EFFECT OF SYSTEMIC RESISTANCE INDUCERS APPLIED PRE- AND POSTHARVEST FOR THE DEVELOPMENT OF A POTENTIAL CONTROL OF *COLLETOTRICHUM GLOEOSPORIOIDES* ON *PERSEA AMERICANA* (MILL.) CV 'FUERTE'

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DECLARATION

I, Ronelle Joy Bosse, declare that the research reported in this thesis, except where otherwise indicated, is my original work. This thesis has not been submitted for any degree or examination at any other university.

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We certify that the above statement is correct.

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To my Lord God, for His blessing and guidance throughout my life.

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THESIS ABSTRACT

Avocados are one of the major food sources in tropical and subtropical regions and are an important horticultural crop in South Africa. Avocados are exported over long distances and may have storage times of up to 30 or more days at temperatures of about 5.5^{o}C. This procedure increases the risk of poor fruit quality, including physiological disorders, early softening and postharvest disease incidence. A major component of the postharvest diseases is Anthracnose caused by *Colletotrichum gloeosporioides*. Anthracnose infects unripe fruit and once infected, the fungus remains dormant in the fruit until ripening begins. This leads to a problem for producers and packers, as the presence of the disease cannot be detected on the pack line, and fruit is not removed. Anthracnose control is normally done through pre-harvest treatment with copper-based fungicides. While effective such treatment needs to be repeated frequently, resulting in copper residues on the avocados.

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The study was conducted to investigate the effects of phosphoric acid and potassium silicate on known antifungal compounds and critical enzymes of the pathways elemental for systemic resistance inducers, so as to evaluate the potential for using them as alternatives to or in conjunction with, copper fungicides in the control of Anthracnose in avocado fruit. The study included storage temperature and time variations, to take account of the logistics in shipping avocado fruit to distant markets.

Pre- and postharvest applications of phosphoric acid and potassium silicate were used, and after harvest, fruit were either ripened at room temperature (22^{o}C) without storage or stored for 28 days at temperatures of 5.5^{o}C or 2^{o}C before analysis.

Concentrations of phenolics, activity of the enzyme phenylalanine ammonia lyase (PAL) and a known antifungal diene were determined in the fruit exocarp. Pre-harvest treatments of phosphoric acid showed that the highest phenolic concentration was found in fruit harvested 14 days after application for fruit stored at room temperature. For fruit stored at 5.5°C it was seen that as fruit softened, phenolic concentrations increased compared with hard fruit immediately after storage, with the highest increase noted for fruit harvested 7 days after application. When comparing the three storage temperatures, phenolic concentrations were enhanced most when fruit was stored at 2°C. Postharvest treatments showed a significant

increase in phenolic concentrations for potassium silicate treated fruit stored at room temperature and 2°C when determined immediately after storage. Fruit stored at 5.5°C showed an increase in phenolic concentrations as it became softer.

When considering PAL enzyme activity, it was found that postharvest treatments of both potassium silicate and phosphoric acid influenced enzyme activity, with potassium silicate having greater effects. Similarly, an increase in PAL activity was noted in the pre-harvest phosphoric acid treatment harvested 14 days after application for fruit ripened immediately as well as fruit stored at 5.5°C. Fruit stored at 2°C showed the highest PAL activity for fruit harvested 7 days after application.

No results were obtained in the analysis of antifungal compounds for both pre- and postharvest treatments. However, it is suggested that the antifungal diene could follow similar trends to those found for phenolics.

It is concluded that applications of both phosphoric acid and potassium silicate do create changes in phenolic concentrations and the activity of the enzyme PAL which is involved in the synthesis of phenolic compounds known to possess antifungal properties. It is therefore possible that phosphoric acid and potassium silicate may be used as part of an integrated programme for Anthracnose control, and should be tested as potential alternatives for high volume copper-based fungicides.

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HYPOTHESIS

The main hypothesis of this study was:

The addition of postharvest dips of potassium silicate and phosphoric acid and pre-harvest sprays of phosphoric acid to 'Fuerte' avocado fruit will enhance the phenolic concentration, the activity of phenylalanine ammonia lyase (PAL) and the production of antifungal compounds, thereby demonstrating the possibility of their use in a new approach to Anthracnose disease control.

AIM

1. To determine the effects of phosphoric acid and potassium silicate postharvest dips and phosphoric acid pre-harvest application on PAL enzyme activity and antifungal phenolic and diene concentrations in 'Fuerte' avocados
2. To determine whether such treatments can induce systemic resistance in fruit and potentially decrease fruit susceptibility to Anthracnose.

OBJECTIVES

The objectives of the study were to:

1. Analyse avocado exocarp for critical compounds related to the quiescent nature of Anthracnose and PAL enzyme activity
2. To investigate possible alterations in relation to the systemically induced resistance to Anthracnose by postharvest dips and pre-harvest sprays.

DEDICATION

This thesis is dedicated to my parents, Vic and Colleen Bosse; without the two of them; this would never have been possible. Thank you!

CHAPTER 1

Literature Review

1. Introduction

Avocado production in South Africa is mainly concentrated in the warm subtropical areas of the Limpopo and Mpumalanga provinces in the North East of the country, with some extension into the cooler, but wetter KwaZulu-Natal Midlands. Of the avocado trees grown in the country, approximately 70% are 'Hass', while the remaining 30% is comprised mainly of 'Fuerte', 'Ryan' and 'Pinkerton' (Donkin, 2007).

The major growing areas of avocado are tropical and subtropical with moist summers. These warm summers result in high disease incidence and fungal infection (Whiley *et al*., 2002). Among the diseases that infect avocados, Anthracnose (*Colletotrichum gloeosporioides*) (Penz. and Sacc) is one of the most important postharvest diseases potentially causing significant losses to the export industry. Pre-and postharvest disease control is essential, without which high losses may occur (Kotzé, 1978).

The world avocado trade is centred on two dynamic markets that are both strong importers. These are the European Union and the United States. Mexico is the largest producer and supplies over one third of the world's total avocado production and over 40%of the world's exports. Chile used to be the largest supplier of avocados to the U.S. import market, but as from 2005, Mexico became the largest as they gained access to 47 states for 'Hass' avocados (De Villiers, 2001). Avocado production areas include Mexico, Chile, Argentina, Peru, California and Florida in the United States, the Dominican Republic, Spain, Israel, South Africa, Australia, and New Zealand (Anonymous, 2006).

South Africa exports avocados over a long distance to the European Union resulting in storage times of up to 30 or more days at temperatures from 7.5°C to 3.5°C as the season progresses (Vorster *et al*., 1990). Due to increased environmental awareness on the use of certain chemicals, new trends towards organic and economically friendly agricultural commodities have been researched in order to preserve postharvest quality of fruit. In addition

to this, cold, heat, irradiation and controlled atmosphere (CA) treatments have been investigated (Wills *et al*., 2007). Low temperature storage is often used in conjunction with CA or 1-Methylcyclopropene (1-MCP). Despite this, fruit still appear on the European market with signs of softening and physiological disorders. The use of 1-MCP or CA is a costly management method in order to maintain high quality fruit.

Anthracnose (*Colletotrichum gloeosporioides*) infects unripe fruit and once infected, the fungus remains dormant until ripening begins (Crane, 2001). This becomes a problem once the fruit has been shipped for a long period, as the fruit may appear healthy at shipping, but on arrival and once ripening has begun, the fruit begins to decay. Anthracnose is usually found in conjunction with other fungi, therefore worsening disease severity (De Villiers, 2001).

Due to the significance of the disease its control, commonly through various copper fungicides (McMillan, 1970), is vital. This, however, can be expensive and difficult due to the latency of the disease.

Copper fungicides have proved to give reasonable control of the disease when applied as pre-harvest sprays of copper oxychloride as well as other formulations (Manicom and Schoeman, 2008). Since copper fungicides are contact fungicides, they need to be used frequently in order to achieve adequate coverage and maintain disease control. This is especially important during the stages of early fruit growth when the surface area is expanding rapidly. This, therefore, results in an increased cost of control. Due to the protective nature of the fungicide, any rainfall can leave the product less effective and therefore not provide adequate control of the disease (Hewitt, 1998). Copper fungicides are applied to leaves and fruit, but do not penetrate into them. As a result, if applied too late, the disease may already have penetrated the fruit and cannot be controlled further with such fungicides.

Although copper fungicides can control Anthracnose on avocados to a large extent, there are various disadvantages. Application of copper fungicides may leave visible residues on harvested fruit. Regular copper reapplication throughout the season in order to control disease outbreak efficiently, results in high labour costs and, therefore the need to investigate alternative disease control measures (Korsten *et al*., 1991).

Further, growing environmental awareness necessitates lowering the dosage of copper fungicides (copper is a heavy metal) to reduce occurrence of residues, not only in the field but also in the packhouse (Manicom and Schoeman, 2008).In order for crops to remain residue-free, to save costs and to prevent resistance to the applied fungicides, products should not be used in isolation but rather in combination, or applied as alternate sprays with other fungicides that have a different mechanism of action. This decreases the chance of pathogen resistance to a certain fungicide while it increases the level of disease control (Lyr, 1995).

Other alternatives to copper fungicides for the control of Anthracnose include the use of Strobilurin fungicides. These fungicides are effective against several different plant pathogenic fungi. Azoxystrobin was the first fungicide in this group registered by EPA (Environmental Protection Agency). Strobilurins have translaminar activity and can therefore move into treated leaves or fruit and, as a result, provide control on upper and lower leaf surfaces, as well asbelowthe fruit epidermis (Hewitt, 1998). Strobilurins are considered environmentally friendly as they are active at low concentrations, have low toxicity to mammals and bees, and are non-persistent in the environment, breaking down readily in soil. They are broad-spectrum fungicides; moreover, they possess slow acting systemic properties that are able to provide long-term disease control (Hewitt, 1998). However, this group of fungicides are expensive, substantially increasing the cost of disease control.

A further group of fungicides that is able to control Anthracnose are triazoles. These fungicides inhibit C_{14}-demethylase, an important enzyme that plays a role in fungal sterol production. Sterols are an integral part of the structure and function of membranes and therefore are essential for the development of functional fungal membranes. The application of triazoles results in abnormal fungal growth and, eventually, fungal death (Hewitt, 1998). Triazoles do not, however, have any effect on spore germination, as spores contain sufficient sterols for the formation of germ tubes. Some spores even have sufficient sterols to produce infection structures; as a result triazoles may not always be effective against infection (Hewitt, 1998).

Particularly in growing regions in close proximity to residential areas and water supplies, increased restrictions are placed on fungicide applications due to environmental and food safety concerns. Although the avocado industry has not been greatly affected by fungicide restrictions to date, it must continue to investigate means to reduce fungicide usage in order to

meet future environmental requirements. Two areas of main concern are: 1) the build-up of copper levels in soil as a result of frequent application of copper-based fungicides during the fruit development phase, and 2) the postharvest application of prochloraz due to increased fungal resistance (Hofman and Ledger, 2001).

The development of new techniques for the control of avocado fruit diseases must take into account the changing face of the industry. With reduced returns becoming a reality, disease management strategies must have a favourable cost-benefit relationship (Hofman and Ledger, 2001).

2. Avocado

2.1. Avocado taxonomy and morphology

Avocados belong to the kingdom Plantae, family Lauraceae, genus *Persea* and the *americana* species (Bergh and Ellstrand, 1986). Avocados are further divided into three races, the Mexican race, *P. americana* var. *drymifolia* (Schlecht.& Cham.), the Guatemalan race, *P. americana* var. *guatemalensis* (L. Wms), and the West Indian race, *P. americana* var. *americana* (C.F. Gaertn) (Ploetz, 2003).

2.2. Avocado cultivars

There are many commercially available cultivars of avocados. Worldwide the most important are 'Hass', 'Fuerte', 'Edranol', 'Pinkerton' and 'Ryan', with 'Hass' being the world's most important commercial avocado cultivar (Whiley *et al*., 2002).

'Hass' fruit are dark green when harvested with a 'bumpy' exocarp, medium-sized, and when ripe, the skin becomes dark, purplish-black. The cultivar is resistant to Cercospora and susceptible to Anthracnose (De Villiers, 2001). 'Fuerte' has a pale-green skin producing medium to large fruit. When ripe, the skin of the fruit yields to gentle pressure (Whiley *et al*., 2002). These avocados are susceptible to Cercospora and Anthracnose (De Villiers, 2001). 'Edranol' avocados have a thick, green skin with small corky speckles and produce medium large fruit (Whiley *et al*., 2002). Similar to 'Fuerte', this cultivar is also susceptible to Cercospora and Anthracnose (De Villiers, 2001). 'Pinkerton' avocados have a medium thick green skin with slight pebbling and a creamy, pale-green flesh. The skin deepens in colour as it ripens (Whiley *et al*., 2002). 'Pinkerton' cultivars are resistant to Cercospora and susceptible to Anthracnose (De Villiers, 2001). 'Ryan' fruit have slightly rough, dark-green skin that remains green when ripe while fruit size of 'Ryan' is large (Whiley *et al*., 2002). 'Ryan' avocados are susceptible to Cercospora and have a slight tolerance to Anthracnose (De Villiers, 2001).

While all avocado cultivars are susceptible to Anthracnose, 'Fuerte' avocados, a particularly important export cultivar for South Africa, are very susceptible to this disease. While growers

are generally more concerned about Anthracnose on the export market, the disease is sometimes also severe on local fruit (Kotzé, 1978).

Kotzé (1978) inoculated ‘Fuerte’, ‘Edranol’, ‘Hass’ and ‘Ryan’ fruit with fresh conidia of Anthracnose through wounds. Results showed ‘Ryan’ to have an exceptional resistance to the spread of infection with wounds healing after inoculation. Towards the end of the avocado season ‘Ryan’ sometimes became infected, while ‘Fuerte’ and ‘Edranol’ were very susceptible to the disease. The infection was seen to spread faster in soft ‘Fuerte’ fruit than in soft fruit of the other cultivars. ‘Hass’ showed greater resistance than ‘Edranol’ and ‘Fuerte’ but when the fruit became soft infections also spread rapidly (Kotzé, 1978).

The green-skin cultivars ‘Fuerte’ and ‘Ryan’ have a thinner exocarp, making them more susceptible to Anthracnose and insect attack (Dirou, 2003). ‘Fuerte’ avocados are the most important green-skin commercial crop, and although becoming less important, remain an important crop in South Africa. ‘Hass’ avocados appear to be less prone to Anthracnose when harvested early, but when harvested later in the season, Anthracnose presence is much more visible due to the mature more state of the fruit at harvest.

2.3. Normal avocado ripening physiology

Fruit ripening was described by Biale (1975) as the process that results from changes in colour, texture and taste and, therefore, makes fruit acceptable for consumption. This process requires many catabolic and anabolic changes and once ripening has begun, it cannot be reversed (Bower and Cutting, 1988). The avocado does not ripen while it is still attached to the tree (Schroeder, 1953); it is thought that a ripening inhibitor exists which prevents on-tree ripening (Tingwa and Young, 1974). Avocados are climacteric fruit and therefore ethylene plays an important part in the ripening process. Blakey (2011) extensively reviewed the literature on avocado ripening behaviour.

Normal avocado ripening occurs only when a certain level of maturity has been reached (Hobson, 1979). Before this stage, only slight softening may occur, predominantly due to shrivelling as a result of water loss (Barmore, 1977); flavour of such fruit is generally poor (Ahmed and Barmore, 1980). Once horticultural maturity measured as fruit water, dry matter or oil percentage (Arpaia *et al*., 2001) has been reached, the rate of postharvest softening

becomes progressively shorter with increasing maturity (Zauberman and Shiffmann-Nadel 1972).

2.3.1. Ethylene

Since avocados are climacteric fruit, a rise in the respiration rate is noticed at the beginning of ripening which is followed by a decline (Figure 1). Ethylene plays a major role in ripening and, therefore, the initiation of ethylene formation is a key process in normal ripening (Bower and Cutting, 1988). Although other factors besides ethylene may be involved, the autocatalytic production of ethylene is of extreme importance to the normal ripening of avocados. Any factors affecting ethylene production could alter the fruit-ripening pattern (Bower and Cutting, 1988).

