treatments consisted of either one (Si x 1), two (Si x 2) or tree (Si x 3) potassium silicate soil drench applications; trees injected with potassium phosphonate (Avoguard®) (PA) and trees receiving no treatment (control). Values in each column followed by different symbols indicate significant differences at a 95% level of significance.



	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	2.0a	2.2a	2.4b	2.3ab	2.4b	2.4b	2.3b	2.7b	2.2b
0.74ml.l^{-1} Si	2.7b	2.2a	2.7c	2.2a	2.3a	2.1a	1.8a	2.5a	2.4c
20ml.l^{-1} Si	2.6b	2.4b	2.5b	2.4b	2.3a	2.7c	2.3b	3.0c	2.3bc
KOH	2.6b	2.2a	2.2a	2.2a	2.3a	2.1a	1.8a	2.5a	2.0a
Rainfall	244	109	11	10	123	588	625	34	5

Figure 4.7: Avocado canopy condition according to the Ciba Geigy disease rating scale recorded over a period of 16 months to determine whether potassium silicate applied as a stem injection to diseased avocado trees, could suppress *Phytophthora cinnamomi* disease severity. Treatments consisted of biannual injections of either 0.74ml.l^{-1} or 20ml.l^{-1} potassium silicate solutions; a KOH solution at pH 10.35 or Potassium phosphonate (Avoguard®) (PA). Values in each column followed by different symbols indicate significant differences at a 95% level of significance.

Table 4.1: Post-harvest disease rating in avocado fruit harvested from trees that were used in a study to determine the efficacy of soluble potassium silicate application to avocado trees on *Phytophthora cinnamomi* disease severity. Treatments consisted of injections of either 0.74ml.l^{-1} or 20ml.l^{-1} potassium silicate solutions (20.7% silicon dioxide); a KOH solution at pH 10.35, one (Si x 1), two (Si x 2) or tree (Si x 3) potassium silicate soil drench applications; trees receiving no treatment (control), or potassium phosphonate (Avoguard®) injected trees (PA). Fruit were stored at 5.5°C for 28 days, left to ripen and rated using a scale where 0 = no incidence of the disease to 3 = a severely infected fruit. Values followed by different symbols within each column for each experiment indicate significant differences at a 95% level of significance.

Treatment	**Disease or Physiological Disorder**								
	Black Cold Damage	Brown Cold Damage	Lenticel Damage	Anthracnose	Stem End Rot	Bruising	Vascular Browning	Pulp spot	Grey Pulp
2005									
PA	0a	1a	0.814bc	0.293b	0.7d	0.214b	0.357d	0a	0a
Si x 1[a]	0a	1.386b	0.507a	0.35c	0.757d	0.357c	0.4d	0a	0a
Si x 2[a]	0a	1.971d	0.714b	0.414c	0.4a	0.486d	0.086a	0a	0a
Si x 3[a]	0a	1.686c	1.021c	0.279b	0.679cd	0.393c	0.143ab	0a	0a
Control	0a	1.6bc	0.721b	0.464d	0.579bc	0.386c	0.379d	0a	0a
0.74ml.l^{-1} [b]	0a	0.843a	0.984c	0.143a	0.484b	0.135a	0.175b	0a	0a
20ml.l^{-1} [b]	0a	1.871cd	1.021c	0.3b	0.593c	0.1a	0.25c	0a	0a
2006									
PA	0.0075a	0.02a	0.195b	0.0275a	0.03a	0.0025a	0.0575a	0a	0.01a
Si x 1[a]	0a	0.01a	0.1675ab	0.0375a	0.095b	0.0225a	0.0925ab	0.0025a	0.055b
Si x 2[a]	0.0075a	0.03a	0.18b	0.025a	0.05a	0.0125a	0.0925ab	0.0025a	0.065bc
Si x 3[a]	0.0158a	0.0368a	0.2b	0.0368a	0.0316a	0a	0.0711ab	0.0026a	0.0947c
Control	0.005a	0.0025a	0.2175b	0.025a	0.06ab	0.0225a	0.075ab	0a	0.04ab
0.74ml.l^{-1} [b]	0a	0a	0.1965b	0.011a	0.032a	0.022a	0.1035b	0a	0.022ab
20 ml.l^{-1} [b]	0a	0.011a	0.138a	0.036a	0.029a	0.012a	0.0615a	0a	0.031ab

[a] Trees treated with a soil drench of potassium silicate

[b] Trees receiving a trunk injection of potassium silicate

Table 4.2: Yield data from avocado trees treated with soluble potassium silicate soil drenches to inhibit *Phytophthora cinnamomi* disease severity. Treatments consisted of one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate soil drench applications per season, trees receiving no treatment as a control treatment, or potassium phosphonate (Avoguard®) injected trees (PA). Each tree was harvested individually, and fruit sent through a pack line to sort according to size. Values within a column in the table with different symbols indicate significant differences at a 95% level of significance.

Treatment		**Yield (Kg/ tree)**	**Fruits/ tree**	**Fruit Count (Kg/ tree)**								
				< 24	24	22	20	18	16	14	12	10
2005	**PA**	57.2ab	294b	13.41a	10.98a	11.03a	8.97a	12.17a	5.17a	3.57a	0.88a	0a
	Si x 1	42.5ab	222.6a	6.86a	8.58a	5.83a	4.37a	8.11a	4.45a	2.87a	0.47a	0a
	Si x 2	39.6a	189.7a	9.61a	3.72a	6.38a	5.06a	7.43a	4.56a	3.93a	0.84a	0a
	Si x 3	45.2ab	253.8ab	10.55a	9.35a	5.47a	5.57a	7.8a	4.51a	4.27a	1.35a	0.05a
	Control	64.4b	348.1b	28.52b	7.11a	7.45a	7.78a	8.3a	5.64a	4.91a	1.39a	0.05a
2006	**PA**	176b	840.8bc	74.45b	12.49a	13.83a	13.44a	7.05a	1.96a	0.46a	0.04a	0a
	Si x 1	135a	648.3a	111c	10.91a	10.56a	7.22a	3.81a	0.42a	0.09a	0a	0a
	Si x 2	146.9a	700a	104.25c	8.04a	13.64a	12.64a	6.04a	4.93a	0.37a	0a	0a
	Si x 3	202.2c	989.2c	158.25d	16.64a	18.11a	17.37a	8.55a	1.85a	0.37a	0.07a	0a
	Control	166.8b	780.5b	16.8a	13.26a	12.33a	11.68a	5.31a	1.3a	0.4a	0.07a	0a

Table 4.3: Yield data from avocado trees treated with soluble potassium silicate as a stem injection to inhibit *Phytophthora cinnamomi* disease severity. Treatments consisted of biannual injections of either 0.74ml.l^{-1} or 20ml.l^{-1} potassium silicate injection solutions (20.7% silicon dioxide); a KOH solution at pH 10.35, or potassium phosphonate (Avoguard®) injections (PA). Each tree was harvested individually, and fruit sent through a pack line to sort according to size. Values within a column in the table with different symbols indicate significant differences at a 95% level of significance.

Treatment	**Yield (Kg/ tree)**	**Fruits/ tree**	**Fruit Count (Kg/ tree)**								
			< 24	24	22	20	18	16	14	12	10
PA	176.058a	846.4a	126a	14.79a	14.74a	14.32a	4.93a	0.9a	0.37a	0a	0a
KOH	184.841a	877.8a	135a	11.22a	13.45a	13.02a	7.52a	3.76a	0.79a	0.07a	0a
0.74 ml.l^{-1} Si	214.578a	1030.2a	159a	45.23a	21.13a	12.39a	5.78a	0.74a	0.3a	0a	0a
20 ml.l^{-1} Si	197.009a	940.8a	150a	14.31a	13.76a	9.74a	7a	1.64a	0.55a	0a	0a

Table 4.4: Avocado leaf nutrient concentrations sampled during July of two consecutive years from avocado trees treated with soluble potassium silicate to inhibit *Phytophthora cinnamomi* disease severity. Treatments analysed consisted of three (Si x 3) potassium silicate soil drench applications per season, biannual injections of either 0.74ml.l^{-1} or 20ml.l^{-1} potassium silicate (20.7% silicon dioxide) injection solutions, trees receiving no treatment as a control, or potassium phosphonate (Avoguard®) injected trees (PA). Standards for nutrient content of avocado tissue were taken from Embleton and Jones (1964), Lahav and Kadman (1980) and Whiley *et al*. (1996a). Values within a column in the table with different symbols indicate significant differences at a 95% level of significance.

LEAF	**N**	**P**	**K**	**Ca**	**Mg**	**Na**	**S**	**Cu**	**Fe**	**Mn**	**Zn**	**B**	**Mo**	**Si**
	%	**%**	**%**	**%**	**%**	**mg/kg**	**%**	**mg/kg**	**Mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**%**
Control July 2005	1.55a	0.10a	0.41a	1.13a	0.83a	18.75a	0.21a	86.25a	200.75a	824.75a	36.25a	29.75a	0.80a	0.23b
PA July 2005	1.45a	0.10a	0.39a	0.94a	0.73a	13.50a	0.21a	73.75a	142.25a	681.75a	31.00a	34.75b	1.47a	0.18b
Si x 3 July 2005[a]	1.58a	0.13b	0.44a	1.04a	0.84a	10.75a	0.24a	125.50a	121.75a	670.50a	33.50a	39.25c	2.30a	0.10a
0.74 ml.l^{-1} Si July 2005[b]	1.95a	0.12b	0.49a	0.92a	0.78a	12.25a	0.24a	115.50a	124.25a	676.50a	32.25a	36b	1.68a	0.11ab
20 ml.l^{-1} Si July 2005[b]	1.53a	0.10a	0.41a	0.92a	0.78a	10.50a	0.22a	95.00a	113.00a	694.75a	35.00a	36b	1.79a	0.15ab
Control July 2006	1.75a	0.12a	0.50a	0.97a	0.75a	18.50a	0.23a	164.50a	134.00a	716.75a	34.00a	33.25a	1.88a	0.24b
PA July 2006	1.80a	0.11a	0.44a	0.99a	0.76a	7.00a	0.25a	116.25a	172.50a	663.25a	33.25a	37.25a	2.77a	0.15ab
Si x 3 July 2006[a]	1.58a	0.13a	0.44a	1.04a	0.84a	10.75a	0.24a	125.50a	121.75a	670.50a	33.00a	39.25a	2.31a	0.30b
0.74 ml.l^{-1} Si July 2006[b]	1.95a	0.12a	0.49a	0.92a	0.78a	12.25a	0.24a	115.50a	124.25a	676.50a	32.25a	36.00a	1.68a	0.12a
20 ml.l^{-1} Si July 2006[b]	1.73a	0.11a	0.45a	1.07a	0.82a	7.50a	0.23a	174.00a	112.75a	81.25a	33.75a	35.25a	1.94a	0.13a
	N	**P**	**K**	**Ca**	**Mg**	**Na**	**S**	**Cu**	**Fe**	**Mn**	**Zn**	**B**		
Deficient	1.60	0.08	0.4	0.50	0.15		0.05	2-3	20-40	10-15	10-15	10-20		
Commercial Range	1.6-2.8	0.08-0.2	0.75-1.5	1-3	0.25-0.8		0.2-0.6	5-15	50-200	30-500	40-80	40-60		
Excess	3.00	0.30	3.00	4.00	1.00	0.25-0.5	1.00	25.0		1000	100	100		

[a] Trees treated with a soil drench of potassium silicate

[b] Trees receiving a trunk injection of potassium silicate

Table 4.5: Avocado root nutrient concentrations for two consecutive years sampled during July from trees used in a study to determine the efficacy of soluble potassium silicate application to avocado trees on *Phytophthora cinnamomi* disease severity. Treatments analysed consisted of three (Si x 3) potassium silicate soil drench applications per season, biannual injections of either 0.74ml.l^{-1} or 20ml.l^{-1} potassium silicate injection solutions (20.7% silicon dioxide), trees receiving no treatment as a control, or potassium phosphonate (Avoguard®) injected trees (PA). Data for 0.74ml.l^{-1} or 20ml.l^{-1} potassium silicate injections in 2005 are not available due to lack of samples taken. Values within a column in the table with different symbols indicate significant differences at a 95% level of significance.

