

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 unsubsized 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 CO₂ 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 mycorrhizal 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, unsubsized 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 from 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)

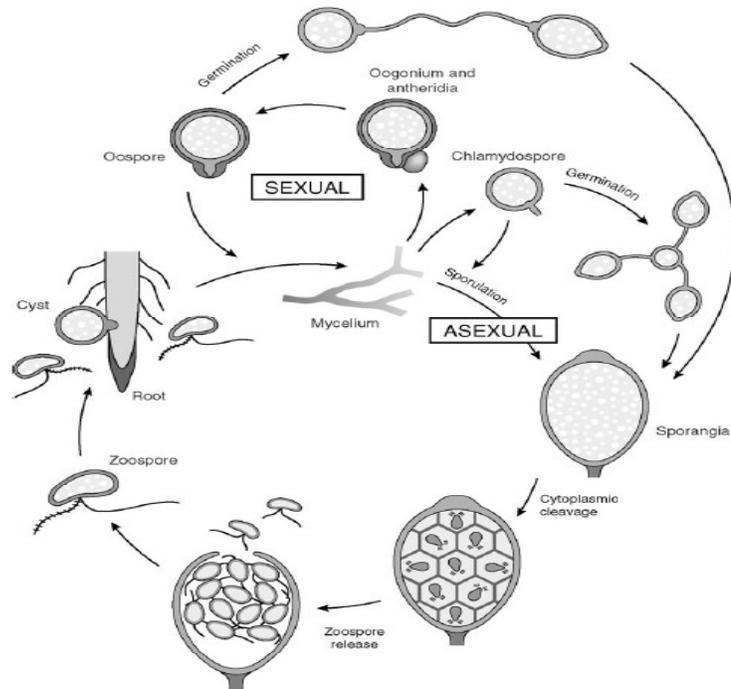


Figure 1.1: Generalized life cycle of *Phytophthora cinnamomi* (Anon, 2004)

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 Chlamyospore Production

Chlamyospores 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, chlamyospores can form readily on nutrient enriched media (Ribeiro, 1983; Pegg *et al.*, 2002). Conflicting reports on chlamyospore formation in liquid media and the type of experiments that have been conducted, make it difficult to evaluate whether chlamyospore formation is due to the aeration effect, water potential, or both (Ribeiro, 1983). Data concerning the effect of pH on chlamyospore development is lacking (Ribeiro, 1983).

1.2.4.4 Chlamyospore Germination

Chlamyospores 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 $0.004\text{mm}\cdot\text{sec}^{-1}$ 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 severe 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 CO₂, 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.

HNO₂ and NH₃ 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 NO₃⁻ 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 CaSO₄ 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 CaCO₃ 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, H₃PO₃), 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 H₃PO₃ and found that isolates of *P. cinnamomi* obtained from trees treated with fosetyl-Al or H₃PO₃ was less affected by fosetyl-Al and H₃PO₃ *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 (Bögge *et al.*, 1997). Microorganism recognition by plant cells depends on the generation of elicitor molecules (including jasmonic acid (JA), salicylic acid (SA), ethylene, ABA, H₂O₂ 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).