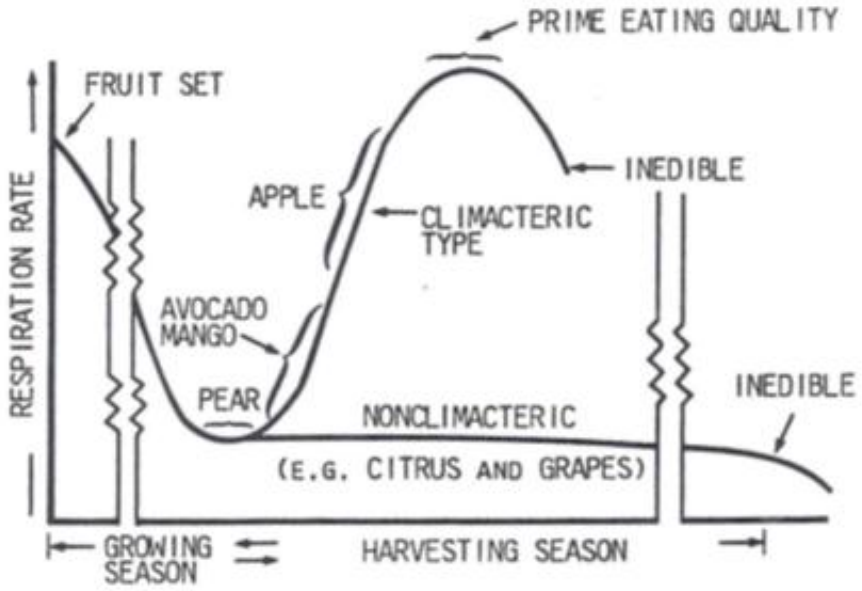

Figure 1.Climacteric and non-climacteric life cycles showing the life cycle of avocado fruit (Wardowski *et al*., 1986)

Ethylene plays an important role in regulating the ripening of avocado fruit. It is therefore important to inhibit ethylene biosynthesis or its action in order to slow the ripening process and extend the postharvest storage life (Jeong, 2002). 1-Methylcyclopropene (1-MCP) is a synthetic cyclopropene that blocks ethylene receptors and prevents the effects of ethylene in plant tissues for extended periods (Sisler *et al*., 1996). It does this by competing with ethylene for the binding site on the ethylene receptor and therefore controls ethylene responses (Sisler

and Wood, 1988). It binds in an apparently irreversible manner to the ethylene receptor and after a certain period the tissue resumes sensitivity to ethylene (Sisler and Serek, 1997).

The first marked change leading to ethylene production is the increase in ACC (1-aminocyclopropane-1-carboxylic acid) synthase activity resulting in an increased ACC level (Figure 2). When still attached to the tree, fruit were found to have low ACC synthase levels (Blumenfield *et al*., 1986).

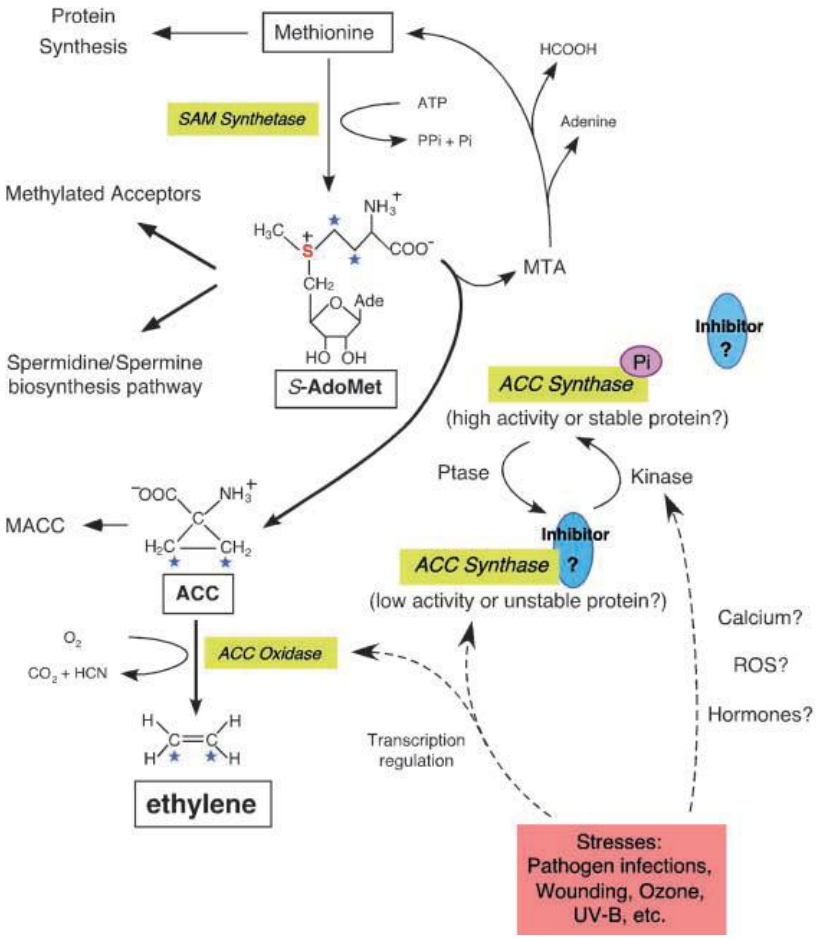

Figure 2. Biosynthetic pathway of ethylene production (Wang *et al*., 2002)

Certain environmental parameters may affect ethylene concentrations. Temperatures above 30°C have adverse effects on avocado ripening (Erickson and Takaake, 1964; Zauberman *et al*., 1977). As temperatures increase from 20°C to 25°C, the rise in ethylene production occurs earlier, while at temperatures higher than 25°C, the peak ethylene concentration decreases (Eaks, 1978).

Water loss also has a profound effect on avocado ripening. Fruit that lose more water after picking ripen faster and the ethylene peak occurs earlier (Adato and Gazit, 1974).

Calcium can affect fruit ripening. Tingwa and Young (1974) found that avocados with higher endogenous levels of calcium had slower ripening rates. The infiltration of calcium into avocados was seen to reduce the ethylene peak and the respiratory rise, resulting in a longer ripening period. Bangerth (1979) described the role of calcium as affecting enzyme activity, membranes, cell walls and interactions with plant growth regulators. All these factors are related to the ripening process.

2.3.2. Enzyme activity and cell wall structure

Alterations in the cell membrane, and in particular the plasma membrane, are important aspects of avocado fruit ripening. Ripening results in a loosening of the cell walls which is followed by cell wall degradation (Platt-Aloia and Thomson, 1981). With the removal of pectin from the matrix of the cell walls, the middle lamella disappears resulting in fruit softening (Bower and Cutting, 1988).

Ferguson (1984) concluded that the important effects of calcium are related to its role in membrane and cell wall structure and function. Calcium ions bind to pectins in the middle lamella of the cell wall and as a result cross-linkages are formed which maintain cell wall structure (Bangerth, 1979).

Calcium is also known to be particularly important in the maintenance of membrane stability and permeability control (Roux and Slocum, 1982; Ferguson, 1984) which could, alone, modify many cellular functions. It is important that high concentrations of calcium are maintained outside the cytosol, acting on the cell walls and plasma membrane. The longer this condition can be maintained, the slower both the ripening and senescence process will be (Ferguson, 1984).

The physiological role of calcium is thought to be of a second messenger (Poovaiah, 1985). When the levels of calcium reach a certain level within the cytosol, it combines with calmodulin. The active calcium-calmodulin complex that is then created combines with a receptor protein in order to create an active enzyme complex (Roux and Slocum, 1982).

Poovaiah (1985) showed that the calcium-calmodulin complex plays a vital role in the growth and development of plants, including the onset and rate of senescence.

Changes in enzyme activities are most probably the cause of changes in the cell wall structure. Cellulose is a major constituent of the cell wall and plays therefore an important role in avocado softening (Scott *et al*., 1963). Cellulase activity was found to increase rapidly with the accompaniment of softening, which was closely correlated with the respiratory climacteric and ethylene (Pesis *et al*., 1978). These authors concluded that ethylene plays a role in controlling cellulase activity; however, it was observed by Tucker and Laties (1984) that cellulase is not the only responsible factor in the solubilisation of cell walls during softening.

Polygalacturonase has also been reported to increase during ripening with a concurrent decrease in pectinesterase activity (Awad and Young, 1979). Polygalacturonase causes the depolymerization of pectin. During the pre-climacteric phase, but after the initiation of ripening, an unknown factor seems to cause pectinmethylesterase to bind to the cell wall (Jansen and Jang, 1960) while at the same time preparing the cell wall substrate for hydrolysis. This occurred in the presence of polygalacturonase. Cellulase appears to be responsible for the early stages of fruit ripening, which is controlled in part by ethylene while polygalaturonase is responsible for final fruit ripening (Bower and Cutting, 1988). It is believed that pectinesterase partially demethylates pectin which makes it suitable for depolymerisation by polygalacturonase (Seymour and Tucker, 1993).

2.3.3. Modification of the ripening process by temperature

Since avocados have a high respiration rate and limited shelf life after harvest, exporters need to decrease the rate in order to allow sufficient time for shipping (Aharoni, 1984). A number of methods are available to reduce the ripening rate of avocados.

Low temperature storage is the most commonly used method. The extent to which the avocado can be chilled depends on the cultivar (Ahmed and Barmore 1980), the temperature of storage, and the period of storage (Eaks, 1976). No evidence of ripening was seen during the storage for ‘Fuerte’ avocados when held at 5°C or lower (Eaks, 1976). Chilling injury occurred when fruit was held at 5°C for a period of longer than two weeks while no injury

was seen at 10°C. Swarts (1982), however, found that temperatures lower than 5°C could be used later in the season. Lütge (2011) found that 'Fuerte' avocados could be stored successfully at 2°C for 28 days without negatively impacting the internal or external quality of fruit and appeared to obtain better results than fruit stored at 5.5°C with 1-MCP.

It was suggested by Zauberman and Jobin-Décor (1995) that 'Hass' avocados could be stored at 2°C, while Kritzinger *et al*. (1998) showed 'Pinkerton' avocados had a higher incidence of mesocarp discolouration when stored at 2°C than at 5°C.

In New Zealand, it was reported that 'Hass' avocados could be stored at 2°C for a period of two weeks and still maintained good fruit quality (Dixon *et al*., 2004). Fruit stored at 0°C or 2°C developed chilling injury symptoms and had a high incidence of rots after being stored for two weeks (Hopkirk *et al*., 1994).

Early season avocado fruit have been found to be more sensitive to chilling injury than fruit picked later in the season (Kritzinger and Kruger, 1997). This is, however, not due to fruit maturity but instead a drop in the pre-harvest ambient orchard temperature to below 17°C (Swarts, 1980). It was therefore suggested that storage temperatures should be higher during the early parts of the season than the latter ones. Vorster *et al*. (1987), therefore, found that 'Fuerte' avocados could be stored at 3.5°C without any damage to the fruit.

Avocados are subtropical fruit and are therefore sensitive to low temperatures. They may, therefore, require mitigating treatments before being placed in cold storage to make the fruit more resistant to low temperatures and to ensure high quality of fruit (Blakey and Bower, 2007).

Woolf *et al.* (1996) found that using a heat treatment of 38°C for 60 minutes reduced chilling injury when 'Hass'fruit stored at 0.5°C and 2°C and suggested that a slightly higher temperature (39°C or 40°C) for 30 to 60 minutes could be as, if not more, effective. Fruit that was pre-heated and then stored at low temperatures were seen to ripen normally, tissue breakdown was significantly reduced; other disorders were slight, and fruit were of acceptable quality.

Another technique that may increase avocado tolerance to low or 'chilling' temperatures is low temperature conditioning. This technique is where cold-sensitive tissue is held at temperatures just above those at which chilling injury occurs in order to induce tolerance to these normally damaging low temperatures (Woolf and Lay-Yee, 1997).

Many studies have shown that low temperature conditioning of fruit and seedlings can reduce chilling injury symptoms (Wang, 1993). The important factors appear to be the temperature difference that occurs between conditioning and storage temperatures, and the duration of the conditioning treatment. Low temperature conditioning treatments that have been found to be effective in reducing chilling injury involve holding fruit, such as zucchini, for 2 days at 15 or 10°C before storage at 5 and 2.5°C, respectively (Buta and Wang, 1993).

2.3.4. Postharvest physiological disorders

Physiological disorders are common where storage for long periods at low temperatures is required. The major physiological disorders of avocados are pulpspot, mesocarp discoloration and vascular browning.

Pulpspot is a blackening of a region which surrounds cut vascular bundles and which is usually localized in nature (Swarts, 1982). This disorder has a much higher incidence early in the season. Mesocarp discolouration results is an overall discolouration of the flesh that is grey to brown in colour and is usually most intense in the distal half of the fruit. This appears to be more of a problem towards the end of the season. Mesocarp discolouration may be indicative of chilling injury, but it may also occur in fruits that have not been stored (Vakis, 1982). Vascular browning is the browning of the vascular strands that run longitudinally through the fruit tissue (Arpaia, 2004). All these disorders involve browning reactions and are a result of the oxidation of o-diphenols to o-quinones by the enzyme polyphenol oxidase (PPO) (Kahn, 1975). Phenolics are also involved in the process (Kahn, 1977).

Polyphenol oxidase or catechol oxidase, phenolase or o-diphenol oxygen oxidoreductase (EC 1.14.18.1) as it is also known, catalyses the oxidation reaction of o-diphenols to the corresponding quinone. The result of this, is that three quinones are irreversibly oxidized to melanin pigments which results in a darkening/browning.

Polyphenol oxidase occurs as a latent form in healthy green tissue and is found in the membranes of chloroplasts, leucoplasts, plastids, thylakoids and amyloplasts (Vaughn and Duke, 1984). In order for PPO to be activated, there needs to be some cellular damage present (Kahn, 1977). Membrane structure and functioning may therefore be a means of PPO activation (Vaughn and Duke, 1984). Polyphenol oxidase is found in at least two forms. The first being a bound or latent form (Kahn, 1977). In this form, the enzyme is attached to the green membranes such as the thylakoids. In the second form, PPO is found as a free or active form where it is immediately available for reaction in the presence of substrate and oxygen (Bower and Cutting, 1988).

Phenolic compounds are precursors for many plant compounds and are found in special tissues or the cell vacuole. Phenolic acids are oxidised atmospherically or ezymatically to quinones if the integrity of the cell membrane is disrupted or upset. The quinones are then polymerised which produces a brown colour (Torres *et al*., 1987). It was found by Cutting *et al*. (1992) that the total amount of phenolics increases as the avocado matures.

Chilling injury is another disorder that can occur. "Chilling injury is the permanent or irreversible physiological damage to plant tissue, cells or organs, which results from the exposure of plants to temperatures below some critical threshold for that species. A chilling temperature is any temperature below the critical threshold temperature (but above freezing) that causes injury" (Lyons and Breidenbach, 1987). Chilling injury symptoms include pitting and blackening of the exocarp, discolouration of the mesocarp and the failure of the fruit to ripen normally (Couey, 1982).

Numerous responses occur as a result of chilling temperatures. These include changes in the membrane structure, function, cessation of cytoplasmic streaming, changes in respiration rates and patterns, changes in ethylene synthesis as well as many biochemical and compositional changes (Morris, 1982).

Symptoms of chilling injury may not be visible when the fruit are in storage but may become visible when the fruit is returned and allowed to ripen at higher temperatures (Couey, 1982).

3. Postharvest diseases

Avocados are transported over long distances in order to get to their final destination. During this time, the fruit is prone to attack by several pathogenic fungi. The severity of attack may be increased by lack of fungicide application or by placing the fruit in incorrect storage temperatures. Since many products, currently used to prevent for the decay of the fruit, are environmentally hazardous as well as leave undesirable residues on the fruit, new techniques need to be implemented to control disease (Eckert and Brown, 1986).

In tropical and wet subtropical environments, the production of avocados is regarded as high risk due to pests and diseases in both the field and postharvest environments. This crop is subject to various diseases throughout the world. Anthracnose occurrence is particularly severe in warm areas with high rainfall (Whiley *et al*., 2002).

Avocados are mature but unripe at the time of harvest and ripening begins after the fruit has been removed from the tree. Unripe fruit are typically free of visible postharvest rots or other internal disorders, but when they are soft enough for consumption, the fruit can be severely affected by disease (Darvas *et al*., 1990). Most postharvest rots in avocado fruit result from latent infections on the fruit which begins on the tree during the growing season (Binyamini and Schiffmann-Nadel, 1972).