ROOTS	**N**	**P**	**K**	**Ca**	**Mg**	**Na**	**S**	**Cu**	**Fe**	**Mn**	**Zn**	**B**	**Mo**	**Si**
	%	**%**	**%**	%	%	mg/kg	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	**%**
Control July 2005	1.1a	0.13a	0.29a	0.62a	0.21a	86a	0.11a	1360a	7800a	593a	204ab	90a	6.53b	2.45a
PA July 2005	1.4b	0.27b	0.31a	1.37a	0.4c	188b	0.17a	2170a	7810a	848b	168a	108b	2.52a	3.35b
Si x 3 July 2005[a]	1.4b	0.3c	0.43b	0.96a	0.31b	155b	0.14a	1460a	9110b	569a	253b	85a	3.14a	3.60b
Control July 2006	1.28a	0.19a	0.29a	0.99a	0.34a	161a	0.14a	1680a	7768a	815c	255.5a	147a	1.52a	4.75b
PA July 2006	1.30a	0.21a	0.33a	0.98a	0.32a	145a	0.15a	1768a	7758a	685a	328a	138a	3.8a	3.18a
Si x 3 July 2006[a]	1.20a	0.18a	0.24a	0.83a	0.29a	159a	0.13a	1693a	9090b	793bc	185a	115a	3.26a	4.75b
0.74 ml.l^{-1} Si July 2006[b]	1.15a	0.15a	0.22a	0.95a	0.26a	94.75a	0.13a	1326a	8073a	653a	211.5a	133a	2.93a	4.69ab
20 ml.l^{-1} Si July 2006[b]	1.28a	0.17a	0.3a	1.13a	0.32a	100.3a	0.15a	1555a	7775a	749b	214.5a	138a	3.49a	4.33ab

[a] Trees treated with a soil drench of potassium silicate

[b] Trees receiving a trunk injection of potassium silicate

Table 4.6: Soil nutrient analysis from an avocado orchard treated with soluble potassium silicate as a soil drench to inhibit *Phytophthora cinnamomi* disease severity. Soil samples analysed were taken from three (Si x 3) potassium silicate soil drench applications per season and trees receiving no treatment (control).

	pH (KCl)	**K**	**Mg**	**Na**	**Resistance**	**Ca**	**Mg**	**Na**	**Ca:Mg**
		mg/kg	mg/kg	mg/kg	Ohms	%	%	%	
November 2004	4.73	250	172	19	3500	36.59	41.94	2.46	0.87
Control July 2005	4.67	100	259	15	3000	62.1	32.92	1.01	1.89
Control July 2006	5.04	108	267	16	1500	62.03	32.79	1.04	1.89
Si x 3 July 2005	5.03	105	210	11	4000	69.37	25.87	0.72	2.68
Si x 3 July 2006	5.28	175	315	12	1500	52.82	39.53	0.8	1.34
	Ca+Mg)/K	**Mg:K**	**S-Value**	**Zn**	**Cu**	**Mn**	**Fe**	**Si**	
			cmol(+)/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	
November 2004	4.13	2.2	3.36	18	34	100	5	8.19	
Control July 2005	23.96	8.3	6.45	42	34	75	5	10.94	
Control July 2006	22.91	7.92	6.67	23	39	87	6	12.33	
Si x 3 July 2005	23.6	6.41	6.65	27	27	53	5	12.89	
Si x 3 July 2006	13.48	5.77	6.53	34	34	68	5.8	18.2	

CHAPTER 5

ACCUMULATION OF TOTAL PHENOLICS DUE TO SILICON APPLICATION IN ROOTS OF AVOCADO TREES INFECTED WITH *PHYTOPHTHORA CINNAMOMI*

5.1 ABSTRACT

The accumulation of soluble and wall-bound phenolics and phenolic polymers in *Persea americana* Mill. roots from thirteen year-old Hass on Edranol trees exposed to the pathogen *Phytophthora cinnamomi*, and treated with water soluble potassium silicate was investigated. Following elicitation, the conjugated and non-conjugated phenolic metabolites present in the induced root tissue were extracted and quantified. From March 2005 to January 2006, three applications (Si x 3) of soluble potassium silicate per season resulted in significantly higher concentrations of crude phenolic compounds in the roots compared to the untreated control. From March to May 2006, the control treatment (133.66µg.l^{-1}; 109.08µg.l^{-1}) resulted in higher crude phenolic levels compared to Si x 3 (94.61µg.l^{-1}; 67.98µg.l^{-1}). Significantly higher crude phenolic concentrations in avocado roots were obtained in Si x 3 during March and May 2006 (94.61µg.l^{-1}; 67.98µg.l^{-1}) when compared to potassium phosphonate (Avoguard®) (49.07µg.l^{-1}; 59.46µg.l^{-1}). Glucoside bound phenolic acid concentrations in trees treated with Si x 3 differed significantly from the untreated control for the period from January to May 2006. Concentrations of glucoside bound phenolic acids obtained with Si x 3 treatment are comparable to that of potassium phosphonate (Avoguard®) with exceptions during March 2005 and May 2006. Three silicon applications per season resulted in significantly lower cell wall bound phenolic acid concentrations on avocado roots compared to the control treatment during May and Sept 2005, and March and May 2006. This trend was negated during January 2006 when a significantly higher cell wall bound phenolic concentration was obtained in Si x 3 (0.71µg.l^{-1}) than the control (0.36µg.l^{-1}). Three silicon treatments per season resulted in significantly lower cell wall bound phenols during May, Jul and Sept 2005 and May 2006 compared to Si x 1, with higher concentrations obtained in Si x 3 only during Jan 2006 (0.71µg.l^{-1} vs. 0.38µg.l^{-1}). Results indicate that potassium silicate application leads to lower cell wall bound phenolics. Silicon treatment of avocado trees resulted in fewer identifiable phenols in avocado roots compared to the untreated control and potassium phosphonate (Avoguard®) treatments. HPLC separation of hydrolysed

phenolic acids extracted from roots revealed all non-conjugated phenolic acid hydrolysed samples to contain 3,4-hydroxibenzoic acid. The glucoside bound samples of both the potassium phosphonate and the untreated control treatments contained 3,4-hydroxibenzoic acid and vanillic acid, while the control also contained syringic acid in the hydrolysed glucoside bound extract. These results indicate that potassium silicate application to avocado trees under *P. cinnamomi* infectious conditions increase total phenolic content of avocado root tissue.

5.2 INTRODUCTION

Due to the threat of infection, plants have evolved a multitude of chemicals and structures that are incorporated into their tissue for the purpose of protection. These defences can repel, deter, or intoxicate including resin-covered or fibrous foliage, resin-filled ducts and cavities, lignified or phenol-impregnated cell walls, and cells containing phenols or hormone analogues (Berryman, 1988).

Various antimicrobial compounds which are synthesized by plants after infection, have been identified. Most phenolic compounds are phenolic phenyl-propanoids that are products of the shikimic acid pathway. Non-pathogenic fungi induce such high levels of toxic compounds in the host, that their establishment is prevented, while pathogenic fungi either induce only non-toxic compounds or quickly degrade the phytoalexins (Macheix *et al*., 1990; De Ascensao and Dubery, 2003). Rapid and early accumulation of phenolic compounds at infection sites is a characteristic of phenolic-based defence responses. This accumulation of toxic phenols may result in effective isolation of the pathogen at the original site of entrance (De Ascensao and Dubery, 2003).

Wehner *et al*. (1982) reported on the sensitivity of pathogens to antifungal substances in avocado tissue. They concluded that no consistent tendencies exist in the antifungal compound concentration in different avocado cultivars, although marked differences were found between plant parts, with avocado leaves containing the highest levels, followed by fruit mesocarp, root, seed and skin extracts.

In avocado some phenolics may act as antioxidants and induce resistance. These phenolic antioxidants are present in plant lipophylic regions. The soluble phenol flavan-3-ol epicatechin is an antioxidant and acts as a trap for free radicles (Vidhyasekaran, 1997). Diene (1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene) inhibits mycelial growth (Prusky *et al*., 1982; Prusky *et al*., 1983) and spore germination (Prusky *et al.,* 1982), and

is degraded by lipoxygenase extracted from avocado peel. An 80% increase in the specific activity of lipoxygenase in peel extracts occurs coincident with a rapid decrease of diene in fruit peel (Prusky *et al.,* 1983).

Epicatechin inhibits lipoxygenase *in vitro*, and may act as a regulator of membrane-bound lipoxygenase. Epicatechin concentration in avocado fruit peel is inversely correlated with lipoxygenase activity and decreases significantly when lipoxygenase increases (Marcus *et al*., 1998). It is suggested that epicatechin plays a role in induced resistance by inhibiting lipoxygenase. Diene decrease is regulated by lipoxygenase activity, which in turn is regulated by a decrease in the antioxidant, epicatechin, concentration (Karni *et al.,* 1989; Prusky *et al.,* 1991). Exposure of avocado fruit to CO_2 for 24h increased diene as well as epicatechin concentrations, while lipoxygenase activity was inhibited (Prusky *et al.,* 1991). Diene has also been isolated from avocado leaves (Carman and Handley, 1999), and appears to accumulate in order of magnitude in Hass ($4.5\mu g.g^{-1}$), Pinkerton, Fuerte, Duke 7 and Edranol ($0.4\mu g.g^{-1}$) avocado leaves.

In addition to diene, numerous other compounds with fungitoxic characteristics are produced in avocado plants. Domergue *et al*. (2000) isolated (E,Z,Z,)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-5,12,15-triene, which inhibited spore germination of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Brune and van Lelyveld (1982) conducted studies on the biochemical composition of avocado leaves and its correlation to susceptibility to root rot caused by *Phytophthora cinnamomi*. They concluded the majority of phenols detected in avocado plant material to be either phenolic acid (C_6-C_1) or cinnamic acid derivatives (C_6-C_3). The possibility exists that avocado plants may convert specific phenolics into coumarins, from which coumarin phytoalexins may be derived.