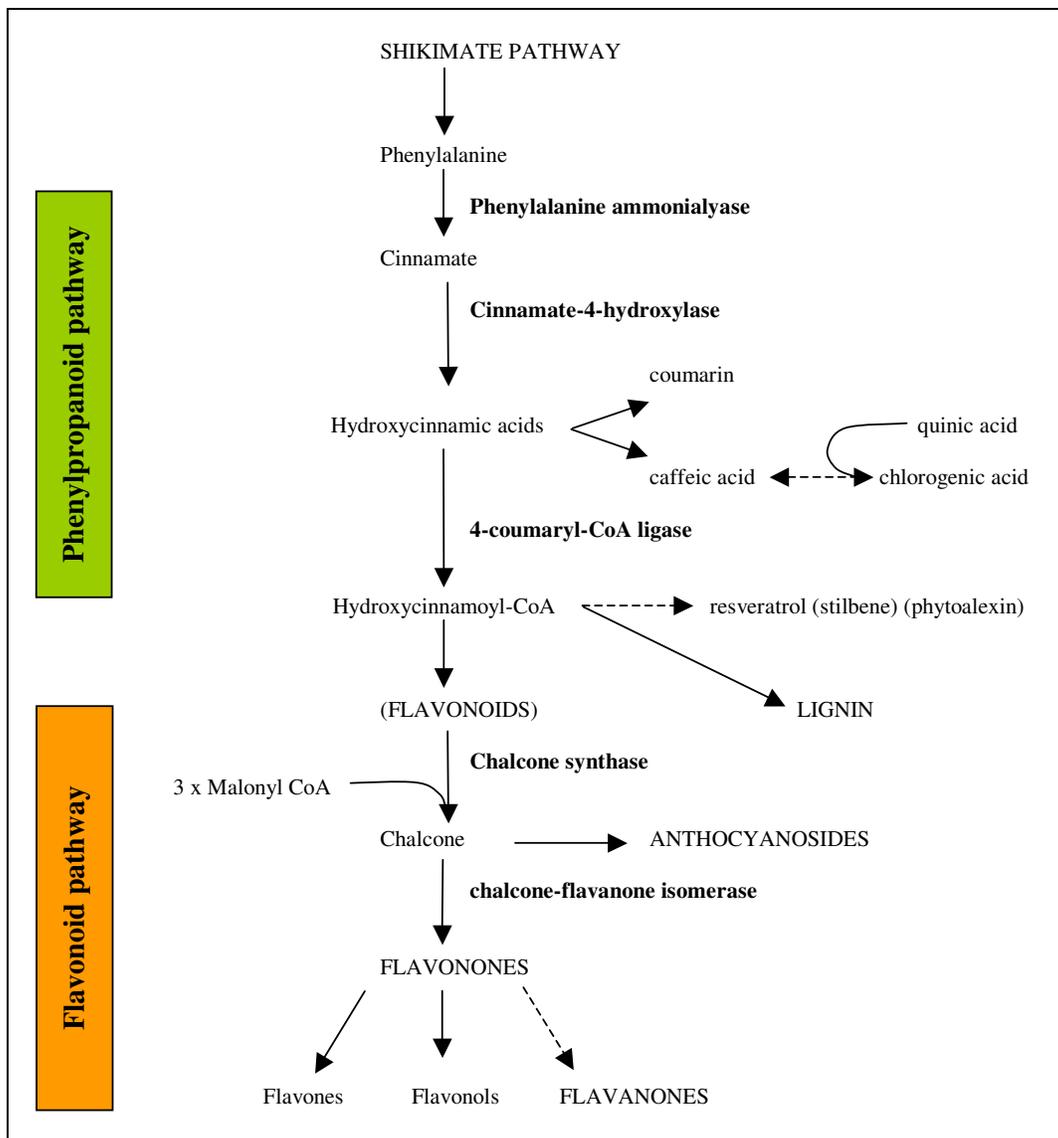


Figure 1.2: Overview of the biochemical pathway along which flavonoids and anthocyanocides are formed. Only key intermediates and enzymes of interest are indicated (Du Plooy, 2006).

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 decompartmentation 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, quercetin, 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 Taubehaus 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 pectinacious barrier (Kolatucky, 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 ($4.5\mu\text{g}\cdot\text{g}^{-1}$), Pinkerton ($2.9\mu\text{g}\cdot\text{g}^{-1}$), Fuerte ($2.5\mu\text{g}\cdot\text{g}^{-1}$), Duke 7 ($0.9\mu\text{g}\cdot\text{g}^{-1}$) and Edranol ($0.4\mu\text{g}\cdot\text{g}^{-1}$).

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 ($\text{C}_6\text{-C}_1$) or cinnamic acid derivatives ($\text{C}_6\text{-C}_3$), 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% SiO₂ 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% SiO₂. 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% K₂O of citrate-soluble potassium, 25% SiO₂ 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 SiO₂, 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 SiO₂, 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 SiO₂ (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 SiO₂ (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 (Adata and Besford, 1986). This fertilizer should contain more than 15% of 0.5N HCl soluble SiO₂ 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 CaCO₃ 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 [(H₄SiO₄)_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) fractionated 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 CaCO₃ 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 (H₂S) 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 $\text{SiO}_2 \cdot n\text{H}_2\text{O}$. These polymerized silicic acids in the cells bind with cellulose, forming a strong bonded silico-cellulose 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* Schltld. 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	<i>Magnaporthe grisea</i> (T.T. Hebert) Barr	Brecht <i>et al.</i> (2004)
Tomato	Bacterial wilt	<i>Ralstonia solanacearum</i> (Smith)	Dannon and Wydra (2004)
Rice	Brown spot	<i>Pyricularia oryzae</i> Cavara	Seebold <i>et al.</i> (2004)
Rice	Neck rot	<i>Bipolaris oryzae</i> (Breda de Haan) Shoemaker	Seebold <i>et al.</i> (2004)
Cucumber	Powdery mildew	<i>Sphaerotheca fuliginea</i> (Schlttl. Fr.) Pollacci	Adatia and Besford (1986)
Peach	Brown rot	<i>Monilinia fructicola</i> (G. Wint) Honey	Biggs <i>et al.</i> (1997)
Avocado	Anthracoise	<i>Colletotrichum gleosporioides</i> (Penz.) Penz. & Sacc.	Anderson <i>et al.</i> (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 SiO₂. 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 *Curvularia*, *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 attributed 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 (Adata 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 SiO₂. 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 H₃PO₃ 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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