3.1. Anthracnose

At the beginning of the past century, Anthracnose disease was recognised as one of the most important diseases in avocados (Stevens, 1922). It is caused by *Colletotrichum gloeosporioides* which infects the fruits early in their development (Yakoby *et al*., 2001).

3.1.1. Host range and dissemination

Anthracnosecan cause large amounts of damage in a variety of crops such as cereals, coffee and legumes (Bailey and Jeger, 1992). An even greater economic loss is due to post harvest Anthracnose disease of tropical and subtropical fruit such as avocado, banana and mango (Jeffries *etal*., 1990). *Colletotrichum* species can also be found on decaying wild fruits (Tang *et al*., 2003).

The conidia are able to spread via rainsplash and can infect virtually all above-ground portions of the host, despite flowers and fruit being the most susceptible. As the fruit, twigs and leaves senesce, they are colonised and new conidia-bearing acervuli are produced (Fitzell, 1987).

3.1.2. Pathogen taxonomy and morphology

Anthracnose is caused by the fungus *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, anamorph of*Colletotrichum gloeosporioides*, belonging to Ascomycota, class Sordariomycetes, subclass Sordariomycetes, order Incertae sedis, family Glomerellaceae, genus *Glomerella* and species *G. cingulata* (Dyko and Mordue, 1979).

The fungus produces conidia on acervuli (Figure 3), which are saucer-shaped fruiting bodies (Fitzell, 1987). Short, simple and colourless conidiophores produce conidia in abundance. Long, brown setae may or may not be produced among conidiophores (Figure 4). These setae are approximately 4-8 x 200 µm, brown, slightly swollen at the base and tapered at the apex (Figure 5). Conidia form on hyaline to lightly brown conidiophores in acervuli which are irregular and roughly 500 µm in diameter. Conidia are colourless when viewed alone, but may also appear pink or salmon coloured. Conidia are approximately 7-20 x 2.5 µm and are hyaline and unicellular. The spores are short, ovoid to cylindrical, and single celled (Figure 6). Conidia may appear slightly curved in some species. *Glomerella cingulata* is the sexual stage of the fungus while *Colletotrichum gloeosporioides* is known asthe asexual stage (Brown, 2008).

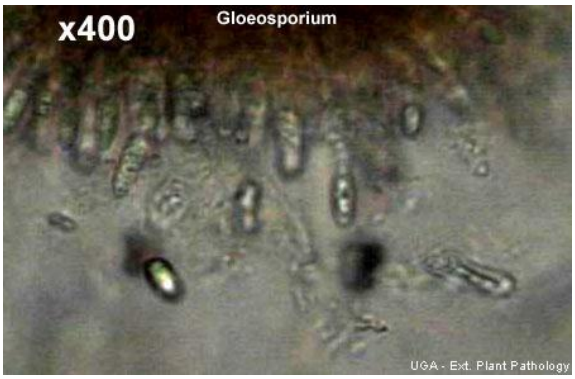

Figure 3. Conidia being discharged from the acervuli of *Colletotrichum gloeosporioides* (Brown, 2008)

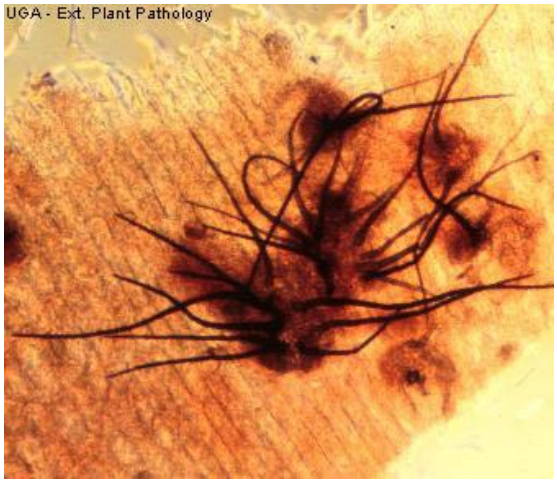

Figure 4. Long, black setae may or may not be produced among conidiophores of *Colletotrichum gloeosporioides* (Brown, 2008)

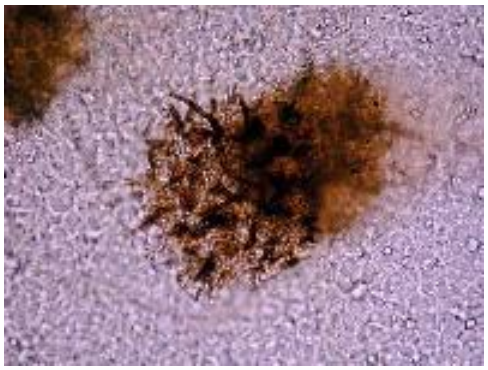

Figure 5. Characteristic setae of *Colletotrichum gloeosporioides* (Palmateer *et al*., 2006)

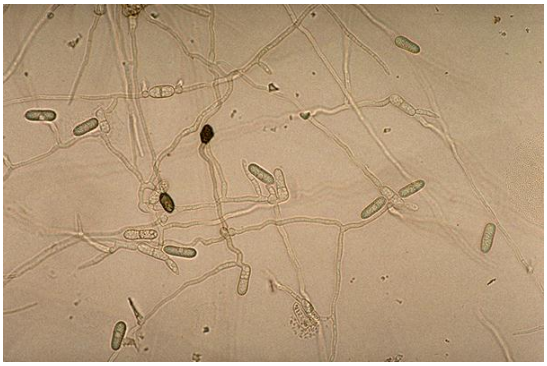

Figure 6. Germinating conidia of *Colletotrichum gloeosporioides* (Brown, 2008)

3.1.3. Environmental factors associated with the disease

Anthracnose is more serious in wetter growing areas and particularly in more susceptible avocado cultivars (Broadley, 1992). Infection and spread of the disease is encouraged by

extended periods of high moisture levels (80% relative humidity) and warm temperatures >18°C (28°C is optimal) (Shabi *et al*., 1994). Infection of the fruit seems to be highly dependent on the numbers of conidia with large numbers result in heavy infection while low numbers result in no damage (Coates, 1991). Anthracnose is usually not important in areas with low summer rainfall.

Disease development is also strongly influenced by ripening temperature. It was seen by Hopkirk *et al*.in1994 that both body and stem rots of "Hass" avocado increased as theripening temperature increased from 20 to 30°C compared with only low levels of disease at 15°C.

3.1.4. Symptoms

Symptoms include dark brown to black lesions (Figure 7) which start off as firm and end up being soft and putrid (Broadley, 1992) and only occur once the fruit begins to ripen. Leaves drop off prematurely and trees can even become defoliated due to severe disease incidence (Crane, 2001). Infection from leaves may pass through the petiole and cause brown to purple lesions on twigs and young branches. Dieback symptoms may occur from the disease entering the woody tissues of the twigs. Flowers that are infected with the disease turn reddish brown, turning dark brown in colour before abscising (Cook, 1975).

Figure 7. *Colletotrichum gloeosporioides* spots on the leaf of an avocado (Broadley, 1992)

Small, light brown spots may appear on the young fruit (Figure 8) and twigs may become infected from the peduncles of infected flowers. The spots on the fruit may enlarge and coalesce and may cover the entire surface (Stevens, 1922). The centres of the spots may also become sunken (Figure 9). The fungus is able to penetrate the fruit epidermal cells directly. The fungus usually penetrates deeply into the flesh in a hemispherical pattern (Figure 10) (Broadley, 1992).

Figure 8. Small, brown lesions on young, ripening avocado fruit infected with *Colletotrichum gloeosporioides* (Nelson, 2008)

Figure 9. *Colletotrichum gloeosporioides* lesions on avocado fruit that are large and sunken (Nelson, 2008)

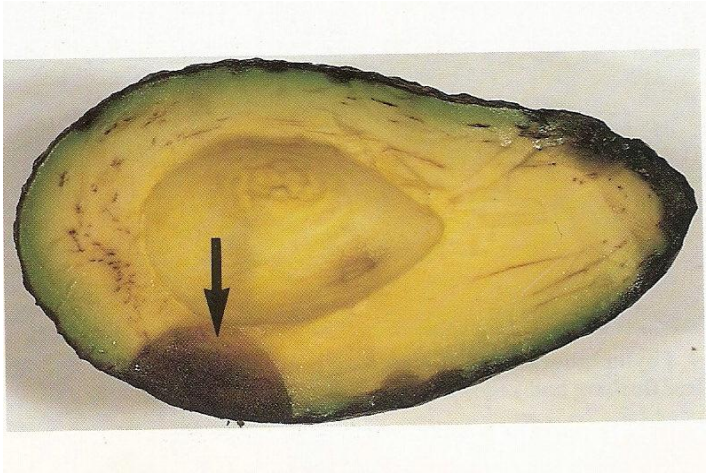

Figure 10. Internal development of the *Colletotrichum gloeosporioides*with hemispherical shape of progressing Anthracnose (Broadley, 1992)

3.1.5. Infection process/disease cycle

The inoculum arises mainly from infected leaves, twigs and mummified fruit. This is a source of large amounts of conidia (Cook, 1975). Spores are spread by rain and wind (Broadley, 1992). In the presence of water, most conidia will germinate within seven hours (Parbery, 1981). Each germinated conidium produces a germ tube. Approximately 5-6 hours after the emergence of the germ tube, the development of a terminal appressorium begins. The germ tube becomes swollen and enlarges. When maximum size is almost reached, the walls of the appressorium thicken and darken and a central germ pore develops (Parbery, 1981).

An infection peg emerges from the germ pore and penetrates the outer wax layer and cuticle of the fruit exocarp. The growth of the infection peg remains in the cuticular region, where it remains dormant until fruit ripening (Figure 11).

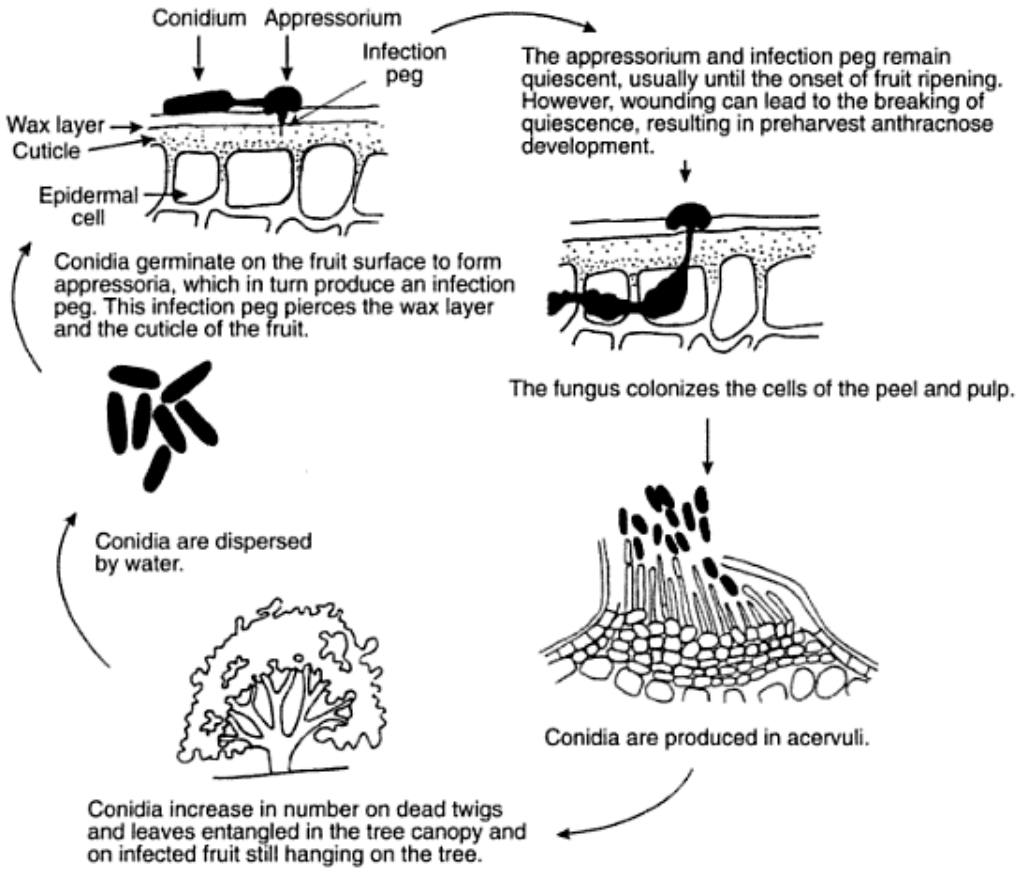

Figure 11. Life cycle of *Colletotrichum gloeosporioides* on avocados (Whiley *et al*., 2002)

3.1.6. Latent infections of *Colletotrichum gloeosporioides* in avocados

Changes in the concentration of antifungal compounds called 'dienes' are thought to be important in the regulation of Anthracnose quiescence in avocado (Prusky, 1996).An antifungal chemical (l-acetoxy-2-hydroxy-4-oxo-heneicosa-12, 15 diene) was isolated from the skin and the flesh of avocado fruit and shown to degrade as the fruit ripen. It is thought to be primarily responsible for latency of infections of immature fruit. Avocado fruits may appear free of blemishes before they ripen, but latent fungal infections quickly result in symptoms when the inhibitory, antifungal compounds present in unripe fruit skins diminish during fruit ripening (Nelson, 2008).Latency of *C. gloeosporioides* in avocados is caused by fungistatic concentrations of these antifungal compounds. When the concentrations of these compounds drop below fungistatic levels during the ripening process, latent infections are activated and hyphae invade the fruit (Prusky *et al*., 1983). Three additional antifungal principles, 1, 2, 4-trihydroxyheptadec-16-yne, 1, 2, 4-trihydroxyheptadec-16-ene and 1-acetoxy-2, 4-dihydroxyheptadec-16-ene, have been isolated from the peel. These, together

with the diene and monoene constitute the total antifungal activity of the unripe fruit peel of the avocado. All these compounds decline in concentration to non-toxic levels during the ripening of the avocado (Adikaram *et al*., 1993).

The antifungal diene compound is concentrated in the outer layers of the fruit and in the skin, and acts as the first line of defence. This reduction in antifungal concentration has been found to correspond with an increase in the fruit's susceptibility to disease. Once the concentration of antifungals has declined past a certain level, Anthracnose is able to resume development to produce symptoms (Prusky *et al*., 1991).

It was observed that the diene levels in the avocado exocarp decrease to subtoxic levels one to two days after harvest, then increase, and then gradually decrease during ripening over the next seven to nine days (Prusky *et al*., 1991). This gradual decline after harvest is thought to be a result of stress in the fruit. Diene levels available to inhibit the fungal growth are higher in the exocarp, however, in the mesocarp dienes are compartmentalised in the oil cells (Kobiler *et al*., 1993; Cummings and Schroeder, 1942).

The exocarp and mesocarp of avocado fruit that are still on the tree contain almost double the diene concentration of fruit postharvest (Prusky *et al*., 1990). Fruit harvested later in the season, being more mature, had lower antifungal diene levels than less mature fruit.

Other factors also play a role in the latency of the fungus. Endopolygalacturonase, a cell macerating enzyme, from the fungus *Colletotrichum gloeosporioides*, was inhibited by the antioxidant and phenolic compound epicatechin (Prusky *et al*., 1989). This phenolic was also seen to inhibit the activity of the fungal enzymes pectate lyase and lipoxygenase (Figure 12) (Wattad *et al*., 1994). This compound could therefore have a direct effect on inhibiting the pathogen on immature fruit (Bowen *et al*., 1995). Lipoxygenase is therefore involved in the transformation of a quiescent infection into an active infection as a result of the decrease of the antifungal diene. This decrease is catalysed by lipoxygenase activity and regulated by the decline of the inhibitor epicatechin. If epicatechin is present in high concentrations, it may result in the inhibition of lipoxygenase activity and result in a delay in the activation of the quiescent infection and decay development (Prusky *et al*., 1992).