The current study was initiated to determine if the application of potassium silicate to avocado trees increases the phenolic concentration in avocado tissue. If possible, specific phenol increases are to be determined, thus confirming the hypothesis that silicon increases the phenolic concentration of host tissues, resulting in the inhibition of Phytophthora root rot severity in avocados.

5.3 MATERIALS AND METHODS

5.3.1 Chemicals

Potassium silicate was obtained from Ineos Silicas (Pty) Ltd, and potassium phosphonate (Avoguard®) from Ocean Agriculture (Johannesburg, South Africa). Analytical grade solvents used in the extractions and HPLC were obtained from Merck Chemicals (Merck, Halfway House, South Africa).

5.3.2 Experimental Layout

An avocado orchard (latitude 23° 43' 60S; longitude 30°10'0E) at an altitude of 847m was selected in the Tzaneen area, South Africa. Trees consisted of thirteen year old "Hass" on "Duke7" seedling rootstocks planted at a density of 204trees.ha^{-1} (7 x 7m spacing). Trees were on a southern facing slope. The trial layout consisted of 50 trees (n) with 10 trees randomly assigned per treatment, and organised in a randomised block design (Appendix B).

5.3.3 Treatments

Treatments consisted of a soil drench with a 20 litre solution of 20ml.l^{-1} soluble potassium silicate (20.7% silicon dioxide) (Bekker *et al*, 2006) per tree either once, twice or three times in a growing season. Trees injected with potassium phosphonate (Avoguard®) were incorporated as a standard fungicide treatment. Untreated trees served as controls. Data was collected from January 2005 to July 2006. Root samples were taken every second month on the northern side of the tree.

5.3.4 Extraction and Quantification of Total Phenolic Compounds

Root samples were freeze dried for 120h. The dried material was ground with an IKA® A11 basic grinder (IKA Werke, GMBH & Co., KG, D-79219 Staufen) to a fine powder. Three extractions were done per sample. One millilitre of a cold mixture of methanol: acetone: water (7:7:1, v:v:v) solution was added to 0.05g powdered plant sample, ultrasonicated for 5min by means of a VWR ultrasonic bath, and centrifuged at 24000g for 1min. No antioxidant (ascorbic acid or $Na_2S_2O_5$) was used, as it would have interfered with the folin-ciocalteau reagent used for total phenol determination (Regnier, 1994). This extraction procedure was repeated twice, and the supernatant fractions pooled. The

solid material left in the eppendorf tube after extraction was saved for cell wall-bound phenolic acid determination. Chlorophyll was removed from the leaf sample solutions by adding 0.5ml chloroform to the supernatant, shaking it for 30s and thereafter centrifuging it for 30s. The organic solvent mixture was evaporated in a laminar flow cabinet at room temperature, whereafter the residue was dissolved in 1ml distilled water. Crude samples were stored at 4°C until extraction.

5.3.5 Non-Conjugated Phenolic Acids

An aliquot of 0.25ml from the crude sample for total soluble phenolic determination was acidified by addition of 25µl 1M HCl before extraction with 1ml anhydrous diethyl ether. The ether extract was reduced to dryness at 4°C and the resulting precipitate was resuspended in 0.25ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

5.3.6 Glycoside-Bound Phenolic Acids

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

5.3.7 Ester-Bound Phenolic Acids

Extraction of soluble ester-bound phenolics took place after hydrolysis under mild conditions. To an aliquot of 0.25ml for total soluble phenolic determination, 0.1ml 2M NaOH was added and the solutions were allowed to stand in the Eppendorf tubes for 3h at room temperature. After hydrolysis 40µl 1M HCl was added and the phenolics extracted with anhydrous diethyl ether. The ether extract was reduced to dryness at 4°C and the resulting precipitate was resuspended in 0.25ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

5.3.8 Cell Wall-Bound Phenolic Acids

The solid material left in the Eppendorf tube after extraction was dried, weighed and resuspended in 0.5M NaOH for 1h at 96°C. Cell wall esterified hydroxycinnamic acid

derivatives were selectively released under these mild saponification conditions. The supernatant was acidified to pH 2 with HCl, centrifuged at 12000g for 5min and then extracted with anhydrous diethyl ether. The extract was reduced to dryness and the precipitate was resuspended in 0.25ml 50% aqueous methanol (De Ascensao and Dubery, 2003).

5.3.9 Quantification of Phenolics by the Folin-Ciocalteau Method

The concentration of phenolic compounds in the various extracts was determined using the folin-ciocalteau reagent (Merck) (Regnier, 1994). The reaction mixture used was reduced proportionally to enable the use of 96-well ELISA plates for the quantification of phenolics. For the quantification of phenolic content, a dilution series (10 – 1000µg.ml^{-1} methanol) was used to prepare standard curves for furellic and gallic acid, which is a modification to the folin-ciocalteau method as described by Regnier and Macheix (1996). The reagent mixture comprised: 170µl distilled water, 5µl standard or plant extract sample, 50µl 20% (v/v) Na_2CO_3 and 25µl folin-ciocalteau reagent. After incubation at 40°C for 30min the absorbance was read at 720nm using an ELISA reader (Multiskan Ascent VI.24354 – 50973 (version 1.3.1)). Spectrometric measurements of the phenolic concentrations in the various extracts was calculated from a standard curve ($y = 0.0013x + 0.0177$, $r^2 = 0.9982$) and expressed as mg gallic acid equivalent per gram of dry weight.

5.3.10 Reverse Phase – High Performance Liquid Chromatography

Extracted phenolic fractions were analysed by means of reverse phase - high performance liquid chromatography (RP-HPLC) (Hewlett Packard Agilent 1100 series) with DAD detection (diode array detector, 280, 325, 340nm). A Luna 3u C-18 (Phenomenex®) reverse phase column (250mm length, 5µm particle size, 4.6mm inner diameter) was used. An excess injection volume of 50µl of each sample was used in a 20µl loop**.** A gradient elution was performed with water (pH 2.6 adjusted with H_3PO_4) and acetonitrile (ACN) as follows: 0min, 7% ACN; 0 – 20min, 20% ACN; 20 – 28min, 23% ACN; 28 – 40min, 27%, ACN; 40 – 45min, 29%, ACN; 45 – 47min, 33%, ACN; 47 – 50min, 80%. The flow rate was 0.7ml.min^{-1}. The identification of the phenolic compounds was carried out by comparing their retention times and UV apex spectrum to those of standards (purchased from Sigma Chemical Company, USA) which included syringic, gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, ferulic, caffeic, and chlorogenic acids. After

each run, the column was re-equilibrated with the initial conditions for 10min. The detector was programmed for peak detection at 280nm, which, although not optimum for ferulic acid and its derivatives, allowed simultaneous detection of hydroxybenzoic and hydroxycinnamic acids and their derivatives (Zhou *et al*., 2004).

5.3.11 Statistical Analysis

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

5.4 RESULTS AND DISCUSSION

Extraction of phenolics in the present study yielded concentrated samples. Apart from crude extract phenolic content determination, four targeted extractions were done to obtain glycoside bound phenolic acids, free phenolic acids, ester bound phenolic acid and cell wall bound phenolic acids. A gallic acid equivalent calibration curve ($y = 0.013x + 0.0177$, $R^2 = 0.9982$) was used to determine the amount of each fraction contained in the sample material. The targeted extract values are representative of the relative amount of each fraction in the crude extract. This is in agreement with phenolic acid functionality as discussed by several authors (Dixon and Paiva, 1995; Beckman, 2000; Zhou *et al*., 2004). Although high concentrations were obtained in the crude extracts, crude extract values do not reflect the combined values of the four other phenolic acid fractions extracted with more specific hydrolysis reactions. This is because phenols are bound to large molecules in the cell cytoplasm, and by hydrolysis, these molecules are split, resulting in the relevant concentrations being measured.

During the harvesting period (July 2005 & 2006), no significant differences were seen between any treatments with regards to crude phenolic concentrations. For the period of March 2005 to January 2006, three silicon applications (Si x 3) per season resulted in significantly higher total phenolic concentrations in root tissue compared to the control (Figure 5.1). From March to May 2006, the control treatment (133.66µg.l^{-1}; 109.08µg.l^{-1}) resulted in higher crude phenolic levels compared to Si x 3 (94.61µg.l^{-1}; 67.98µg.l^{-1}). Although this data does not correlate with any of the parameters of the phenological model proposed by Kaiser (1993), it is proposed that the lower metabolic plant levels are as a result of lowered physiological activity in the plant, due to lower temperatures, leading to sub-optimal photosynthesis. Although Si x 3 resulted in significantly higher

phenolic concentrations in avocado roots only during March and May 2006 (94.61µg.l^{-1}; 67.98µg.l^{-1}) compared to potassium phosphonate (Avoguard®) (49.07µg.l^{-1}; 59.46µg.l^{-1}), Si x 3 is statistically comparable to the current control method implemented to suppress *Phytophthora* infection. Statistically similar total phenol concentrations in avocado roots were obtained by two silicon applications per season (Si x 2) throughout the duration of the experiment except for March 2005 compared to Si x 3. Two (Si x 2) silicon applications per season mostly resulted in significantly higher phenol concentrations in avocado tissue compared to the control, except for July 2005, Sept 2005, March 2006 and Jul 2006.

Glucoside bound phenolic acid concentrations (Figure 5.2) for Si x 3 differed significantly from the control for the period January to May 2006. Significant differences between these two treatments prior to Jan 2006 were only detected during May 2005. This could possibly be related to the dry period experienced during that time (Appendix B). It is expected that a significant difference will be seen between Si x 3 and the control treatment under conditions where the trees are subjected to environmental stress. Concentrations of glucose bound phenolic acids obtained with Si x 3 treatment were comparable to that of the potassium phosphonate (Avoguard®) treatment with exceptions during March 2005 and May 2006.

Three silicon applications per season resulted in significantly lower cell wall bound phenolic acid concentrations in avocado roots (Figure 5.3) compared to the control treatment during May and Sept 2005, and March and May 2006. This trend was negated during January 2006 when a significantly higher cell wall bound phenolic concentration was obtained in Si x 3 (0.71µg.l^{-1}) than the control (0.36µg.l^{-1}). Although the trend was not consistent compared to Si x 3, the potassium phosphonate (Avoguard®) treatment did not differ from the control throughout the tested period. Three silicon treatments per season resulted in significantly lower cell wall bound phenols during May, Jul and Sept 2005 and May 2006 compared to Si x 1, with higher concentrations obtained in Si x 3 only during Jan 2006 (0.71µg.l^{-1} vs. 0.38µg.l^{-1}). No significant difference was obtained between Si x 1 and Si x 3, except during Jan 2006, when Si x 3 (0.71µg.l^{-1}) resulted in higher cell wall bound phenols compared to Si x 2 (0.35µg.l^{-1}). Results indicate that potassium silicate application leads to lower cell wall bound phenolics. If the hypothesis of silicon being build into cell walls as part of a physical barrier is correct, it is possibly that silicon replaces phenol-binding molecules, or is bound in the place of phenolics, resulting in lower cell wall bound phenols. Epstein (2001) reported an accumulation of

phenolic compounds in the epidermis of silicon-deprived plants inoculated with a phytopathogenic fungus. It was accounted by Carver *et al*. (1998) that silicon-deprived leaves have been shown to exhibit higher phenylalanine ammonia lyase (PAL) activity compared to silicon-replete leaves, concluding that silicon deprivation may have been compensated for by the rise in PAL activity, in turn contributing to plant fungal resistance. Menzies *et al*. (1991) reported an extreme change in defence response expression of infected silicon-fertilized epidermal plant cells. Their results indicated that silicon accumulation was subsequent to phenol appearance in infected tissue, challenging the physical barrier-hypothesis that silicon accumulation in plant cell walls in close contact with the pathogen confers resistance to fungal penetration by physical means.

No significant differences were seen between treatments for ester bound phenolic concentrations throughout the duration of the trial (Figure 5.4).

Non-conjugated phenolic concentrations did not differ significantly between treatments during March, Jul and Sept 2005, and Jan and March 2006. Three silicon applications per season (1.62µg.l^{-1}) and potassium phosphonate (Avoguard®) (2.44µg.l^{-1}) resulted in significantly lower non-conjugated phenol concentrations compared to that of the control (2.80µg.l^{-1}) only during Nov 2005 (Figure 5.5), while the concentrations between Si x 3 and potassium phosphonate (Avoguard®) were statistically similar.

After silicon is taken up by a plant, it goes through a silicification process, and is either deposited in the cell wall, cell lumen, or intercellular spaces (Epstein, 1999; Sangster *et al.,* 2001). Silicon possesses a strong affinity for organic poly-hydroxyl compounds which participate in lignin synthesis. This partly explains its tendency to accumulate in cell walls during plant maturation or pathogen attack, which both corresponds to a radical change in cell wall constitution, with the apposition of lignin (Jones and Handreck, 1967; Inanaga and Okasaka, 1995). Electron microscopy and dispersive x-ray analysis led Samuels *et al.* (1991) and Chérif *et al*. (1992a) to conclude that enhanced defence reactions in the cucumber plant to *Pythium ultimum* Trow. and *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci appear to be the result of silicon present in the plants' transpiration stream, and not because it becomes bound to the plant cell wall. Although Menzies *et al*. (1992) and Chérif *et al*. (1992b) deemed the possibility of silicification of cell walls as not to be completely discarded, silicon is more likely to affect signalling between the host and pathogen, resulting in more rapid activation of a hosts' defence mechanisms. Heath (1976, 1979, 1981) and Chong and Harder (1980, 1982) investigated the effect of silicon on haustoria formation, and concluded that heavy silicon deposition

in the haustorial mother cells located at or near the centres of infection colonies was a protective mechanism of the plant to pathogen penetration. This mechanism acted as a permeability barrier to minimize passage of deleterious cell breakdown products to the rest of the pathogen mycelia. Heath (1979) reported that silicon accumulation as a response to infection is not limited to silicon accumulating plants (Epstein, 1999). Heath (1981) reported silicon accumulation not to be related to haustoria formation and, although uncertain on the significance of silicon in the cell walls and necrotic cytoplasm, suggested silicon accumulation to reflect a passive secondary association of silicon with phenolic compounds present in the disorganized host cell. Heath and Stumpf (1986) suggested the high levels of wall-associated phenolics in silicon-depleted tissue to result in faster inhibition of fungal enzymes involved in fungal-penetrating peg formation. In untreated tissue, the presence of silicon in the cell walls acted to 1) restrict substance flow to the haustorial mother cell, 2) reduce the interchange between the fungus and plant, so lesser amounts of phenolics are produced by the host, and 3) acted as a physical barrier to the penetration peg if it reached the cell wall (Heath, 1981). Results from the current study indicate that potassium silicate application to avocado trees leads to higher crude extract phenolic concentrations but lower cell wall bound phenolics compared to the control. Silicon accumulation was subsequent to phenol appearance in infected tissue, challenging the physical barrier-hypothesis that silicon accumulation in plant cell walls in close contact with the pathogen confers resistance to fungal penetration by physical means.

The accumulation of crude phenols in avocado roots treated with potassium silicate corresponds to higher root densities and lower canopy ratings (Chapter 4). The accumulation of phenols in avocado roots due to potassium silicate treatment could therefore be responsible for the increased resistance to *Phytophthora* observed in nursery trees and avocado orchards.

Phenols derived from cinnamic and ferulic acid would be polar (hydrophilic), while polymeric phenolics would be less polar (hydrophobic), and co-extracted biopolymers possibly present (e.g. terpenes) would be very hydrophobic (Regnier and Macheix, 1996). In the current study effective separation by gradient extraction was achieved by tapping the differences in the hydrophobic/hydrophilic nature of mobile phase components, as well as extracted molecule polarity. Phenolics were adsorbed onto the stationary phase at low solvent strength through Van der Waals forces and, according to their decreasing ability to participate in hydrogen bonding at distinct solvent concentrations, selectively

released (Cunico *et al*., 1998). Results from the RP-HPLC investigation in the current study could not be quantified to satisfaction and are therefore presented qualitatively only (Figure 5.6). Representative chromatograms for potassium phosphonate (Avoguard®), Si x 3 and control treatments are included.

Crude extracts of avocado roots used for determination of total phenol concentration were separated using HPLC, but, although separated peaks were obtained, the compounds were unidentifiable as phenols were present as glycosides. Nuutila *et al*. (2002) reported that, for quantitative determination of individual flavonoid glycosides to occur, glycosides need to be hydrolyzed and the resulting aglycones are then identified and quantified. Chérif *et al*. (1994) reported the fungitoxicity of these compounds to be apparent only after acid hydrolysis of the plant extracts. Hydrolysis of samples was therefore imperative to determine specific compounds within the phenol constitution of avocado root extracts. After hydrolysis, peak sizes reduced dramatically. Phenolic compounds were however identified on the basis of peak shape and retention time.

Silicon application to avocado trees resulted in fewer identifiable phenols in avocado roots compared to the control and potassium phosphonate treatments. HPLC separation of hydrolysed phenolic acids extracted from roots revealed that all non-conjugated phenolic acid samples contain 3,4-hydroxibenzoic acid [retention times of potassium phosphonate, Si x 3 and control treatments being R_t = 14.693, R_t =14.878 and R_t = 14.984, respectively]. The hydrolysed glucoside bound samples of both the potassium phosphonate and control treatments also contained 3,4-hydroxibenzoic acid (R_t = 14.693; R_t = 14.881, respectively) and vanillic acid (R_t = 22.326; R_t = 22.621, respectively). The control treatment contained syringic acid (R_t = 23.154) in the hydrolysed glucoside bound extract.

Nuutila *et al*. (2002) reported that phenol based defence responses are characterised by an accumulation of phenolic compounds within host cell walls, as well as the synthesis and deposition of the phenolic polymer, lignin. Esterification of phenolic cell wall materials is a common occurrence in expression of resistance (Cunico *et al*., 1998). Phenols in the cell wall has been suggested to act as a template for further lignin deposition, indicating esterification and lignification to be contigious rather than separate processes. Lignin formation takes place as a result of cell damage due to mechanical puncturing or infectional penetration (De Ascensao and Dubery, 2003).

5.5 CONCLUSION

The accumulation of phenols and phenolic polymers in *Persea americana* roots exposed to cell wall derived elicitors from the pathogen *Phytophthora cinnamomi*, and treated with water soluble potassium silicate, was investigated. These findings support the hypothesis that silicon application results in heightened resistance against *P. cinnamomi* infection via an elevation of phenolic levels in the roots. Although crude phenolic concentrations differed between treatments and no clear deduction may be made concerning the effect of potassium silicate on the phenolic content of avocado roots in the presence of *P. cinnamomi*, it is clear that similar or higher crude phenolic concentrations are obtained in avocado roots with three silicate applications per season compared to potassium phosphonate treated trees. This was also true for glucoside bound phenolic concentrations in roots from trees treated three time per season with potassium silicon (Si x 3) compared to potassium phosphonate treated trees.

In this study the potassium silicate application lead to lower cell wall bound phenolics. The possibility that silicon replaces phenol-binding molecules is not fully understood. However, this study indicates that the accumulation of silicon was subsequent to phenol appearance in infected tissue; challenging the physical barrier and conferring to the cell wall in close contact with the pathogen some resistance to fungal penetration by physical means. The future search on the use of silicon therefore can be upheld with this strategy to control plant disease in general and avocado root diseases in particular.