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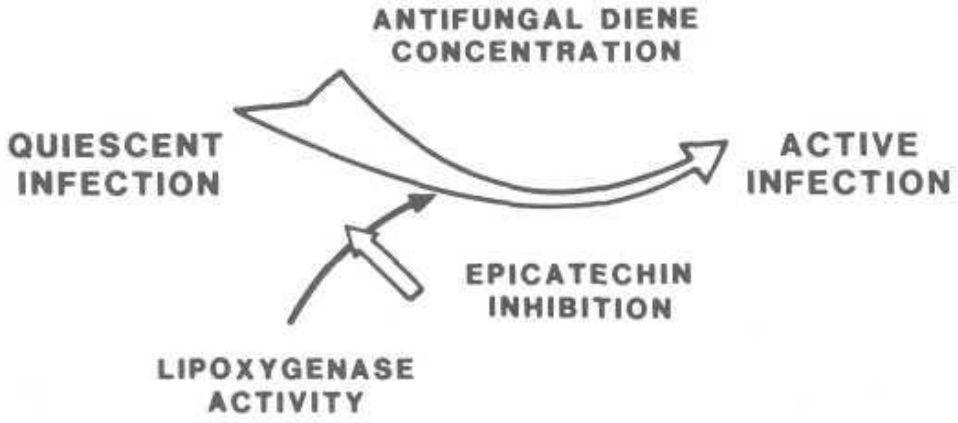

Figure 12. Breakdown of the antifungal diene catalysed by lipoxygenase which is inhibited by epicatechin (Prusky *et al*., 1992)

Increased diene concentrations are associated with an increase in epicatechin biosynthesis genes (Beno and Prusky, 2000). The genes encoding 9-denaturase, 12-denaturase and elongase are activated and other genes of the phenylpropanoid pathway are also transcribed. These genes include phenylalanine ammonia-lyase (PAL) thereby increasing the resistance of avocado fruit to Anthracnose. It was also seen that reactive oxygen species (ROS) increase significantly in the avocado fruit after inoculation with Anthracnose, suggesting that ROS activate the phenylpropanoid pathway as well as diene synthesis (Yakoby *et al*., 2002).

Most antifungal compounds are phenolic in nature and are produced via phenylalanine in the phenylpropanoid pathway (Figure 13) (Hahlbrock and Scheel, 1989).

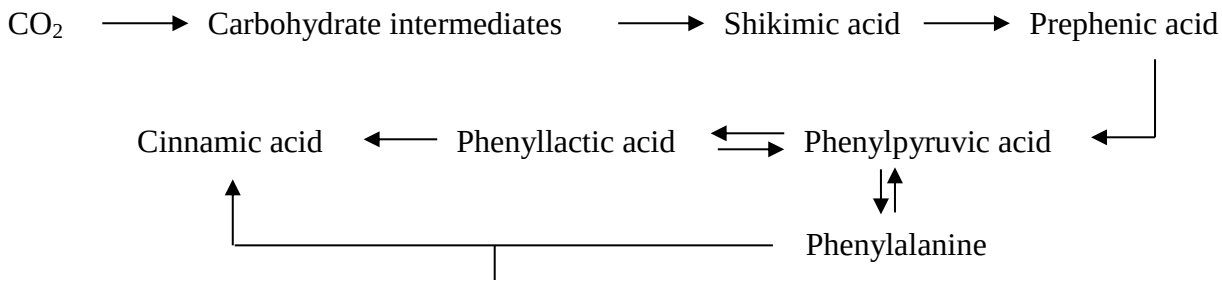

Figure 13. Phenolic synthesis via the phenylpropanoid pathway (Mohr, 1995)

The oxidation of phenols is a common event in host–pathogen interactions. This is shown by the browning of cells and tissues. The oxidation of phenols leads to formation of quinones and free radicals that can inactivate enzymes which may be part of the collection of weapons used by the pathogen. In addition, these oxidized phenolic species have enhanced antimicrobial activity and as a result may be directly involved in stopping pathogen development (Hammerschmidt, 2005). Some studies have shown that the synthesis of phenolic compounds

in plant tissues is generally related to phenylalanine ammonia-lyase activity (Artes *et al*., 1998).

Phenylalanine is produced via the shikimic acid pathway (Biehn *et al*., 1968). Shikimic acid is produced from carbohydrate intermediates and the supply to each fruit depends on the sink strength, source supply, photosynthesis, vegetative-reproductive competition as well as fruit growth. All these factors therefore affect the shikimic acid pathway and hence the eventual production of phenylalanine (Bower, 1990).

3.1.7. Disease management

Both pre- and postharvest treatments can be used in order to treat *Colletotrichum gloeosporioides* on avocados. Pre-harvest treatments include application of various fungicides to avocado trees. These can include copper-based fungicides, such as copper hydroxide and copper oxychloride, which are applied from fruit-set to the time of harvest and can be applied at 14-day intervals. Recommended application rates for conventional orchards are 3-6 kg/ha per application to foliage at four times per season, starting in October and ending in February, with four week intervals. This can account for between 30-450 mg/kg copper input into the top 1 cm of the soil profile per year (Van Zwieten *et al*., 2004). These fungicides can also assist in the prevention of other infections of avocados from other diseases such as pepper spot and sooty blotch. Copper fungicides do, however, have limitations (Willingham, 2001). They provide protection only on the fruit surface and therefore need to be applied often, resulting inhigh labour cost. Applications can also leave copper residues on the exocarp. Copper fungicides act by allowing Cu^{2+} to form complexes with enzymes that possess hydroxyl, sulphydryl, amino or carboxyl groups. This complex formation inactivates the enzymes and leads to a general disruption of the metabolism and breakdown of the cell wall (Hewitt, 1998).

Azoxystrobin and Trilogy® can also be used for Anthracnose control (Palmateer *et al*., 2006). It is a broad spectrum fungicide and can provide protection on both upper and lower leaf surfaces, but is expensive and therefore increases the cost of disease control.

Plant husbandry such as ensuring good ventilation, good sanitation by pruning dead leaves, twigs and fruit before infection can spread, handling the fruit with care and controlling pests and insects, can also reduce disease appearance (Broadley, 1992).

Fungal diseases are a result of the interaction between the environment, inoculum load and the tree health. The expression of the disease can be minimized by manipulating the environment, tree health or by the direct reduction of inoculum. Biological Control Agents (BCA) have an advantage because they are found naturally and when they are found in a higher than natural concentration on the exterior of fruit such as avocados, is unlikely to be harmful as the fruit is not consumed (Everett, 1997). Postharvest biological control has been achieved with several bacteria and yeasts which may provide suitable alternatives, in the future, for fungicide treatments (Korsten *et al*., 1995).

A Bacillus-based biocontrol agent, Avogreen, has been implemented in some cases (De Villiers, 2001). The problem with this BCA is that there are problems with the survival and multiplication of the bacteria under certain orchard conditions and therefore is not used anymore. An environmentally sustainable bacterial BCA hasshown promise for controlling avocado rots in New Zealand (Everett, 1996). The bacterium *Bacillus subtilis* QST713 is commercially available in New Zealand and other bacteria are under investigation (Everett, 1997).

Treatments with antioxidants that delay the breakdown of dienes, and treatments that use CO_2 or non-pathogenic strains of *Colletotrichum* to help stimulate diene production have also been effective in Israel (Prusky, 1988).

Postharvest treatments aim at maintaining fruit quality. This can be achieved by spraying fruit with recommended fungicides and cooling fruit as quickly as possible after harvest (Broadley, 1992). Prochloraz application can also be used (Darvas, 1985). Fruit temperature needs to be controlled to reduce fungal development and fruit should be held at low temperatures (<4°C) once they are near ripe (Hofman and Ledger, 2001).

With the loss of several fungicides for post-harvest disease control and the decreasing residue tolerance of various fungicides (Wisniewski and Wilson, 1992), sanitation of both fruit and packhouse surfaces could become an important disease management tool (Roberts and Reymond, 1994). Detergents and/or sanitizers have been used on a routine basis in food processing and dairy industries to reduce inoculum of spoilage organisms (Park *et al*.,1991). This is not, however, effective against Anthracnose as it is present as a latent infection in immature fruit long before harvest.

4. Systemic resistance inducers

All plants possess their own systems of defence against pathogens, whether it might be against fungi, bacteria or viri. Without such defence mechanisms, plants would succumb to an even greater number of diseases.

The two most clearly defined forms of induced resistance are SAR (systemic acquired resistance) and ISR (induced systemic resistance), the latter is potentiated by plant growth-promoting rhizo-bacteria (PGPR) (Elad *et al*., 2010). In induced systemic resistance the antagonist induces systemic resistance in the host plant. The antagonist therefore causes changes in the plant's physiology that are only expressed later on when the host plant is under stress due to attack by the pathogen (Whipps *et al*., 1988).

When a pathogen comes into contact with a plant, the plant responds by setting off a number of signals which leads to the induction of defence responses. Some defences may be structural (such as cell wall thickening), others are biochemical, e.g. phenolics, phytoalexins and enzymes such as glucanases or chitinases which break down fungal cell walls (Agrios, 1988). If defences are not induced quickly enough, disease may develop.

The alteration of a plant's response to pathogen attack can be enhanced by increasing the synthesis of toxins, such as phytoalexins, acting as inhibitory and repellent substances. Phytoalexins are also able to promote the formation of biochemical barriers (Marschner, 1995). These are molecules inhibiting the development of a fungus on hypersensitive tissue. The formation of such phytoalexins is driven by host plant cells coming into contact with a pathogen (Agrios, 1988).

Phytotoxins can often also be toxic to the host. When these compounds are accumulated at a rapid rate and in very high concentrations within the infection court, resistance to a pathogen can be observed, and as a result, the pathogen dies (Fosket, 1994). Some flavonoids found in legumes such as soybeans or peas have the ability to act as suppressors of certain fungal pathogens. These flavonoids are considered phytoalexins (Dixon, 1986). The increase in flavonoid concentration that can result in plant disease resistance is triggered by the application and accumulation of silicon (Cherif *et al*., 1992). Silicon can, however, also induce resistance against pathogens.

Induced systemic resistance relies on pathways regulated by jasmonate and ethylene (Pieterse *et al*., 1998; Knoester *et al*., 1999; Yan *et al*., 2002). Systemic acquired resistance is effective across a wide array of plant species, whereas there is demonstrated specificity in the ability of PGPR strains to elicit ISR on certain plant species and genotypes (van Wees *et al*., 1997; Yan *et al*., 2002).

Chemical inducers of systemic resistance include the synthetic salicylic acid (SA) analogues 2,6-dichloroisonicotinic acid (INA) and acibenzolar-S-methane (ASM), methyl jasmonate, chitin and chitosan, laminarin, and β-aminobutyric acid (BABA). Phosphate salts, silicon, amino acids, fatty acids, and cell wall fragments can also stimulate systemic resistance.

The future use of SAR and ISR in conventional agriculture seems promising. Synthetic elicitors, in general, do not exhibit any direct antimicrobial activity, unlike traditional pesticides/fungicides. In addition, the use of synthetic elicitors and PGPR strains seems to be environmentally benign. SAR and ISR and other forms of induced resistance are an attractive approach for managing crop pests and diseases in a sustainable manner within the scope of a conventional agriculture system.

4.1. Silicon

Silicon is taken up by plants in the same manner in which macronutrients are absorbed (Epstein, 1999). In certain soil types the application of silicate compounds has resulted in an increase in yield in certain monocot crops including sugarcane, rice and barley (Williams and Vlamis, 1957). Plants do, however, alter the chemical form and the availability of Si in the soil (Epstein, 1999). Silicon's mechanism of action seems to be as a result of both physical and physiological alterations.

Silicon is an important component in plant resistance to biotic stresses, e.g. insects and fungi (Keeping and Kvedaras, 2008). Silicon is also used in sugarcane in South Africa to reduce the effect of the sugarcane stalk borer, *Eldana saccharina* (Meyer and Keeping, 2005). There are not many publications on the effect of Si on nematodes but Walker and Morey (1999) found that the levels of *Tylenchulus semipenetrans* were reduced with applications of potassium silicate.

The susceptibility of plants to fungal diseases can be altered by Si applications. Depositions of Si into the epidermal cells of plants may form an effective mechanical barrier against fungal penetration. Plants harden as a result of Si accumulation and therefore plants receive extra protection, preventing insects and fungi from entering the cells (Kim *et al*., 2002). Silicon can also act in the host tissue whereby it alters the signals that are sent between the host and the pathogen. This results in the activation of a much faster and more extensive plant defence mechanism. Silicon can accumulate at sites near pathogen entry points (Fawe *et al*., 2001) and can therefore act against pathogens by increasing their mechanical resistance. This phenomenon has been reported for cucumber (*Cucumis sativus*) plants (Samuels *et al*., 1991; Marschner, 1995).

Silicon has the ability to form complexes with phenolic compounds. The presence of these phenolic compounds into the infection courts acts as a defence mechanism against these fungal pathogens and their attack. This resistance mechanism is facilitated by the presence of soluble silicon (Menzies *et al*., 1991). Several studies showed that lower disease severity in the Si-treated plants is in line with higher activity of protective enzymes (PPO and PAL) in leaves of rice, wheat and cucumber (Cai *et al*., 2008). These enzymes have important roles regulating the production and accumulation of antifungal compounds such as phenolic metabolism products (lignin), phytoalexins and pathogenesis-related proteins in plants. Silicon application can induce the production of antifungal compounds after the penetration of pathogens into the epidermal cell (Inanaga*et al*., 1995).

Soybean and cucumber are examples of broad-leaf plants that can also contain high Si leaf/stem concentrations. This accumulation results from an active uptake of Si by the plant (Epstein, 1999). Plants which contain considerable amounts of the silicon take up high concentrations of this element from the soil. Plants are able to transport silicon from the roots to the canopy (Miyake and Takahashi, 1995).

In the 2003/2004 New Zealand avocado season it was found that ‘Hass’ fruit from trees injected with soluble silicon eight and twelve weeks prior to harvest had significantly less Anthracnose than untreated control trees (Anderson *et al*., 2004). There was a 50% reduction in the number of fruit affected with Anthracnose eight weeks after treatment with soluble silicon.

Soybean stem canker can be used as an example demonstrating the positive effects of Si in disease control. This disease is caused by a fungus with two developmental phases, the anamorph is found in infected tissues, and the telemorph (Morgan-Jones, 1989), responsible for the first infection occurring in the subsequent growing season. An increase in the resistance of soybeans to soybean stem canker was noted by Juliatti *et al*. (1996) when calcium silicate was used, as both a control and a nutrient source. Silicon reduces the effect of powdery mildew on barley (Rodriguez *et al*., 2003).

4.2. Phosphoric acid

Phosphoric acid (H_3PO_3) is translocated in plants both acropetally and basipetally (Zentmyer, 1979). It is initially translocated to the leaves via the xylem and then with the photosynthates to the roots via the phloem (Piccone *et al*., 1987). Kotzé *et al*. (1987) suggested that phosphoric acid acts indirectly by activating plant defence mechanisms against pathogens.

When phosphoric acid is added at high concentrations, it acts like a fungicide and inhibits fungal growth and disrupts lipid and phosphorus metabolism. At low concentrations, however, phosphoric acid acts as a defence elicitor inducing hypersensitive cell death (apoptosis), prevention of the spread of the pathogen, lignification and causing phytoalexin accumulation (Guest *et al.*, 1995).

Scoparone is a phytoalexin associated with the resistance of citrus to *Phytophthora citrophora*, accumulating in citrus bark after inoculation with the pathogen. It was found that in some cases, this phytoalexin accumulated to higher levels in infected bark treated with phosphoric acid than in untreated infected bark (Afek and Sztejnberg, 1986).

Phosphoric acid compounds such as Phosetyl-Al have been extensively applied to control root rot of avocados caused by *Phytophthora cinnamomi*. Phosetyl-Al degrades to ethanol and phosphite in plant tissues and the latter is the toxophore (toxic chemical) that acts either by activating the defence mechanisms in the plant or by acting directly on the fungus by restricting its development (Fenn and Coffey, 1984).

Phosetyl-Al has the important characteristic of basipetal systemicity. The compound has the ability to move from the leaves to the root independent of root depth or distance from the

point of application. This natural distribution of phosetyl-Al by internal means to the soil provides protection within any part of the plant where *Phytophthora cinnamomi* can develop. No other soil treatment can achieve such an efficient localisation of a product (Bezuidenhout *et al*., 1987).

Phosphonate acts systemically in avocado trees and high concentrations may accumulate in developing fruit, shoot and root tips. Phosphonate is believed to work against Phytophthora root rot by inhibiting the growth of *Phytophthora cinnamomi*; the compound was found to retard fungal growth at high concentrations and but not to kill the fungus. It also indirectly stimulates plant defence mechanisms. By doing this, it causes the natural plant defence systems to bring the invasion under control (Giblin *et al*., 2005).