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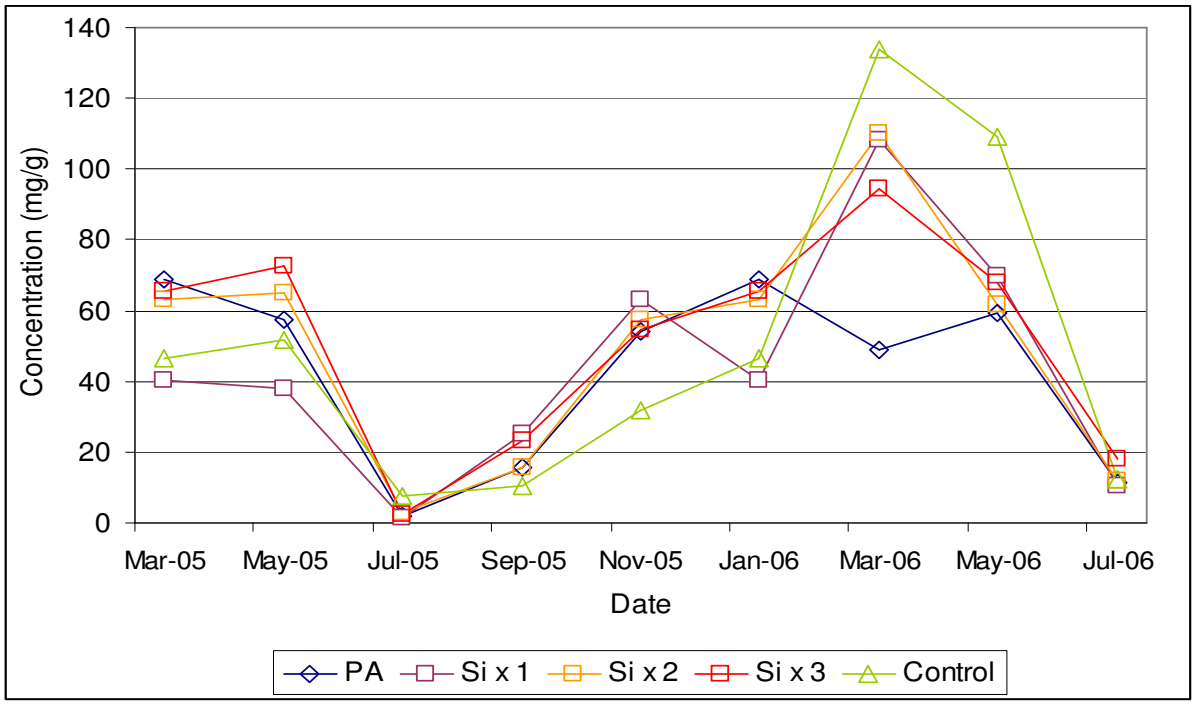
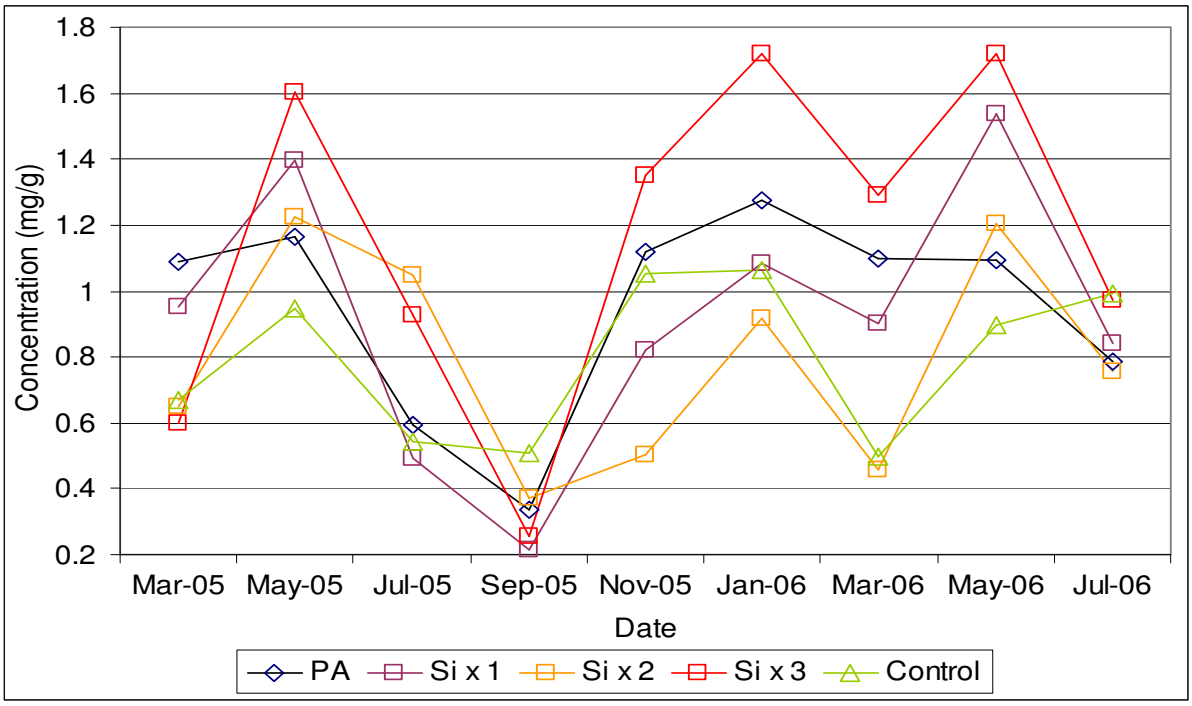


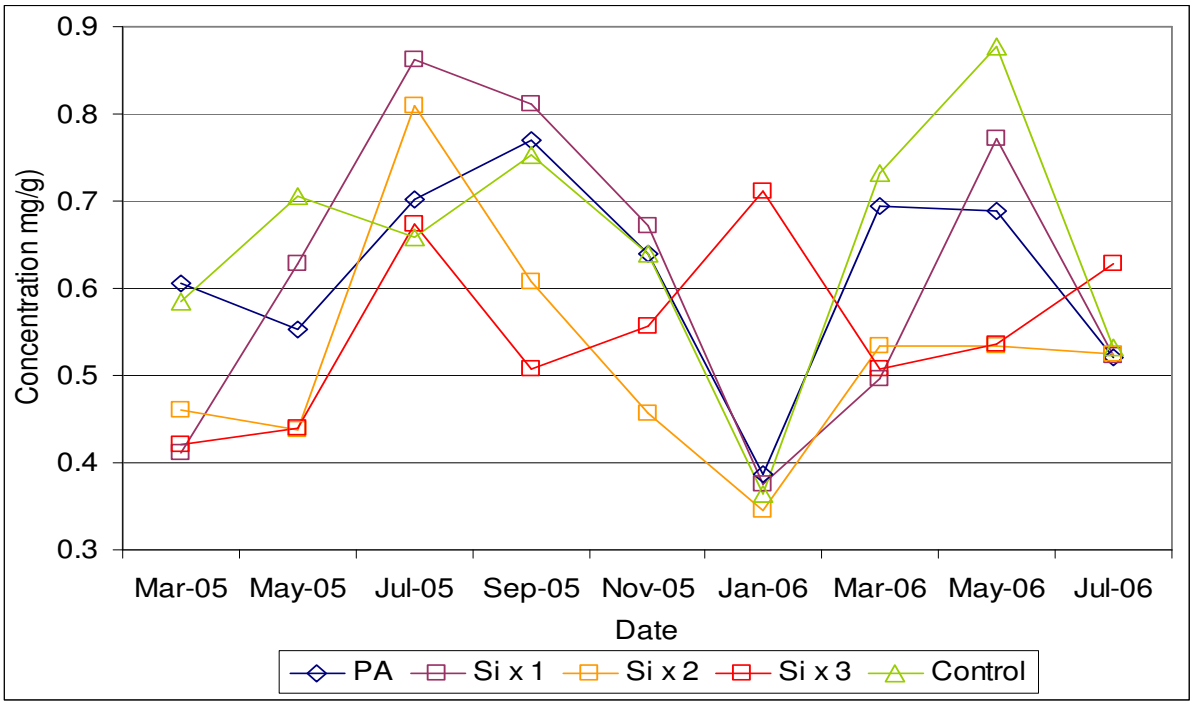
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	67.77b	57.26bc	1.94a	15.81ab	53.94b	68.77b	49.07a	59.46a	11.25a
Si x 1	45.42a	37.82a	1.66a	25.34b	62.94c	40.42a	108.23c	69.64b	10.61a
Si x 2	63.38b	65.19c	2.93a	15.77ab	57.56bc	63.08b	110.25c	61.62ab	11.94a
Si x 3	65.32b	72.62c	2.5a	23.18b	54.8bc	65.32b	94.61b	67.98b	17.92a
Control	46.34a	51.62b	7.7a	10.41a	31.94a	46.34a	133.66c	109.08c	12.28a

Figure 5.1: Total soluble phenolic content of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA). Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.



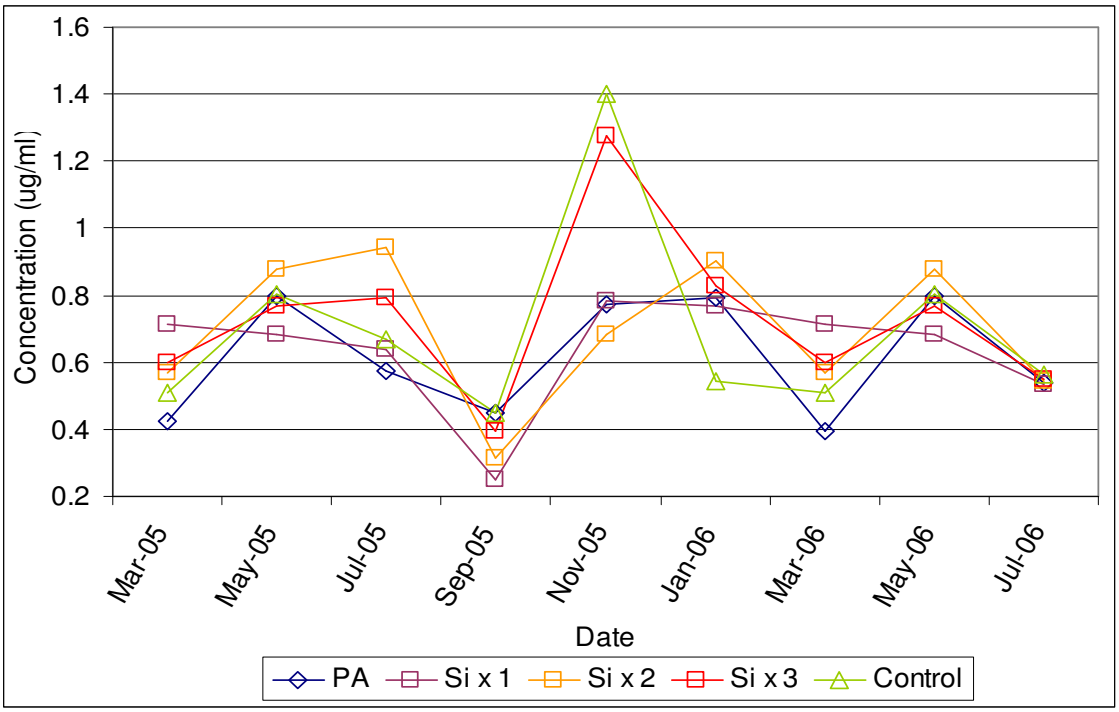
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	1.09b	1.16ab	0.59ab	0.34a	1.12b	1.27ab	1.09b	1.09a	0.79a
Si x 1	0.95ab	1.39b	0.49a	0.21a	0.82ab	1.08a	0.90b	1.54b	0.84a
Si x 2	0.65a	1.23ab	1.05b	0.37a	0.50a	0.92a	0.46a	1.21ab	0.75a
Si x 3	0.59a	1.60b	0.93b	0.26a	1.35b	1.72b	1.29b	1.72b	0.97a
Control	0.67a	0.95a	0.54ab	0.51a	1.05b	1.06a	0.49a	0.89a	0.99a

Figure 5.2: Total concentration of glucoside bound phenolic acid after hydrolysis of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA). Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.



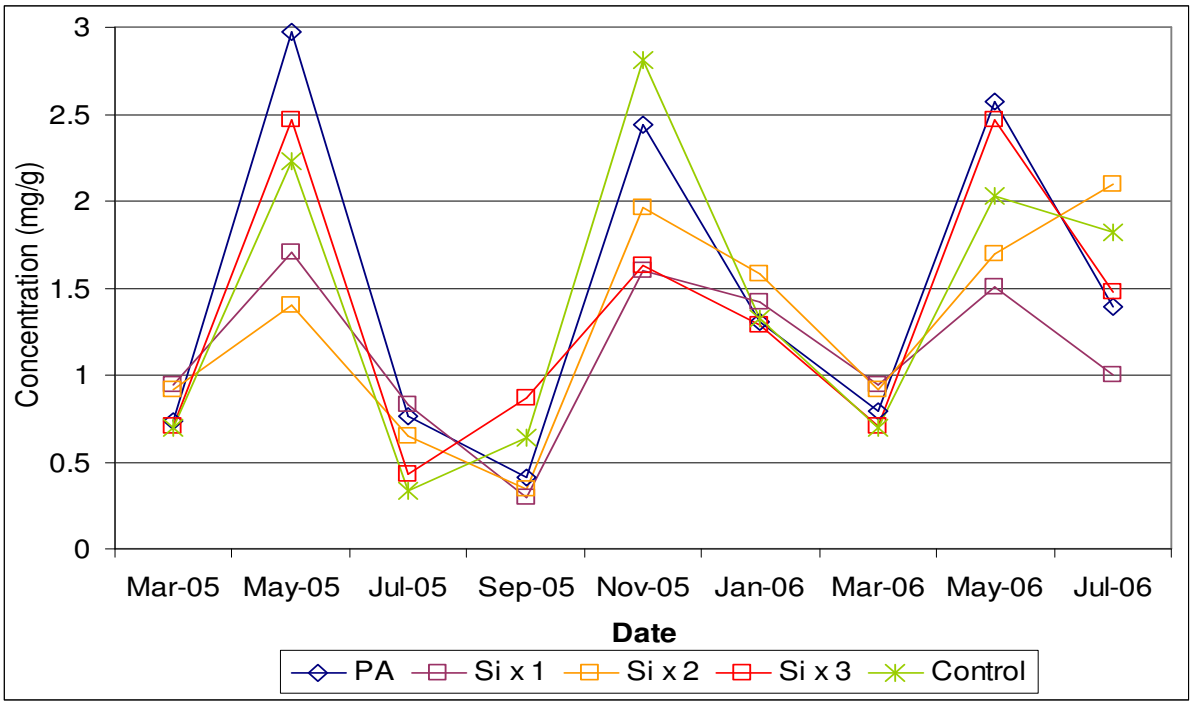
	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	0.61b	0.55ab	0.70ab	0.77b	0.64b	0.39a	0.69b	0.68ab	0.52a
Si x 1	0.41a	0.63b	0.86b	0.81b	0.67b	0.38a	0.49a	0.77b	0.52a
Si x 2	0.46ab	0.44a	0.81ab	0.61ab	0.46a	0.35a	0.53ab	0.53a	0.52a
Si x 3	0.42a	0.44a	0.67a	0.51a	0.56ab	0.71b	0.51a	0.54a	0.63a
Control	0.58ab	0.71b	0.66a	0.75b	0.64b	0.36a	0.73b	0.88b	0.53a

Figure 5.3: Total concentration of cell wall bound phenolic acid after hydrolysis of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA). Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.



	Mar-05	May-05	Jul-05	Sep-05	Nov-05	Jan-06	Mar-06	May-06	Jul-06
PA	0.42a	0.79a	0.57a	0.45a	0.77a	0.79a	0.39a	0.79a	0.54a
Si x 1	0.72a	0.68a	0.64a	0.25a	0.78a	0.77a	0.72a	0.68a	0.54a
Si x 2	0.57a	0.88a	0.94a	0.31a	0.68a	0.90a	0.57a	0.88a	0.54a
Si x 3	0.59a	0.77a	0.79a	0.39a	1.27a	0.83a	0.59a	0.77a	0.55a
Control	0.51a	0.80a	0.67a	0.45a	1.40a	0.54a	0.51a	0.80a	0.56a

Figure 5.4: Total concentration of ester bound phenolic acid after hydrolysis of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA. Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.



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

Figure 5.5: Total concentration of non-conjugated phenolics acid after hydrolysis of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees, which were either untreated (controls) or treated with potassium silicate as a soil drench. Treatments consisted of either one (Si x 1), two (Si x 2) or three (Si x 3) potassium silicate applications per season; trees injected with potassium phosphonate (PA). Values in table within a column with different symbols indicate significant differences at a 95% level of significance (student t-test). Phenolic concentration expressed as mg gallic acid equivalent per gram of dry weight.

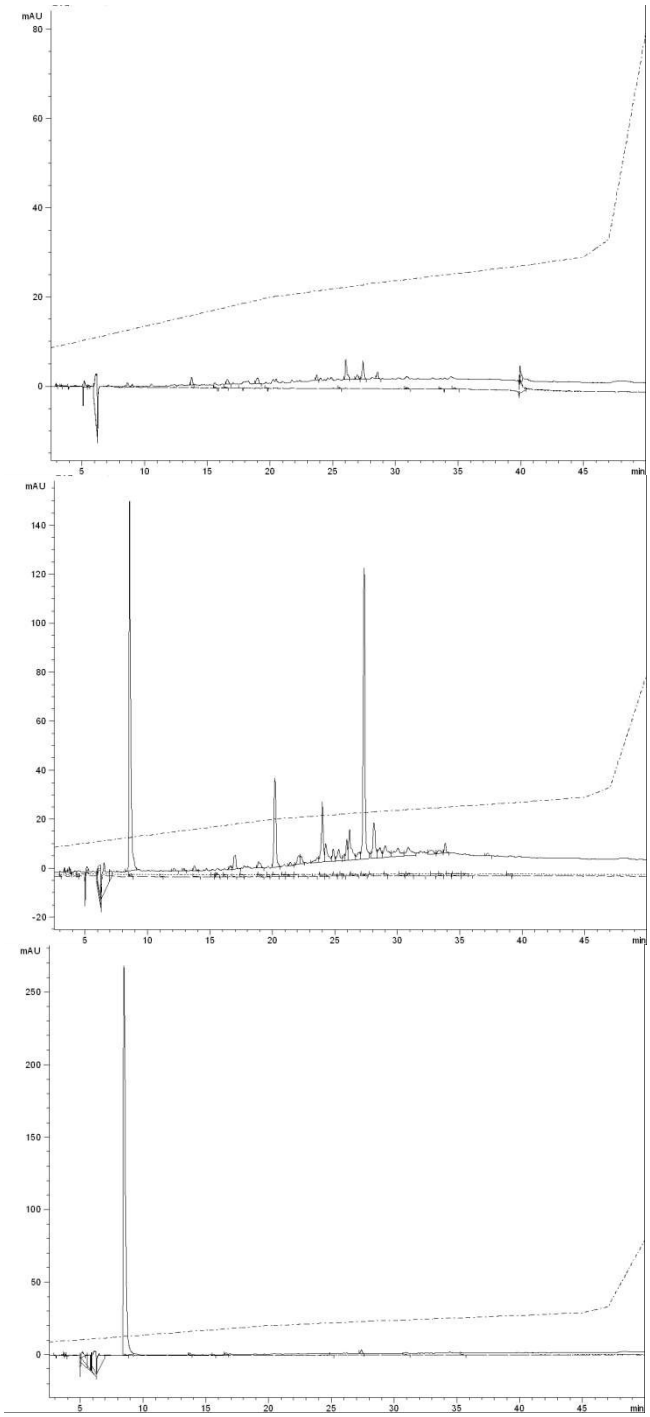


Figure 5.6: Chromatographs of avocado roots recovered over a period of 18 months in *P. cinnamomi* infected trees. Treatments consisted of trees receiving no treatment as a control treatment (a), trees injected with potassium phosphonate (PA) (b) or three (Si x 3) potassium silicate applications per season (a).

GENERAL DISCUSSION AND CONCLUSIONS

The avocado (*Persea americana* Mill.) is a tropical fruit produced in almost all tropical and subtropical climatic regions, and exported worldwide (Knight, 2002). In South Africa, avocado production is confined to the Limpopo and Mpumalanga provinces in the north and north-east, and to a lesser extent in the frost free lowland coastal belts and cooler midlands of KwaZulu Natal (Lovegrove and Hooley, 2000). Currently the area planted with avocado trees in South Africa amounts to 12400ha, with approximately 3015000 trees in production, which could amount to more than 50 thousand tons, of which 36thousand tons (9 million cartons) are destined for the export market (Retief, 2007).

Phytophthora root rot, caused by the fungus *Phytophthora cinnamomi* Rands, is the most important pre-harvest disease of avocado trees and attacks trees of all ages, including nursery trees, leading to tree death by destruction of the feeder roots (Hardy *et al.,* 2001). *Phytophthora* root rot has been the main economic factor limiting successful avocado production in countries such as Australia, South Africa and the USA (Coffey, 1987).

The prevention of *Phytophthora* root rot relies on limited non-chemical practices including implemented cultural practices (Ohr and Zentmyer, 1991), biological control with the use of *Trichoderma* isolates (Duvenhage and Kotze, 1993; Casale, 1990; Pegg, 1977), and host resistance (Coffey 1987). Chemical control however remains the most important control measure, and to this end, phosphate-based fungicides play a major role. Duvenhage (1994, 1999) however concluded that the possibility of resistance against fosetyl-Al, the most commonly used fungicide, does exist, which would pose a serious threat to the avocado industry.

The need therefore exists to find a viable alternative to phosphonate fungicides. The suppressive effects of silicon on plant diseases have been indicated by various authors (Ma and Takahashi, 2002; Epstein, 1999). Potassium silicate was therefore investigated as a viable alternative treatment against *P. cinnamomi* infection of avocado trees. The objective of this study was to determine whether the application of soluble silicon from potassium silicate to *P. cinnamomi* infected trees would suppress the disease.

The first objective was to assess the affect of potassium silicate *in vitro* on fungal growth to establish if potassium silicate has any direct effect on fungal growth. In the current study potassium silicate (20.7% SiO_2) induced a total inhibition of *P. cinnamomi*

mycelial growth at all concentrations tested, with effective inhibition at 5ml.l^{-1}. This was however not true for all fungi investigated, and a total inhibition for all fungi tested (*Alternaria solani, Colletotrichum gloeosporioides, Curvularia lunata, Drechslera* sp., *Fusarium oxysporum, Fusarium solani, Glomerella cingulata, Lasiodiplodia theobromae, Mucor pusillus, Natrassia* sp., *Pestalotiopsis maculans, Phomopsis perniciosa, Phytophthora capsicii, Phytophthora cinnamomi, Pythium* F-group, *Sclerotinia sclerotiorum, Sclerotium rolfsii, Stemphylium herbarum* and *Verticillium tricorpus*) was only attained at a concentration of 40ml.l^{-1} and higher. Soluble potassium silicate, having a pH of 12.7, raised the pH of agar from 5.6 to 10.3 and 11.7 at concentrations of 5 and 80ml.l^{-1} agar respectively. Fungal growth was however only partially inhibited at these high pH values. Clearly, potassium silicate has an inhibitory effect on fungal growth *in vitro* and this was mostly fungicidal rather than attributed to a pH effect.

The effect of such a high concentration of silicon, as those required to suppress fungal growth *in vitro*, on beneficial micro-organisms in the soil is yet to be determined, and a concentration of 20ml.l^{-1} consequently led to a suppression of root rot development in both greenhouse and field trials. If silicon inhibits spore formation, inoculum concentration in soils may be reduced to such an extend that lower fungicidal rates will be necessary, resulting in an overall reduction in production costs and a reduced pressure on resistance development.