Pegg *et al*. (1987) used phosphoric acid on avocado trees as soil drenches, tree trunk injections and foliar sprays to control Phytopthora on *Persea indica* seedlings growing in pots. These authors found that both foliar sprays and soil drenches were very effective in controlling the root rot disease. However, the authors also reported that foliar sprays may cause borderline leaf and basal burn on fruit if residues of copper fungicides are present when phosphoric acid was applied.

5. Conclusions

The presently used control measures for postharvest diseases of the Anthracnose complex consist mainly of various copper formulations. Usage of copper, as a heavy metal, is met with environmental concerns, especially with respect to build-up of copper levels in avocado orchards. While reductions in dosage rates may be possible with improved formulations and application methods, such reductions are unlikely to be substantial. In addition, as copper fungicides are contact fungicides, it will still be necessary to ensure adequate fruit coverage during the infection period. Other alternatives, which are systemic in nature and would solve this problem, are costly. Therefore, it is necessary to find alternatives which will not have adverse environmental or cost implications. It is known that the avocado fruit have an effective antifungal system, which prevents the Anthracnose organisms from developing after initial infection until this resistance is lost during ripening. If this loss of resistance can be prevented or slowed down, it may be possible for fruit to ripen before the infection is able to develop to the extent of adversely affecting fruit quality, not impacting on consumer acceptance.

The mechanism of resistance to Anthracnose is basically understood, and therefore the target for a different disease control strategy is known. There are numerous compounds which have an effect on the resistance of plants to diseases. Formulations containing silicon and phosphoric acid have been successfully applied to various crops resulting in disease control via eliciting induced plant resistance. The potential to use silicon for this purpose in avocado fruit exists, and phosphoric acid is extensively used as an effective control against Phytophthora. However, an understanding of the mode of action of both compounds with respect to the physiology of resistance of the avocado fruit to Anthracnose will be necessary before their potential as disease control methods can be determined.

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CHAPTER 2

Effects of systemic resistance inducers applied pre- and post-harvest on total phenolics of *Persea americana* (Mill.) cv 'Fuerte' fruit

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Avocado exports from South Africa require extended low temperature shipping often enhancing postharvest disease incidence. Anthracnose, (*Colletotrichum gloeosporioides*) as a latent infection, may cause considerable losses due to the decline of antifungal compounds in the fruit during fruit ripening. The objective of the study was to use systemic resistance inducers pre- and postharvest to enhance phenolics as possible antifungal compounds and thus potentially decrease postharvest diseases. Pre-harvest Avoguard® 500 SL (500 ppm phosphoric acid a.i.) was applied and fruit were harvested on the application day as well as 7, 14, and 21 days thereafter. Fruit were also treated postharvest with Phosguard 400 SL (500 ppm phosphoric acid a.i.) and AgriSil™ K50 (1000 ppm silicon a.i.). Fruit were either ripened immediately at room temperature or stored for 28 days at either 5.5°C or 2°C before ripening. After removal from storage, exocarp was sampled at five distinct softening stages and fruit condition was evaluated. Exocarp samples were analysed for total phenolics. Pre-harvest phosphoric acid showed highest phenolic concentrations at 14 days after application for fruit ripened immediately and 7 days after harvest for 2°C. Postharvest treatments showed potassium silicate to increase phenolic concentrations for fruit stored at room temperature and 2°C. Both treatments also influenced phenolic concentrations, with potassium silicate having greater effects. It is concluded that these compounds may be used as part of a postharvest disease control strategy.

1. Introduction

In tropical and wet subtropical environments, the production of avocados is regarded as 'high risk' due to potential pests and disease infestations pre- and postharvest. Anthracnose is particularly problematic in warm areas that have a high rainfall (Whiley *et al*., 2002), typical of many South African growing conditions.

Anthracnose, caused by *Colletotrichum gloeosporioides*, infects fruit early in the growth cycle, but becomes latent until ripening begins after harvest. A number of factors play a role in the latency of the fungus. *Colletotrichum gloeosporioides* is inhibited by the antioxidant and phenolic epicatechin (Prusky *et al*., 1989); this phenolic compound may therefore inhibit the pathogen on immature fruit (Wattad *et al*., 1994).

The responses of plant-to-pathogen interactions have been characterised by the early accumulation of phenolic compounds at the interaction site and as a result there is limited development of the pathogen due to rapid cell death (Fernandez and Heath, 1989). It is thought that rapid accumulation of phenols results in apoptosis and therefore the effective isolation of the pathogen (Friend, 1981).

Most phenolic compounds are phenylpropanoids derived from the shikimic acid pathway. Following microbial attacks phenolics often accumulate mostly as phytoalexins, inducible low-molecular-weight compounds. These molecules might be present constitutively at low concentrations in the plant, but accumulate rapidly upon attack (Strack, 1997). Pathogenic fungi can either induce only non-toxic compounds or quickly degrade phytoalexins, while non-pathogenic fungi induce such high levels of toxic compounds in their host so that their establishment is prevented (De Ascensao and Dubery, 2003).

Rapid and early accumulation of phenolic compounds at infection sites is a characteristic of phenolic-based defence responses. This may result in the isolation of the pathogen at the original site of entrance as a result of the rapid and early accumulation of phenolic compounds (De Ascensao and Dubery, 2003).

Silicon (Si) has long been associated with disease resistance in plants. Early studies showed Si accumulating at sites near pathogen entry points (Fawe *et al*., 2001). These observations

led researchers to believe that silicon acted against pathogens by increasing the mechanical resistance of plants. More recent work showed that silicon can also induce defence responses. Dann and Muir (2002) found that growing pea plants in silicon amended potting mix increased the production of defence proteins (chitinase and glucanase).

Phosphoric acid fungicides have been proven to be especially effective against Oomycete pathogens, such as *Phytophthora*, *Phythium*, and Downy mildews in a number of crops. Phosphoric acid has both a direct and indirect effect on these pathogens. The indirect effect is considered to be through stimulating the plant's natural defence response against pathogen attack which can be seen against Anthracnose in blueberries (Schilder, 2005).

Phosphoric acid which is used for the control of Phytophthora root rot in avocado induces plant defences. It has been found that phosphoric acid fungicides are especially effective against oomycete pathogens, such as Phytophthora, Pythium, and Downy Mildew in a number of crops as well as against Anthracnose in strawberries (Guest *et al*., 1995).

The aim of the experiment was to investigate if the known systemic resistance inducers potassium silicate and phosphoric acid (Yakoby *et al*., 2002) when applied pre-harvest or postharvest to 'Fuerte' avocado trees/fruit can enhance rind phenolics, thereby potentially delaying Anthracnose symptom development.

2. Material and Methods

2.1. Fruit

Fruit were obtained from two locations in KwaZulu-Natal, Wartburg (29°27'S, 30°40'E) and Howick (29°45'S, 30°25'E). Pre-harvest applications were carried out on Wartburg fruit and postharvest treatments were applied to fruit obtained from Howick. The Howick site was a certified organic orchard, and the fruit had therefore not been exposed to the compounds pre-harvest through the use of phosphoric acid for Phytophthora root rot control. Postharvest dips of 500 mgL^{-1} Phosguard 400 SL and 1000 mgL^{-1} AgriSil™ K50 were applied to fruit after collection from the packhouse. Avoguard® 500 SL was also applied to trees (May/June) at the Wartburg site and fruit were harvested at the time of treatment as well as 7, 14 and 21 days thereafter. Fruit were then either stored at 5.5°C or 2°C for 28 days (to simulate a typical cold storage period of South African fruit exported to Europe) before ripening or remained to ripen immediately at room temperature without storage.

2.2. Fruit softness

Fruit softness was determined using a hand-held densimeter (Bareiss, Oberdischingen, Germany) with a 5 mm tip (Eaks, 1966). Fruit softness was measured on a scale of 0 to 100 whereby fruit was deemed hard and unripe at 90-100, medium at 68-75 and soft (eating ripe) at 50-55. Three equally spaced readings were taken around the circumference of each fruit and the average reading recorded.

2.3 Fruit sampling

Exocarp was sampled by removal of strips(taken from the top to the bottom of the avocado (lengthways) and approximately three to four cm in width) at three stages of fruit softness readings of the densimeter (>85, 70-75, 52-54). Three strips were taken at each softness level. Mesocarp was then protected from desiccation by Vaseline and fruit allowed to continue ripening (Blakey, 2011). After removal, strips of exocarp were immediately snap-frozen in liquid nitrogen, freeze-dried and finely ground before analysis.

2.4. Extraction and analysis of total phenolics

The extraction of both bound and soluble, free phenolic compounds was carried out according to Böhm *et al*.(2006).Ground, freeze-dried exocarp (0.1 g) was weighed into glass tubes. An amount of 1mL1 M hydrochloric acid was mixed with the samples which were then incubated for 30 min at 37°C. Thereafter, 1mL 2 M NaOH (in 75% methanol) was used for alkaline hydrolysis, and the mixture again incubated for 30 min at 37°C. Thereafter, 1 mL0.75 M meta-phosphoric acid was added and samples were centrifuged (Sorvall RC-5C Plus, Newtown, CT, USA) at 3000x g for 5 min. At the end of centrifugation 1mL acetone/water (1:1) was added (Serafini *et al*., 1998) and the mixture re-centrifuged at 3000 x g for 5 min. This extraction with acetone/water was repeated twice. Combined supernatant were made up to 10 mL with acetone/water.

The rind total phenolic concentration was determined using the Folin-Ciocalteu assay. Nano-pure water (5 mL), 1.0 mL sample extract and 1.0 mL Folin-Ciocalteu reagent were added to a 25 mL volumetric flask. The content was mixed and allowed to stand for 5-8 min at room temperature, thereafter, 10 mL 7% sodium carbonate solution was added, followed by the addition of nano-pure water to volume. Solutions were mixed and allowed to stand at room temperature for two hours, and the absorbance determined at 750 nm. The total phenolic content was expressed against gallic acid (Singleton and Rossi, 1965). The linearity range for this assay was determined as 0.5-5.0 mgL^{-1} GAE, giving an absorbance range of 0.050-0.555 AU.

2.5. Ethylene production rate

In order to check ripening physiology of treated and untreated fruit, individual fruit were incubated in 1 L containers for 30 min, into which a 20 mL GC vial was placed. Ethylene was measured using a GC-FID (DANI 1000, DANI Instruments, Monzese, Italy). A stainless steel alumina G1 column was used to determine ethylene by comparison with an ethylene standard curve. The mobile phase was instrument grade nitrogen gas at 35 kPa. The ethylene production rate was calculated taking into account the mass and volume of fruit as well as the free space in the jar using the formula:

$$\text{Ethylene} = \frac{\text{x}\mu\text{L}CO_2}{1\ \text{L}_{\text{air}}}\text{x}\ \frac{\text{V}_{\text{headspace}}}{1}\text{x}\frac{1}{\text{m}_{\text{fruit}}}\text{x}\frac{1}{\text{t}}$$

2.6. Respiration rate

In order to check ripening physiology, treated and untreated fruit respiration rate was determined by measuring the rate of carbon dioxide evolution using an infrared gas analyser (EGM-1, PP Systems, Hitchin, UK). Individual fruit were incubated in 1 L containers for 12 min. The headspace carbon dioxide concentration was converted to respiration rate taking into account the volume and mass of fruit, as well as the free space in the jar and the ambient carbon dioxide concentration. The following formula was used to determine he respiration rate:

$$\text{Net } CO_2(\mu L) = \frac{x\mu L CO_2}{1\ L_{air}} x\ \frac{V_{headspace}}{1} x \frac{1}{m_{fruit}} x \frac{1}{t} - \text{ambient } CO_2$$

2.7. Statistical analysis

Data were analysed as a factorial design with each treatment consisting of 20 replicates. A general analysis of variance was performed using Genstat 14th edition (VSN International, Hemel Hempstead, UK).Least significant difference (LSD) at the 5% level was used to separate treatment means.

3. Results

Ethylene production and respiration rates indicated a normal ripening pattern for the fruit. However, actual values of CO_2 and ethylene between untouched and touched (exocarp removal) fruit differed. The reason for this was that the exocarp had been removed from the avocado and therefore the control of gas movement and diffusion from the avocado fruit appeared to have been changed.

3.1. Visual observations

Anthracnose was not observed in fruit but slight bruising was seen (Figures 1 and 2).

3.2. Effect of pre-harvest treatments on total phenolic concentrations

Fruit stored at room temperature showed the greatest differences in exocarp phenolics of treated and control fruit harvested 14 days after treatment. For fruit harvested 21 days after treatment, the same differences were observed; however, phenolic concentrations were more similar at this stage. Highest phenolic concentration was observed for fruit harvested 14 days after treatment and prior to softening (Figure 3).

Storage temperature appeared to influence the total phenolic levels. Cold storage at 5.5°C resulted in less variation in phenolic concentration compared to fruit stored at room temperature. Storage time did not affect phenolic concentration of hard fruit independent of the time after treatment. As fruit softened phenolic concentrations increased in fruit harvested 7 days after treatment and similar differences were observed for soft fruit harvested 14 days after treatment. Differences observed for fruit harvested after 21 days were not observed for 5.5°C storage as in fruit stored at room temperature (Figure 4).

Fruit harvested after 7 days and stored at 2°C was seen to have the greatest (significant) difference in phenolic concentrations of treated fruit, while this effect could no longer be observed for fruit harvested after 14 and 21 days. It was also observed that phenolic concentration declined over time. When comparing the three storage temperatures, there was a tendency for fruit stored at 2°C to enhance phenolic concentration the most (Figure 5).

3.3. Effect of postharvest treatments on total phenolic concentrations

Total phenolic concentration changes during ripening are shown in Figures 6, 7 and 8.Alterations in the phenolic concentrations were observed during ripening. Fruit stored at 5.5°C showed an increase in phenolic concentration with softness in potassium silicate treated fruit. Once soft, the levels appeared to decrease to those of hard, unripened fruit. Fruit stored at 2°C showed an increase in phenolic concentrations during ripening for control fruit, while phosphoric acid treated fruit only showed an increase when fruit was fully ripe. Potassium silicate treated fruit showed an initial decrease in phenolic concentration which was followed by a significant increase during ripening.

4. Discussion

The maturity of fruit plays an important role in the fight against fungal attack as it influences the natural resistance of fruit (Prusky, 1996). Prusky *et al*. (1982) stated that the presence of dienes in the peel of avocados is adequate to prevent the growth of Anthracnose in unripe fruit. This appears to decrease with fruit ripening (softening).

Some phenolic compounds are known to impart antifungal properties (Hahlbrock and Scheel, 1989). Phosphoric acid(as a systemic resistance inducer) (Bower *et al*., 2003) was applied to determine whether it could increase the concentration of phenolic compounds present at later ripening stages and thereby potentially decreasing disease incidence, especially where fruit is stored for a long period at low temperatures. A tendency towards an increase in phenolic compounds was indeed observed. It was, however, notable that the effect was not immediate. It appeared that phosphoric acid increased phenolic concentrations the greatest at an optimum harvest of 14 days after spraying, for fruit ripened immediately after harvest at room temperature and when fruit was stored at 5.5°C. This was not seen for fruit stored at 2°C where optimum harvest day appeared to be 7 days after spraying.

While it therefore appears that some time is needed after the phosphoric acid application for the effect to be realised, the reason for a difference if storage was conducted at lower temperature, is not known. It is, however, suggested that the mechanism is complex, perhaps involving a change in gene regulation in the shikimic acid pathway. The results imply that not only is the time the fruit remains on the tree after the application of phosphoric acid important, but postharvest conditions prior to ripening also appear to play a role. Storing fruit at low temperatures affects membrane function and activity and the position of membrane-associated enzymes (Lyons and Raison, 1970). Thus, initial changes taking place on the tree, may not be realised in the same manner if storage temperatures are sufficiently low to alter critical enzyme function. Further work will be needed to elucidate the possible mechanisms.

The results of Bekker *et al*. (2007) showed similar trends to those found in this study when analysing total phenolic concentrations in avocado trees, after the application of potassium silicate.