Phytophthora root rot of avocado nursery trees can be inhibited successfully by potassium silicate application as seen from the glasshouse trials conducted. The effectiveness of silicon application, however, depends on repeated applications to infected trees. Samuels *et al*. (1993), using the powdery mildew-cucumber pathogen system, showed that within a short period of time after Si application ceased, prophylactic effects receded. Interruption of silicon application, or if applications are too far apart, may lead to reduced disease control and according to the mechanical barrier hypothesis, the protection against fungal haustoria penetration may expire. The timing of reapplication will be determined by, among other factors, soil structure, as silicon in solution leaches easily, rendering the applied silicon unreachable for plant root uptake. Sandy soils will therefore necessitate more regular applications of silicon to maintain the level of disease suppression in the host plant. Root rot suppression in silicon treated trees was comparable to, or even better than root rot suppression in inoculated, potassium phosphonate (Avoguard®) treated trees. These findings are of paramount importance as this implies that potassium silicate may

be proposed as a possible alternative control measure to inhibit the effects of *P. cinnamomi* on avocado nursery trees.

The application of silicon to nursery trees seems to impart some form of protection as inoculated, silicon treated trees rendered the highest fresh and dry root mass compared to all other treatments. This implies that silicon either stimulates plant growth or imparts some form of protection to avocado roots if applied prior to *P. cinnamomi* inoculation. This may be due to mechanical barriers (Chérif *et al.,* 1994; Datnoff *et al.,* 1997), induction of plant enzymes (Chérif *et al.,* 1992) and a hastened expression thereof (Remus-Borel *et al*., 2005); or the induction of fungitoxic metabolites in the plant (Fawe *et al*., 2001). Although worldwide research is being conducted on the effect of silicon on other crops, future research on avocado should focus on the possible mode of action that silicon imposes to defend avocado tissue from infection. The accumulation of amorphous tissue in roots, and especially around sites of infection, should be examined microscopically. Datnoff *et al.* (1997) stated that disease suppression is made possible by the increased silicification of the epidermal cells, as the link between silicon deposition and pathogen resistance stems from the fact that Si accumulates at sites of infection (Fauteux *et al.,* 2005).

The economic viability and effective implementation of a new method to suppress Phytophthora root rot is only proven during a trial on field grown avocado trees in full production. In the current study, higher root densities were obtained throughout the trial period with potassium silicate application comparing to that of potassium phosphonate (Avoguard®) injections and untreated control trees. Differences in root density between treatments were affected by the availability of soil moisture, i.e. rainfall received throughout the season, although seasonal growth flushes and timing of silicon application also played a role. To provide maximum protection, and therefore minimize disease development, Bowen *et al.* (1992) suggested silicon be applied continuously. Our results indicate this to be true, as found in the nursery tree trials; and as three applications of silicon resulted in the best disease suppression and stimulation of new root growth. These results correlated well with tree canopy ratings, as trees that received silicon frequently, showed better canopy conditions compared to the untreated control treatments. It is proposed that silicon application should be timed according to the phenological model (Kaiser, 1993) with the first application during the period of flowering and fruit set (September); the second to occur before the fruit drop

(November); and the third application to be applied before the root flush during February to March.

Phenological cycling, rather than rainfall, was the determining factor in canopy health. Under conditions of limited drought stress, tree canopies showed less symptoms of disease stress, whereas during dry conditions, canopy condition deteriorated dramatically. All potassium silicate soil drench treatments resulted in better canopy ratings over the 18 month period of data collection compared to the untreated control. This indicates that potassium silicate soil drench treatments reduced drought stress, apart from reducing disease stress. This was reiterated by Gong *et al.* (2005) who reported that silicon improves the water status of drought stressed wheat plants compared to untreated plants.

The effect of potassium silicate as a stem injection to control *P. cinnamomi* severity was not evident in differences in tree root densities, or canopy health ratings. Potassium silicate injections did not show any significant trends throughout the trial period.

Potassium silicate injections did not increased tree health to such an extent that it had an effect on canopy condition and no clear trends were observed. Anderson *et al.* (2004) injected avocado trees, with a disease rating of 5.5, with a 200ppm (0.74ml potassium silicate) solution. They reported stimulation of epicormic buds, with "an eventual significant increase in canopy density", and a 31% mean tree health improvement. In the current study, no epicormic bud bursts were observed, and no simultaneous increase in canopy density detected. No mention is made as to when epicormic bud bursts were observed with relation to phenological cycling, and it is proposed that the cycling observed by Anderson *et al.* (2004) was due to tree phenology, rather than due to a siliceous effect.

In the current 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 *et al.* (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 cucumber plants.

The application of potassium silicate to avocado trees to suppress Phytophthora root rot seems to be most effective when applied as a soil drench. Menzies *et al.* (1992) reported that foliar applications of potassium silicate at 17mM Si are as effective as a 1.7mM root application. This will however most probably not be as effective in avocado trees as data from nutrient analysis suggests that silicon is not actively and efficiently translocated in avocado tissue. The possibility of physical barrier formation in roots will therefore be

limited, and the expression of phenolic and other fungitoxic compounds confined to plant parts receiving silicon. Application of Si to Si-deficient soils also creates the possibility of reducing fertiliser rates to be applied in successive years after Si application, and reduced fungicide applications (Seebold *et al*., 2004).

Although the current study's focus was not on the effect of silicon on post harvest disease incidence of avocado, data were collected to determine possible benefits of silicon, over and above that of inhibiting Phytophthora root rot. Anthracnose severity during the 2004/2005 season was lower in fruit from trees treated with silicon. No significant differences were seen during the 2005/2006 season with regards to anthracnose incidence between treatments. Results obtained by Anderson *et al*. (2004) however confirmed that silicon injection may be a possible preventative measure to control anthracnose incidence and severity in avocado fruit if applied separately from phosphorous acid treatments. This was reiterated by Anderson *et al.* (2005) who stated that a silicon-phosphorous acid mixed application lead to no control of anthracnose as a result of a lower silicon solubility rate at a lower pH, deeming silicon to be unavailable to plants.

Although some level of inhibition of stem end rot was obtained in fruit from trees receiving silicon as a soil drench, results were not consistent, and fruit from silicon injected trees did not differ significantly from the control. This confirms results obtained by Anderson *et al.* (2005) who found that stem end rot incidence and severity thereof in fruit from silicon injected trees did not differ significantly from fruit harvested from uninjected trees. There was however a decrease in vascular browning in fruit harvested from trees receiving two and three silicon applications.

There seems to be a link between the application of silicon to avocado trees and a decrease in severity and incidence of the two most important post harvest diseases threatening the avocado industry. Although no significant differences were observed between treatments, copper concentrations in avocado leaves in some instances exceeded the permissible standard by a factor eleven. Some authors (Boshoff *et al*., 1996; Schoeman and Manicom, 2002) have reported on the beneficial effects of copper sprays on post harvest disease incidence, in particular *Colletotrichum gloeosporioides*. This, however, leads to a build up of copper in not only soils, but also in avocado tissue, possible leading to toxic levels in plants. Future research should focus on the possible optimisation of silicon application to inhibit post harvest disease development in fruit. Silicon may be a valuable alternative to copper application, especially in the light of

copper build-up in soils, and residues on fruit being unfavourable for the European export market (Duvenhage, 2002).

The application of potassium silicate to trees as a soil drench led to higher yields compared to the control treatment. It is possible that improved tree condition due to a lower root rot severity led to lower flower/fruit drop resulting in higher yields compared to the control treatment. This was also true for the number of fruit per tree. Results from both total yield per tree and the number of fruit per tree indicate that Si x 3 is effective in, if not increasing yield and fruit number, sustaining tree health to a productive level. It is imperative to determine whether the timing at which the third application was employed with regards to the tree phenological model, is the determining factor in increasing yields and number of fruit per tree.

Silicon applications to the soil also appeared to affect nutrient concentrations in avocado trees.

Three silicon applications resulted in higher boron concentrations in leaves compared to all other treatments and it thus appears that silicon application increases the boron uptake of avocado plants. Whiley *et al*. (1996) reported that boron application may increase fruit set and quality. If this does indeed occur, it may result in additional benefits of silicon application to the avocado plant.

It does not appear as if three silicon applications per season to avocado trees as a soil drench increase the silicon translocation to avocado leaves, and contrary to the expected outcome, silicon concentrations were the lowest in avocado leaves from plants receiving three silicon applications during 2005, and only marginally higher during 2006. However, significantly higher levels of silicon were obtained in avocado roots from trees receiving three silicon applications. This indicates that avocado roots absorb silicon, but this silicon is not effectively translocated in the plant to leaf tissue. High levels of silicon were also obtained in potassium phosphonate (Avoguard®) treated root tissues. It would be interesting to note whether phosphorous acid treatment of avocado trees increase the plants uptake of silicon. The mode of action of potassium phosphonate (Avoguard®) may not only be a direct, fungitoxic and indirect, enzyme releasing function, but may also alter plant nutrient composition, implementing the numerous functions ascribed to silicon.

In addition to suppression of disease, potassium silicate application to avocado trees as a soil drench also leads to an increase in soil pH. This is an especially important additional benefit of silicon application as it is known that most avocado producing areas of South

Africa have low pH values partly due to high rainfall and low CEC (cation exchange capacity) of the soil in which avocado is cultivated.

The accumulation of phenols and phenolic polymers in *Persea americana* roots exposed to *P. cinnamomi*, and treated with water soluble potassium silicate, was investigated. Similar or higher total phenolic concentrations were obtained in avocado roots receiving three silicate applications compared to roots from potassium phosphonate (Avoguard®) treated trees. This was also true for glucoside bound phenolic concentrations in the Si x 3 treatment compared to the potassium phosphonate (Avoguard®) treatment. Results indicate that potassium silicate application leads to lower cell wall bound phenolics. It is possible that silicon replaces phenol-binding molecules, or is bound in the place of phenolics, resulting in lower cell wall bound phenols. Results indicated that silicon accumulation was subsequent to phenol appearance in infected tissue, challenging the physical barrier-hypothesis that silicon accumulation in plant cell walls in close contact with the pathogen confers resistance to fungal penetration by physical means.

Future research is paramount to effectively determine the role silicon application does play in disease suppression. Results from the *in vitro* study opens up a wide scope for research into the effect of application of silicon to suppress post harvest disease development. Biggs *et al.* (1997) reported a 65% *in vitro* fungal inhibition of the brown rot pathogen *Monilinia fructicola* (G. Wint) Honey of peach fruit, and attained similar growth inhibition of fungal colonies on fruit dipped in a calcium silicate solution. Although infection of avocado fruit with the anthracnose and stem end rot complexes occurs before fruit are harvested (Anderson *et al*., 2005), post-harvest dips may inhibit lesion development.

Future research should be focused on the effect of silicon application on soil micro-fauna, especially those involved in biological control of *P. cinnamomi* including *Trichoderma* isolates (McLeod *et al.,* 1995), as applied silicon may affect the effectiveness of such a control method. It is also imperative to determine whether silicon application only inhibits mycelial growth, or if it has an effect on the sexual reproduction of the fungus.

Molecular work on gene activation due to silicon application is to be undertaken. The activation of a gene regulation system by Si has long been proposed by Wingate *et al*. (1988). The presence or absence of these genes does however not determine resistance or susceptibility, but the magnitude and speed with which the gene information is expressed is important (Chérif *et al.,* 1992). The effect of silicon on the speed and magnitude of expression are to be considered.

Although fungitoxic metabolites such as borbonol (Zaki *et al.,* 1980; Wehner and Apostolides, 1981) and diene (Chang *et al*., 1975; Prusky *et al*., 1982) have been reported in avocado tissue, their presence and heightened expression have not been linked to Phytophthora root rot. The possibility of heightened expression of these and other toxic compounds due to silicon application needs to be examined.

Phytophthora cinnamomi inoculum is present in the majority of avocado producing soils throughout the world. The control of root rot is therefore imperative for the continual and economically viable production of an avocado crop. The application of silicon to avocado trees to inhibit root rot results in an effective decrease in disease severity and aid in the maintenance of a healthy canopy condition under diseased circumstances.

The results of the current study support the hypothesis that silicon application, through an elevation of the total phenolic levels, causes an increase of resistance against *P. cinnamomi* root rot in avocados.

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APPENDIX A

Greenhouse environmental data

Experiment 1

Trees were grown in a controlled environment greenhouse under diurnal temperature fluctuations of 7º to 32ºC and relative humidity between 30 and 90%.

Experiment 2 & 3

Table A 1: Environmental data presented as monthly averages collected at the height of the pot rim from the glasshouse for experiments 2 & 3.

	Av. Temp.	**Max temp**	**Min temp**	**RH**	**Dew point**	**Light intensity**
April	20.06	36.94	11.31	9.70	9.01	152.37
May	20.58	28.19	12.15	8.44	6.21	80.60
June	22.01	25.04	21.78	12.58	5.30	33.63
July	26.93	21.69	30.50	16.59	5.63	74.92
August	34.67	25.51	26.42	14.31	5.52	148.64
September	34.67	25.51	26.42	14.52	7.82	174.56
October	33.39	25.92	25.56	15.43	23.79	281.78
November	25.14	30.57	19.54	11.80	4.06	25.08

Experiment 4

Table A 2: Environmental data presented as monthly averages collected at the height of the pot rim the glasshouse for experiments 4.

	Av. Temp	**Max temp**	**Min temp**	**RH**	**Dew point**	**Light intensity**
April	21.60	26.83	18.28	9.49	8.86	30.60
May	20.45	23.35	16.71	12.26	7.79	29.28
June	21.08	26.34	14.56	9.69	6.12	47.08
July	26.93	28.72	16.44	9.56	5.63	74.92
August	34.67	30.55	16.34	9.27	5.52	148.64
September	34.67	30.55	16.34	9.27	5.52	148.65
October	33.29	30.00	17.10	9.60	5.46	144.92
November	23.73	29.30	18.24	10.36	5.58	150.86

APPENDIX B

D	E	A	B	A	C	D	B	C	E
C	B	D	E	B	E	A	C	D	A
B	D	C	D	A	E	A	B	E	C
B	E	B	D	D	C	A	C	E	A
B	D	B	A	C	A	E	D	E	C

A Potassium phosphonate (Avoguard®)

B Si x 1

C Si x 2

D Si x 3

E Control

Figure B 1: The randomized block resign of avocado trees treated with potassium silicate as a soil drench to inhibit *Phytophthora cinnamomi* disease severity. Treatments consisted of either one (Si x 1), two (Si x 2) or tree (Si x 3) potassium silicate soil drench applications; trees injected with potassium phosphonate (Avoguard®) (PA) and trees receiving no treatment (control)

C	B	D	A	A
A	D	B	C	B
D	A	C	D	C
B	C	A	B	D

A Potassium phosphonate (Avoguard®)

B KOH

C 0.74ml Si

D 20ml Si

Figure B 2: The randomized block resign of avocado trees treated with potassium silicate as a stem injection to inhibit *Phytophthora cinnamomi* disease severity.
Treatments consisted of biannual injections of either $0.74ml.l^{-1}$ or $20ml.l^{-1}$ potassium silicate solutions (20.7% silicon dioxide); a KOH solution at pH 10.35 or potassium phosphonate (Avoguard®) (PA).

APPENDIX C

Date	Product	Active ingredient		Dosage
17/02/2003	Demildex	copper oxychloride	0.3 kg	/100 L Hi
25/08/2003	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
7/11/2003	Avoguard 500SL	Phosphorous acid	0.05 ml	/ha
12/11/2003	Demildex	copper oxychloride	0.3 kg	/100 L Hi
2/02/2004	Demildex	copper oxychloride	0.3 kg	/100 L Hi
9/02/2004	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
4/08/2004	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
9/11/2004	Demildex	copper oxychloride	0.3 kg	/100 L Hi
29/11/2004	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
21/12/2004	Demildex	copper oxychloride	0.3 kg	/100 L Hi
21/01/2005	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant
16/11/2005	Avoguard 500SL	Phosphorous acid	0.08 ml	/plant
21/01/2006	Demildex	copper oxychloride	0.3 kg	/100 L Hi
16/02/2006	Avoguard 500SL	Phosphorous acid	0.05 ml	/plant

Table C 1: Standard spraying program implemented in the orchards used the a field trial to determine the efficacy of potassium silicate as either a sol drench or stem injection to inhibit *Phytophthora cinnamomi* disease severity.

APPENDIX D

Figure D 1: Ciba Geigy avocado tree rating scale from 0 to 10 where 0 = healthy looking tree and 10 = dead tree.

EFFICACY OF WATER SOLUBLE SILICON FOR CONTROL OF *PHYTOPHTHORA CINNAMOMI* ROOT ROT OF AVOCADO

by

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RESUMÉ

In the current study potassium silicate (20.7% SiO_2) induced a 100% inhibition of *P. cinnamomi* mycelial growth at all concentrations tested. Total inhibition for all fungi tested (*Alternaria solani, Colletotrichum gloeosporioides, Curvularia lunata, Drechslera* sp., *Fusarium oxysporum, Fusarium solani, Glomerella cingulata, Lasiodiplodia theobromae, Mucor pusillus, Natrassia* sp., *Pestalotiopsis maculans, Phomopsis perniciosa, Phytophthora capsicii, Phytophthora cinnamomi, Pythium* F-group, *Sclerotinia sclerotiorum, Sclerotium rolfsii, Stemphylium herbarum* and *Verticillium tricorpus*) was attained at a concentration of 40ml.l^{-1} and higher. Although the high pH of potassium silicate solutions does contribute to the inhibition of fungal growth, the inhibitory effect of potassium silicate on fungal growth *in vitro* is mostly fungicidal rather than attributed to a pH effect. Phytophthora root rot of avocado nursery trees can be inhibited successfully by potassium silicate application. The effectiveness of potassium silicate application depends however on the repetition of applications. These findings are of paramount importance as this implies that potassium silicate may be a alternative control measure to inhibit the effects of *P. cinnamomi* on avocado nursery

trees. Silicon either stimulates plant growth or imparts some form of protection to avocado roots if applied prior to *P. cinnamomi* inoculation.

Potassium silicate applied as a soil drench resulted in higher root densities compared to that of potassium phosphonate (Avoguard®) injections and untreated control trees. Reapplication again resulted in the best disease suppression and stimulation of new root growth. These results correlated well with tree canopy ratings, as trees that received silicon frequently, showed better canopy conditions compared to the untreated control treatments. Potassium silicate application leads to effective inhibition of *Phytophthora cinnamomi* infection in avocado orchards.

Potassium silicate application resulted in an increase of crude phenols and phenolic polymers in avocado roots cells to similar levels to that obtained in roots from potassium phosphonate (Avoguard®) treated trees. Potassium silicate application leads to lower cell wall bound phenolics.

The results of the current study support the hypothesis that silicon application, through an elevation of the total phenolic levels, causes an increase of resistance against *P. cinnamomi* root rot in avocados.

EFFEKTIWITEIT VAN WATER OPLOSBARE SILIKON VIR *PHYTOPHTHORA CINNAMOMI* WORTEL VROT BEHEER IN AVOKADO

deur

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SAMEVATTING

Kaliumsilikaat (20.7% SiO_2) induseer ‘n 100% inhibisie van *P. cinnamomi* groei by alle getoetste konsentrasies. Totale inhibisie van alle swamme getoets (*Alternaria solani, Colletotrichum gloeosporioides, Curvularia lunata, Drechslera* sp., *Fusarium oxysporum, Fusarium solani, Glomerella cingulata, Lasiodiplodia theobromae, Mucor pusillus, Natrassia* sp., *Pestalotiopsis maculans, Phomopsis perniciosa, Phytophthora capsicii, Phytophthora cinnamomi, Pythium* F-groep, *Sclerotinia sclerotiorum, Sclerotium rolfsii, Stemphylium herbarum* en *Verticillium tricorpus*) was verkry by ‘n konsentrasie van 40ml.l^{-1} en hoër. Alhoewel die hoë pH van kaliumsilikaat wel ‘n inhiberende uitwerking het op swamgroei, is die suksesvolle inhibisie van swamgroei grootliks toe te skryf aan die swamwerende effek van kaliumsilikaat op swamgroei *in vitro* eerder as ‘n pH effek.

Phytophthora wortelvrot van avokado kwekelinge kan suksesvol onderdruk word met kaliumsilikaat toediening. Die effektiwiteit van die toediening hang wel af van die hertoediening daarvan. Hierdie bevindinge is van kardinale belang aangesien dit impliseer dat kalium silikaat ‘n alternatiewe beheer middel is om *Phytophthora*

wortelvrot te inhibeer in avokado kwekelinge. Of silikon stimuleer plant groei, of dit induseer ‘n vorm van beskerming in avokado wortels voor infeksie plaasvind.

Kaliumsilikaat toediening as ‘n grond-benatter lei tot hoër worteldigthede in vergelyking met kaliumfosfaat (Avoguard®) staminspuitings en onbehandelde kontrole bome. Hertoediening lewer die beste resultate, maar drie toedienings per seisoen is voldoende. Worteldigdheid verhoging na silikaat toedienings korreleer goed met blaredak gesondheid, aangesien bome wat gereeld behandel is met silikon beter blaredekking getoon het in vergelyking met die kontrole bome. Kaliumsilikaat toediening lei to effektiewe inhibisie van *Phytophthora cinnamomi* infeksie in avokado boorde.

Kaliumsilikaat toediening lei tot ‘n toename in totale fenole en fenoliese polimere in avokado wortel selle tot soortgelyke vlakke soos gevind in avokado weefsel vanaf kaliumfosfaat behandelde bome. terselfdertyd lei silikaat toediening tot laer selwand gebinde fenole.

Hierdie resultate ondersteun die hipotese dat kaliumsilikaat toediening, deur die verhoging van oplosbare fenole in avokado wortelselle, die plant se weerstand verhoog, en die effek wat *Phytophthora* wortelvrot het op avokado plante inhibeer.