Silicon application to diseased plants has been shown to control disease development and spread with various degrees of success (Epstein, 1999). It is generally accepted that plants with higher silica content are more resistant to phytopathogenic fungi than those plants that have lower silica content (Ishiguro, 2001). After silicon is taken up by a plant, a silicification process occurs, and the silicon is either deposited in the cell wall, cell lumen, or intercellular spaces resulting in a physical barrier to fungal infections (Epstein, 1999). In this study, the silicon was only applied after harvest, thus allowing little time for this process. Nevertheless, there did appear to be some effect in stimulating production of new phenolic compounds. It is again suggested that this process is a multi-step one, affected by the temperature of storage as well as the fruit ripening process.

Phosphoric acid, used for the control of Phytophthora root rot in avocado, can induce plant defence systems. At high concentrations phosphoric acid has been seen to act like a fungicide by inhibiting fungal growth and disrupting lipid and phosphorus metabolism (Guest *et al*., 1995). However, there is also evidence of lignin formation (Cahill *et al*., 1993). This would require stimulation of phenolic compound synthesis. Due to increased phenolic concentrations, it can be assumed that phosphoric acid is capable of inducing resistance in avocado, possibly by increasing compounds derived from the pathway, such as the antifungal dienes.

5. Conclusions

Both phosphoric acid and potassium silicate were found to alter the total phenolic compound concentrations in fruit during the ripening phase. However, the temperature of storage affected the process, and a pre-harvest application of phosphoric acid appeared to be more effective, especially if fruit was left on the trees for 14 days after application. While there was no significant indication of Anthracnose infection of the fruit, and the efficacy of this approach to postharvest decay control could not be tested, there is evidence that such an approach could be useful. However, before any recommendation can be made, it will be necessary to study the changes in individual phenolics, as significant changes in antifungal compounds within the phenolic group may be hidden within the totals measured in this study. A better understanding of the process of phenolic antifungal compound stimulation at the genetic transcription level, especially as affected by storage, including temperature of storage, would also be useful.

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7. Figures

Figure 1. ‘Fuerte’ avocado fruit showing no signs of Anthracnose presence or bruising.

Figure 2. ‘Fuerte’ avocado fruit showing slight bruising.

Figure 3. Phenolic concentration at specific fruit softness during ripening of ‘Fuerte’ avocados stored at room temperature (22°C). Trees were sprayed pre-harvest with phosphoric acid (500 mgL^{-1}) and harvested at 0, 7, 14 and 21 days after application. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.052.

Figure 4. Phenolic concentration at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at 5.5°C for 28 days. Trees were sprayed pre-harvest with phosphoric acid (500 mgL^{-1}) and harvested after 0, 7, 14 and 21 days after application. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.072.

Figure 5. Phenolic concentration at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at 2°C for 28 days. Trees were sprayed pre-harvest with phosphoric acid (500 mgL^{-1}) and harvested after 0, 7, 14 and 21 days after application. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.055.

Figure 6. Phenolic concentration at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at room temperature (22°C). Fruit were dipped with potassium silicate (1000 mgL^{-1}) or phosphoric acid (500 mgL^{-1}) on arrival at the laboratory. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.048.

Figure 7. Phenolic concentration at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at 5.5°C for 28 days. Fruit were dipped with potassium silicate (1000 mgL^{-1}) or phosphoric acid (500 mgL^{-1}) on arrival at the laboratory. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.041.

Figure 8. Phenolic concentration at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at 2°C for 28 days. Fruit were dipped with potassium silicate (1000 mgL^{-1}) or

phosphoric acid (500 mgL^{-1}) on arrival at the laboratory. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.059.

Figure 1.

Figure 2.

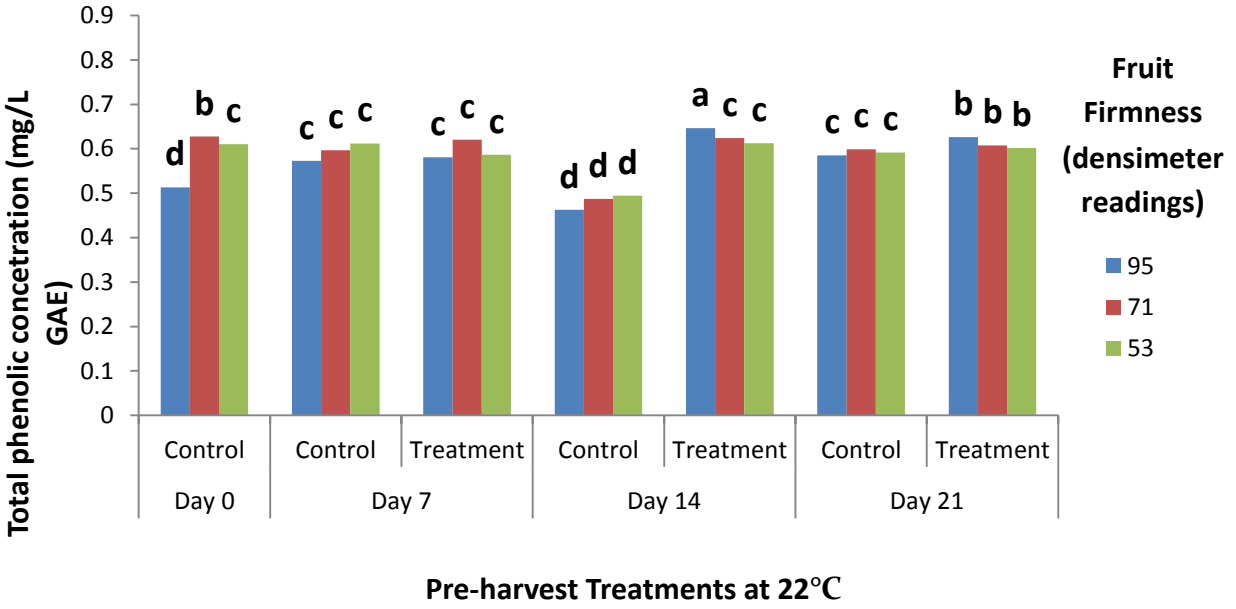


Figure 3.

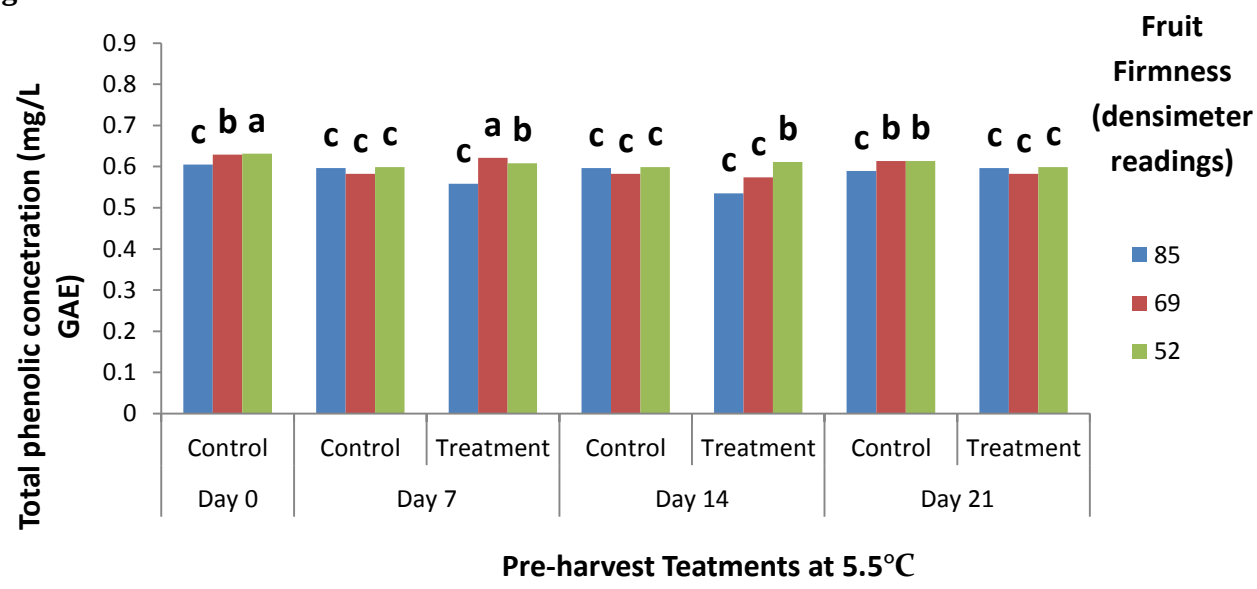
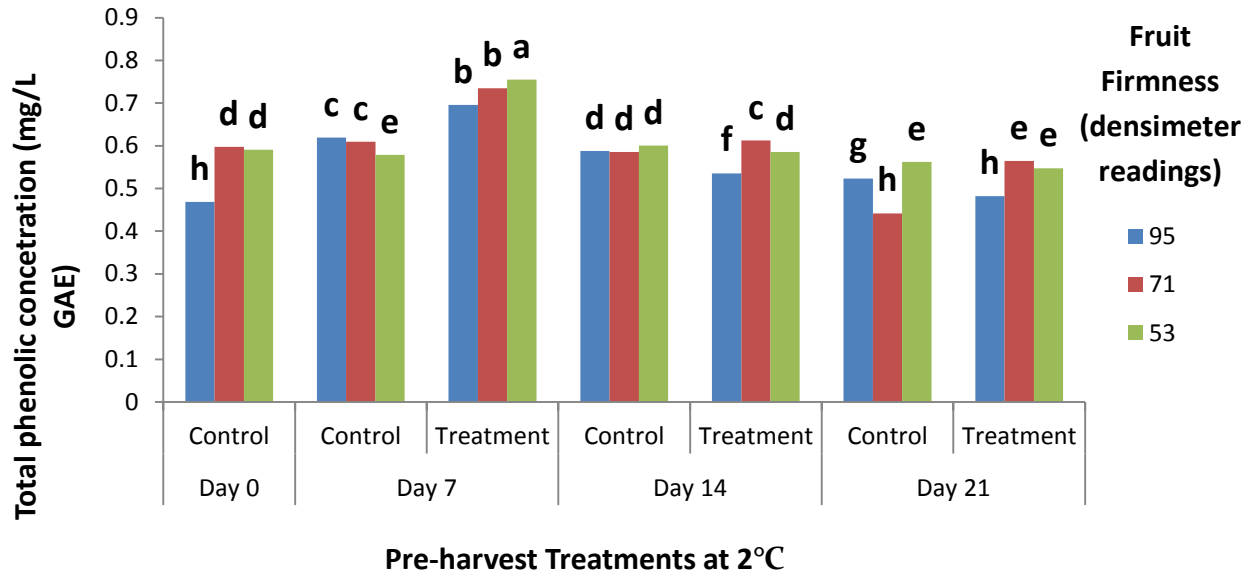


Figure 4.



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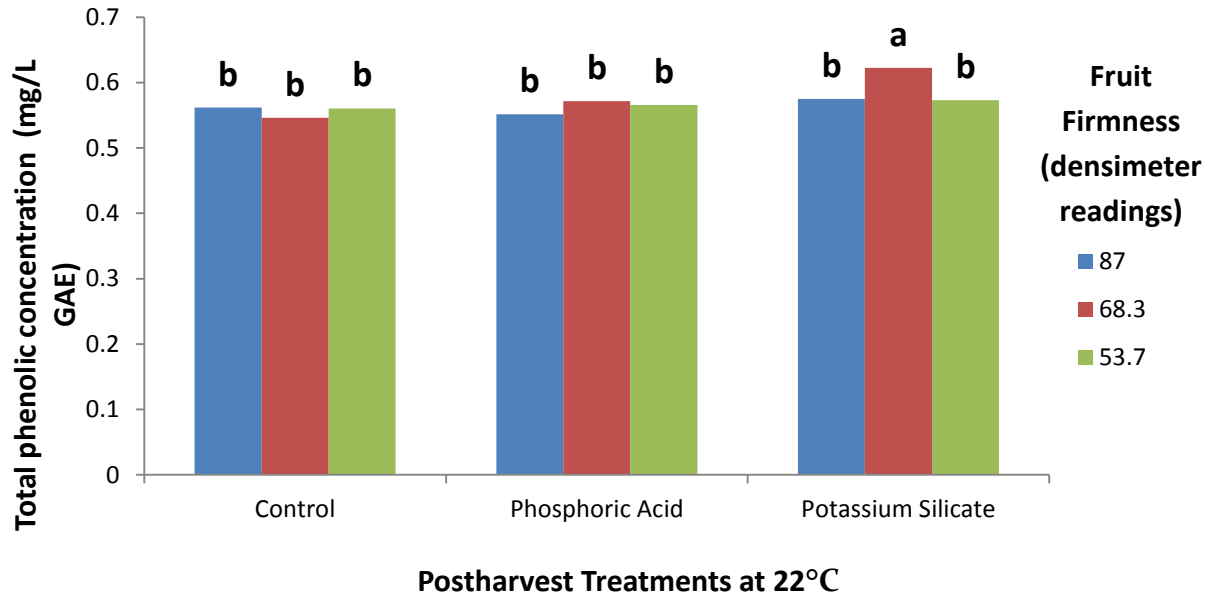

Figure 6.

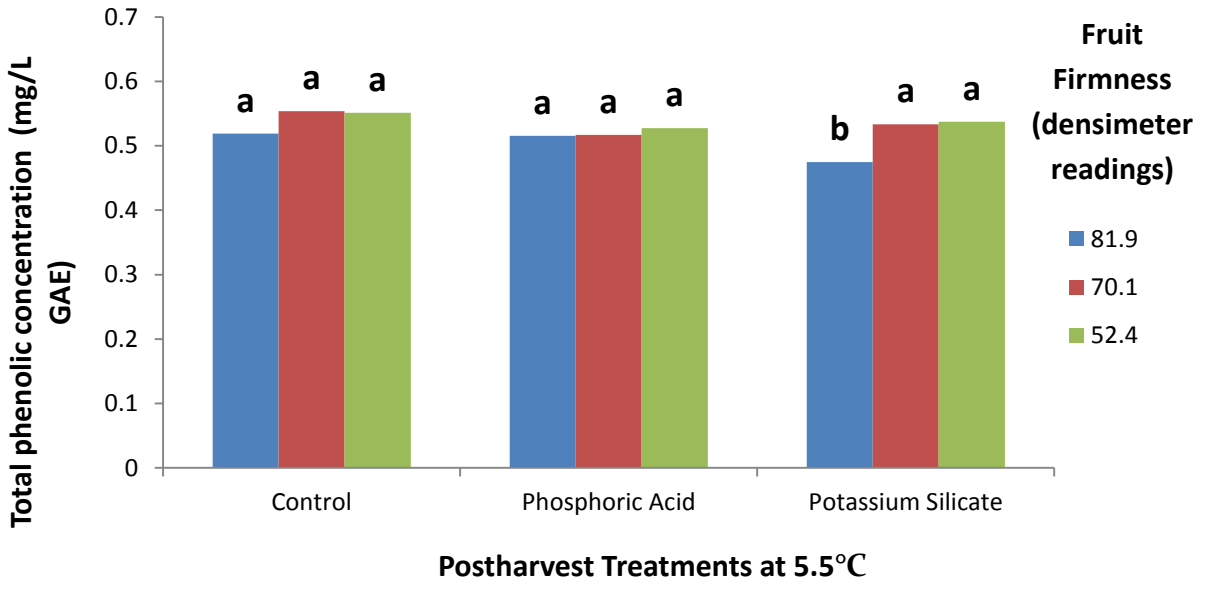

Figure 7.

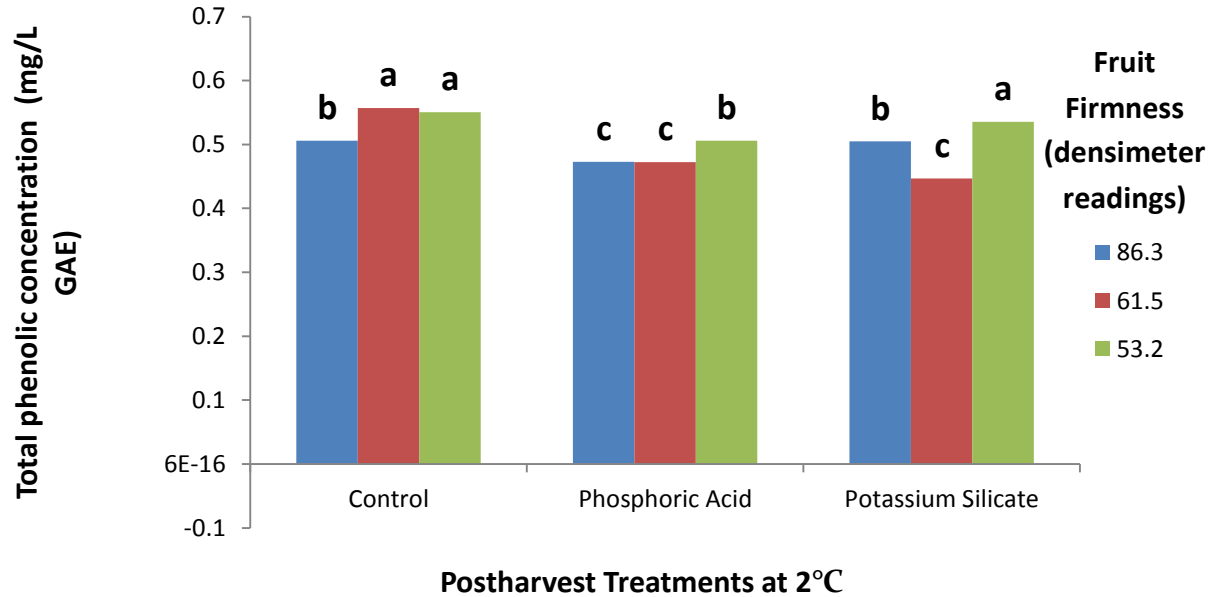

Figure 8.

CHAPTER 3

Effects of systemic resistance inducers applied pre- and post-harvest on enzymatic activity of*Persea americana* (Mill.) cv 'Fuerte' fruit

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Colletotrichum gloeosporioides is an important postharvest pathogen that attacks a wide range of tropical and subtropical fruits, amongst these avocados. The importance of the crop necessitates effective control of the disease, without harming the environment. The objective of the study was to use systemic resistance inducers to enhance antifungal compounds in the fruit exocarp and thus potentially decrease Anthracnose. Pre-harvest sprays of Avoguard® 500 SL (500 ppm phosphoric acid a.i.) were also applied, and fruit were harvested on the day of spraying and 7, 14, and 21 days thereafter. Fruit were treated postharvest with Phosguard 400 SL (500 ppm phosphoric acid a.i.) and AgriSil™ K50(1000 ppm silicon a.i.). Fruit were ripened immediately or stored for 28 days at either 5.5°C or 2°C before ripening. Exocarp was sampled during ripening and fruit condition was evaluated. Tissue was analysed for phenylalanine ammonia-lyase (PAL) activity. Pre-harvest applications with phosphoric acid showed highest PAL activity for fruit harvested 14 days after application, for fruit ripened immediately, as well as fruit stored at 5.5°C. Postharvest treatments showed both potassium silicate and phosphoric acid to decrease Anthracnose in stored fruit. Both compounds also influenced PAL activity, with potassium silicate having greater effects.

1. Introduction

The sale of high quality fruit to consumers is an important challenge for the avocado industry. Unfortunately surveys have consistently identified a number of quality defects in avocados, with postharvest diseases being one of the major quality problems (Hofman and Ledger, 2001).

Latency of *C. gloeosporioides* in avocados is caused by fungistatic concentrations of antifungal compounds known as dienes. When the concentrations of these compounds decreases below fungistatic levels during the ripening process, latent infections are activated (Prusky *et al*., 1983). Increased diene concentrations have been linked to an up regulation of epicatechin biosynthesis genes (Beno and Prusky, 2000). Activation of genes such as for phenylalanine ammonia-lyase, (PAL), increases the resistance of avocado fruit to Anthracnose (Yakoby *et al*., 2002).

Most antifungal compounds are phenolic in nature and are produced via the phenylpropanoid pathway. Phenylalanine ammonia-lyase is an essential enzyme in the resistance-related phenylpropanoid pathway in avocados (Prusky, 1994; Ardi *et al*., 1998). Activity of PAL is highly correlatedto the increase in epicatechin concentration, a phenolic providing resistance to pathogen attack. The induction of PAL activity in avocados can be used as a measure of effective disease reduction by an increase in resistance of avocado fruit to the pathogen (Prusky, 1994). Epicatechin, a cinnamic acid derivative, is a central molecule in this pathway, and many steps affect its final concentration and that of its derivatives (Hahlbrock and Scheel, 1989).

Induced systemic resistance is a phenomenon describing an antagonist inducing systemic resistance in the host plant. This antagonist causes changes in the plant's physiology that are only expressed later on when the host plant is under stress due to attack by a pathogen (Whipps *et al*., 1988). Silicon can act in the host tissue by altering the signals that are sent between the host and the pathogen. This results in the plant defence mechanism being activated, much faster and more extensively, as demonstrated in cucumber plants (*Cucumis sativus*) (Samuels *et al*., 1991; Marschner, 1995).

The aim of this investigation was to use potassium silicate and phosphoric acid, known systemic resistance inducer compounds (Yakoby *et al*., 2002) as postharvest applications and the latter also as a pre-harvest spray on 'Fuerte' avocado trees and fruit in order to enhance PAL activity, to delay Anthracnose symptom development.

2. Material and Methods

2.1. Fruit

Fruit were obtained from two locations in KwaZulu-Natal, Wartburg (29°27'S, 30°40'E) and Howick (29°45'S, 30°25'E). Pre-harvest applications were carried out on Wartburg fruit and postharvest treatments were applied to fruit obtained from Howick. The Howick site was a certified organic orchard, and the fruit had therefore not been exposed to the compounds pre-harvest through the use of phosphoric acid for Phytophthora root rot control. Postharvest dips of 500 mgL^{-1} Phosguard 400 SL and 1000 mgL^{-1} AgriSil™ K50 were applied to fruit after collection from the packhouse. Avoguard® 500 SL was also applied to trees (May/June) at the Wartburg site and fruit were harvested at the time of treatment as well as 7, 14 and 21 days thereafter. Fruit were then either stored at 5.5°C or 2°C for 28 days (to simulate a typical cold storage period of South African fruit exported to Europe) before ripening or left to ripen immediately at room temperature without storage.

2.2. Fruit softness

Fruit softness was determined using a hand-held densimeter (Bareiss, Oberdischingen, Germany) with a 5 mm tip (Eaks, 1966). Fruit softness was measured on a scale of 0 to 100 whereby fruit was deemed hard and unripe at 90-100, medium at 68-75 and soft (eating ripe) at 50-55. Three equally spaced readings were taken around the circumference of each fruit and the average reading recorded.

2.3. Fruit sampling

Exocarp was sampled by removal of strips(taken from the top to the bottom of the avocado (lengthways) and approximately three to four cm in width) at three stages of fruit

softnessreadings of the densimeter (>85, 70-75, 52-54). Three strips were taken at each softness level. Mesocarp was then protected from desiccation by Vaseline and fruit allowed to continue ripening (Blakey, 2011). After removal, strips of exocarp were immediately snap-frozen in liquid nitrogen, freeze-dried and finely ground before later analysis.

2.4. Extraction and analysis of phenylalanine ammonia-lyase

2.4.1. Season 1

Phenylalanine ammonia-lyase was extracted according to the method of Lister *et al*. (1996) which was modified slightly. All steps were performed at 4˚C. Ground exocarp tissues (0.1 g) of avocado at the different ripening stages were used. A volume of 5 mL 0.05 M sodium borate buffer (pH 8.8) containing 5 mM mercaptoethanol and 1% of PVP was added to the sample. The mixture was vortexed and placed on a shaker for 25 min. The enzyme extract was centrifuged for 20 min at 12 000 x g (Sorvall RC-5C Plus, Newtown, CT, USA) and the supernatant was collected for determination of PAL activity.

Phenylalanine ammonia-lyase activity was assayed by the methods of Jiang and Joyce (2003). A reaction mixture of 1.9 mL 0.05 M sodium borate buffer (pH 8.8) containing 1 mL20 mM L-phenylalanine and 0.1 mL enzyme solution was used. The samples were incubated for 1 hour at 37˚C. In the control sample, the enzyme extract was replaced by 1 mL 0.05 M sodium borate buffer (pH 8.8). The reaction was stopped by adding 0.2 mL6 M trichloroacetic acid (TCA). Enzymatic activity was expressed as the amount of enzyme causing a change of 0.01 in absorbance at 290 nm per hour. The Bradford microassay was used to determine the protein concentration of the samples (Bradford, 1976), by reading the samples spectrophotometrically at 595 nm andcomparing the value with a standard curve prepared against bovine serum albumin. Final enzyme activity was expressed as specific activity, taking the protein content into account.

2.4.2. Season 2 – Method improvement

Extraction of PAL was carried out according to the method of Lister *et al*. (1996) with slight modification.All steps were performed at 4˚C. Ground exocarp tissue (0.04 g) from avocado at different ripening stages was used. A volume of 2 mL 0.05 M sodium borate buffer (pH

8.8) containing 5 mM mercaptoethanol and 1% of PVP was added to the sample in Eppendorf tubes. The mixture was vortexed and placed on a shaker for 25 min. The enzyme extract was centrifuged for 20 min at 12 000 x g (HealForce, Neofuge 13R, High Speed Refrigerated Bench-top Centrifuge) and the supernatant was collected for determination of PAL activity which was assayed by the methods of Jiang and Joyce (2003) as described in 3.1.4.1.

2.5. Ethylene production rate

In order to check ripening physiology of treated and untreated fruit, individual fruit were incubated in 1 L containers for 30 min, into which a 20 mL GC vial was placed. Ethylene was measured using a GC-FID (DANI 1000, DANI Instruments, Monzese, Italy). A stainless steel alumina G1 column was used to determine ethylene by comparison with an ethylene standard curve. The mobile phase was instrument grade N_2 at 35 kPa. The ethylene production rate was calculated taking into account the mass and volume of fruit as well as the free space in the jar using the formula:

$$\text{Ethylene} = \frac{\text{x}\mu\text{L}CO_2}{1\ \text{L}_{\text{air}}}\text{x}\ \frac{\text{V}_{\text{headspace}}}{1}\text{x}\frac{1}{\text{m}_{\text{fruit}}}\text{x}\frac{1}{\text{t}}$$

2.6. Respiration rate

In order to check ripening physiology, treated and untreated fruit respiration rates were determined by measuring the rate of carbon dioxide evolution using an infrared gas analyser (EGM-1, PP Systems, Hitchin, UK). Individual fruit were incubated in 1 L containers for 12 min. The headspace carbon dioxide concentration was converted to respiration rate taking into account the volume and mass of fruit, as well as the free space in the jar and the ambient carbon dioxide concentration. The following formula was used to determine the respiration rate:

$$\text{Net CO}_2(\mu\text{L}) = \frac{\text{x}\mu\text{L}CO_2}{1\ \text{L}_{\text{air}}}\text{x}\ \frac{\text{V}_{\text{headspace}}}{1}\text{x}\frac{1}{\text{m}_{\text{fruit}}}\text{x}\frac{1}{\text{t}} - \text{ambient CO}_2$$

2.7. Statistical analysis

Data were analysed as a factorial design with each treatment consisting of 20 fruit replicates. A general analysis of variance was performed using Genstat 14th edition (VSN International,

Hemel Hempstead, UK).Least significant difference (LSD) was used to separate treatment means. The data represent the average of two seasons.

3. Results

Ethylene production and respiration rates indicated a normal ripening pattern for the fruit. However, actual values of CO_2 and ethylene between untouched and touched (exocarp removal) fruit differed. The reason for this was that the exocarp had been removed from the avocado and therefore the control of gas movement and diffusion from the avocado fruit appeared to have been changed.

3.1. Visual observations

Anthracnose was not observed in fruit but only slight bruising (Figures 1 and 2).

3.2. Effect of pre-harvest treatments on PAL activity

Fruit stored at room temperature showed an increase in PAL activity for fruit harvested 7 days after treatment but only at the softening stage. The greatest differences were found for fruit harvested 14 days after treatment with the highest PAL activity in soft control fruit followed by soft treated fruit. An increase in PAL activity with softening was observed for treated fruit harvested after 14 days. Activity decreased for fruit harvested after 21 days (Figure 3).

For fruit stored at 5.5^{o}C, the harvest at 14 days after phosphoric acid application appeared to have enhanced PAL activity. However, this fruit also had a significant enhancement of activity during ripening. This was the only harvest at which phosphoric acid appeared to influence PAL activity. Fruit harvested 21 days after treatment had the lowest activity (Figure 4).

Reducing storage temperature to 2°C showed an earlier increase in PAL activity for treated fruit harvested 7 days after treatment with similar effects seen for treated fruit harvested 14 days after treatment (Figure 5). For all storage temperatures, control fruit at day 0 (day of treatment) appeared to increase in activity with softening.

3.3. Effect of postharvest treatments on PAL activity

Phenylalanine ammonia-lyase activity of fruit ripened immediately after harvest showed a significant increase during softening for both the phosphoric acid and potassium silicate treatments. Phosphoric acid had much higher activity than potassium silicate while the latter appeared to have a greater influence in the changes in activity. The combination of the treatments had no effect on PAL activity, although some stimulation during ripening did occur (Figure 6).

Fruit stored at 5.5°C had slightly different patterns of activity. Phosphoric acid and potassium silicate enhanced PAL activity at all ripening stages with soft fruit displaying the highest PAL activity. This was also seen for the combination treated fruit (Figure 7).

In fruit stored at 2°C PAL activity was suppressed and no significant differences in fruit softening could be determined. The combination of the two treatments appeared to result in a tendency for somewhat higher activity of PAL at the end of storage (hard fruit), but this declined as fruit ripened (Figure 8).

4. Discussion

The maturity of fruit influences their natural resistance against fungal attacks (Prusky, 1996). In order to identify why more mature fruit seem to be more susceptible to pathogen attack rather than younger fruit,one needs to determine the biochemical changes that occur during fruit aging. The same can be said of fruit as it ripens, the process moving towards greater cell ageing and senescence.

Most antifungal compounds are phenolic in nature and are produced via phenylalanine in the phenylpropanoid pathway (Hahlbrock and Scheel, 1989). PAL is an essential enzyme in this pathway; high activity of PAL would result in high phenolic concentrations and as a result increase antifungal compounds thereby potentially reducing Anthracnose development. This may show that PAL might have some inhibitory effects on the growth of the fungus and temperature is known to have different effects on Anthracnose development which therefore might explain why different results are seen at the colder temperatures (Adikaram *et al*., 1993).

Phosphoric acidacts as a fungal growth inhibitor at high concentrations while at low concentrations it can act as a defence elicitor (Guest *et al*., 1995). Silicon has been found to offer protection against fungal infections in various crops by strengthening cell walls and thus making it difficult for the fungal hyphae to penetrate and colonise the plant (Fawe *et al*., 2001). It is also thought that silicon plays an important role in enhancing host resistance to plant diseases by stimulating defence reaction mechanisms (Kaluwa *et al*., 2010). The results of this study indicate that the mechanism through which these compounds may enhance antifungal activity, could be through at least in part, an enhancement of PAL activity, which in turn alters phenolic content, and in particular, epicatechin. The mechanism is probably complex, involving modification of gene activity as well as enzyme activity. It is of note that when applied pre-harvest, phosphoric acid appears to take 7 to 14 days to exert maximal effect. This explains why better results appear to have been obtained with pre-harvest rather than postharvest applications. Storage of fruit and the temperature of the storage also appear to affect the activity of PAL after application of phosphoric acid and potassium silicate.

Temperature *per se* also plays an important role in disease incidence. Fungal rots develop once ripening has begun due to the decrease in antifungal compounds (Prusky *et al*., 1983).

The rate at which fruit ripens depends on the temperatures at which fruit is subjected. The higher the temperature, the faster fruit ripens and, therefore, fungal development increases (Hopkirk *et al*., 1994). By reducing storage temperature, fruit ripening will be slowed and rot development may be inhibited due to the continued presence of antifungal compounds. Application of elicitors such as phosphoric acid and potassium silicate could enhance antifungal compounds through stimulation of PAL activity, and combined with optimal storage and ripening temperatures, it is suggested could play a significant role in postharvest decay management.

5. Conclusions

Both phosphoric acid and potassium silicate have clearly been shown to alter PAL activity. It appears that some time is needed for enhancement of activity after the application, as shown by the optimal period of 7 to 14 days after phosphoric acid application. Postharvest conditions such as temperature also affect changes in activity. However, there appears to be sufficient evidence to suggest that further work to evaluate the concentration of specific phenolics, and epicatechin in particular and the identified avocado antifungal diene will be useful. Following this, it is suggested that development of the use of systemic resistance inducers such as phosphoric acid and potassium silicate as part of a postharvest decay control programme is possible. Once the mechanisms of decay control are better understood, it may also be possible to predict the potential for decay in any particular batch of fruit.

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7. Figures

Figure 1.‘Fuerte’ avocado fruit with no signs of Anthracnose presence or bruising.

Figure 2. ‘Fuerte’ avocado fruit with slight bruising.

Figure 3. Phenylalanine ammonia-lyase activity at specific fruit softness during ripening of ‘Fuerte’ avocados stored at room temperature (22°C). Trees were sprayed pre-harvest with phosphoric acid (500 mgL^{-1}) and harvested at 0, 7, 14 and 21 days after application. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.062.

Figure 4. Phenylalanine ammonia-lyase activity at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at 5.5°C for 28 days. Trees were sprayed pre-harvest with phosphoric acid (500 mgL^{-1}) and harvested after 0, 7, 14 and 21 days after application. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.073.

Figure 5. Phenylalanine ammonia-lyase activity at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at 2°C for 28 days. Trees were sprayed pre-harvest withphosphoric acid (500 mgL^{-1}) and harvested after 0, 7, 14 and 21 days after application. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.135.

Figure 6. Phenylalanine ammonia-lyase activity at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at room temperature (22°C). Fruit were dipped in potassium silicate (1000 mgL^{-1}) or phosphoric acid (500 mgL^{-1}) on arrival at the laboratory. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.170.

Figure 7. Phenylalanine ammonia-lyase activity at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at 5.5°C for 28 days. Fruit were dipped in potassium silicate (1000 mgL^{-1}) or phosphoric acid (500 mgL^{-1}) on arrival at the laboratory. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD = 0.183.

Figure 8. Phenylalanine ammonia-lyase activity at specific fruit softness during ripening of ‘Fuerte’ avocados after storage at 2°C for 28 days. Fruit were dipped in potassium silicate

(1000 mgL^{-1}) or phosphoric acid (500 mgL^{-1}) on arrival at the laboratory. Different letters above columns represent statistical differences at $p \leq 0.05$. LSD 0.138.

Figure 1.

Figure 2.

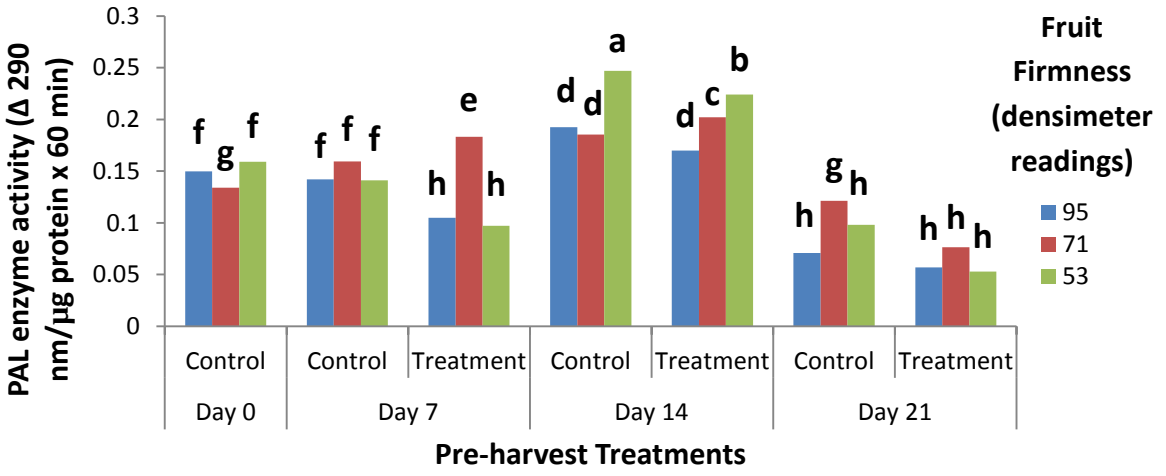


Figure 3.

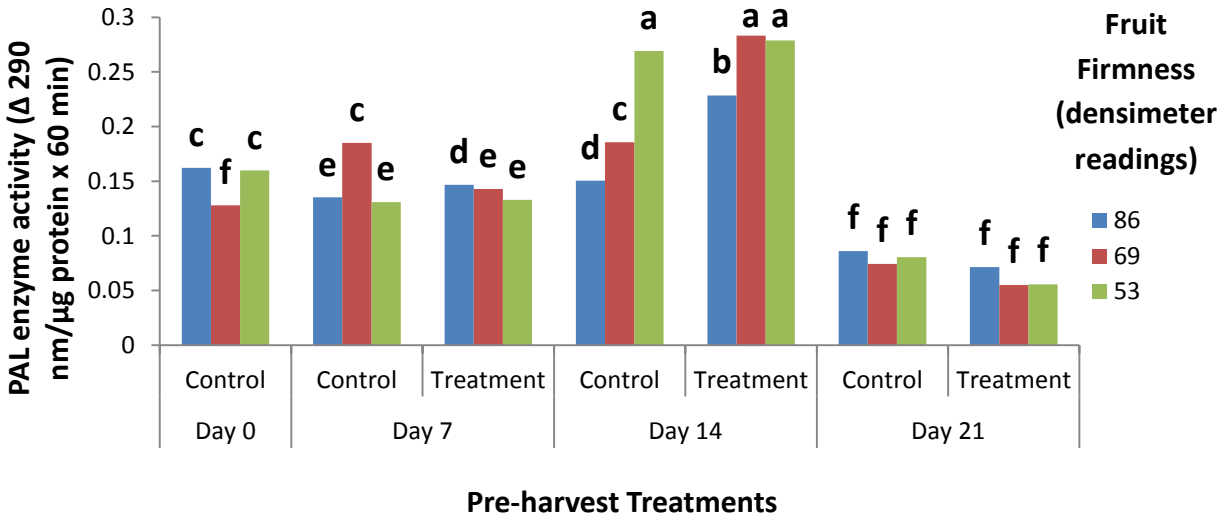
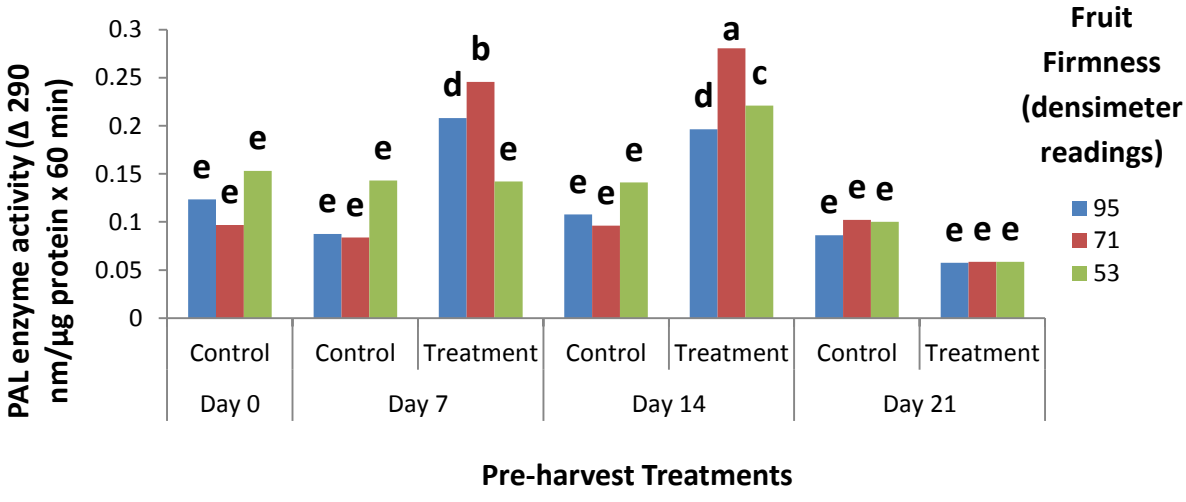


Figure 4.



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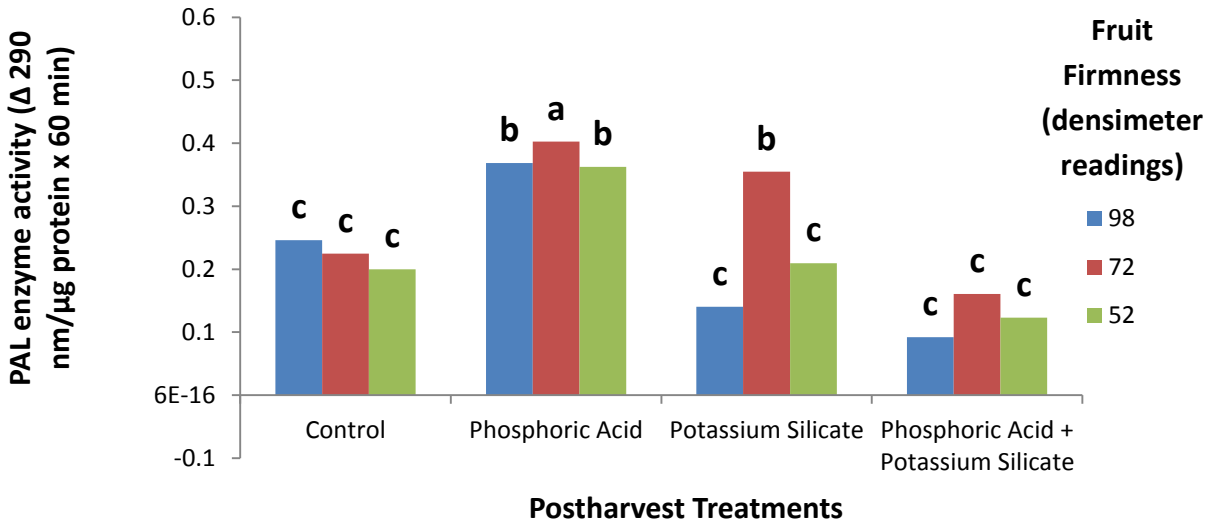

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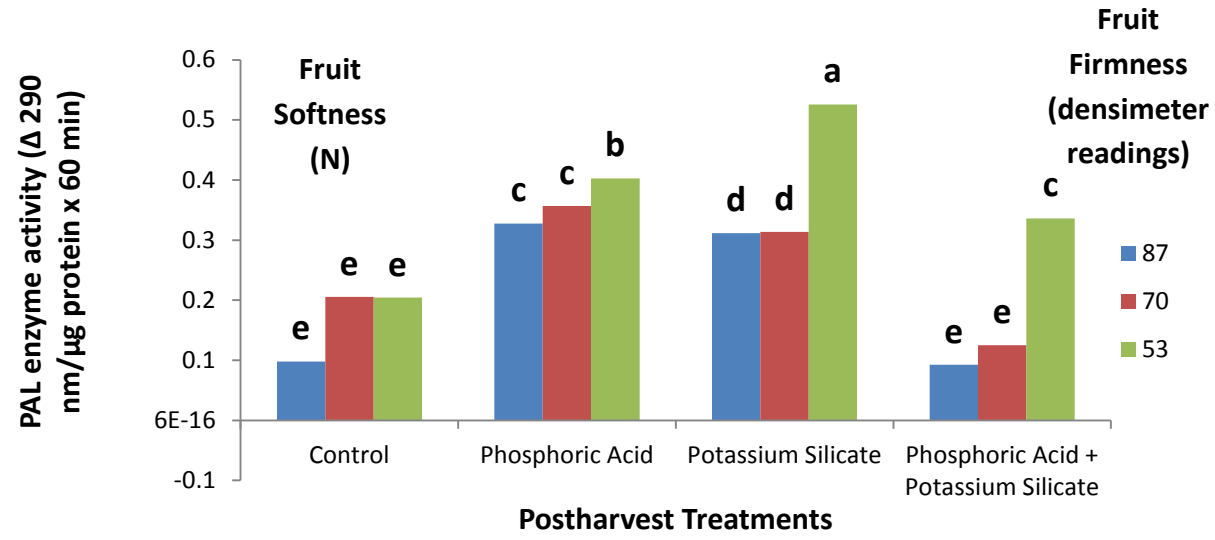

Figure 7.

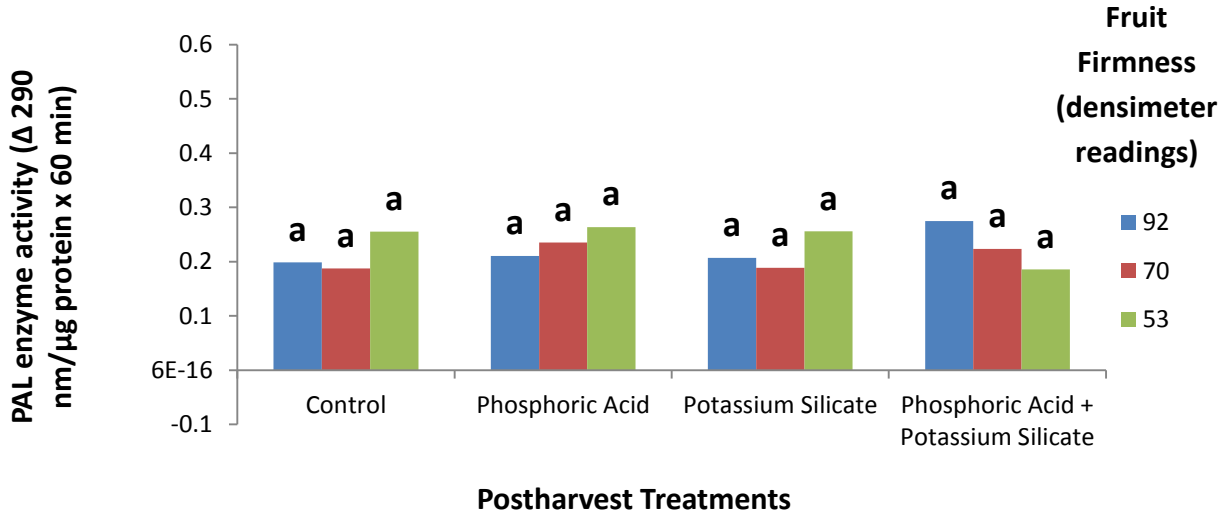

Figure 8.

CHAPTER 4

The effects of systemic resistance inducers applied pre- and post-harvest on antifungal dienes in *Persea americana* (Mill.) cv 'Fuerte' fruit

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World avocado production has consistently increased over the past decade. Long shipping periods are common to consistently provide distant markets with fruit, necessitating extended cold storage which often results in poor fruit quality and early softening. This may enhance occurrence of postharvest disease incidence. Anthracnose (*Colletotrichum gloeosporioides*), as a latent infection, may cause considerable losses. Antifungal compounds in the fruit decline during avocado fruit ripening. The objective of this study was to use systemic resistance inducers to enhance the concentration of antifungal dienes and thus decrease postharvest diseases, reducing the need for other chemical control measures, such as copper sprays. Pre-harvest sprays of Avoguard® 500 SL (500 ppm phosphoric acid a.i.) were applied to fruit trees, and fruit were harvested on the day of spraying as well as 7, 14, and 21 days thereafter. Postharvest dips of Phosguard 400 SL (500 ppm phosphoric acid a.i.) and AgriSil™ K50(1000 ppm silicon a.i.) were applied to fruit. Fruit were either ripened immediately at ambient temperature or stored for 28 days at either 5.5°C or 2°C before ripening. The general fruit appearance was evaluated during ripening and exocarp samples analysed for potential antifungal compounds. Due to possible malfunctioning of the equipment no interpretable results on antifungal compound concentrations could be obtained. However, based on results obtained in other studies, it is suggested that further work should be conducted.

1. Introduction

South Africa exports avocados over a long distance and fruit may have storage times of up to 30 days or more at temperatures of about 5.5°C. This extended storage period increases the potential for poor fruit quality that includes physiological disorders such as grey pulp and vascular browning (Leclereq, 1990). An additional problem often occurring is early softening, which can increase postharvest disease incidence (Eksteen, 1990).

Anthracnose (*Colletotrichum gloeosporioides*) infects unripe fruit and once infected, the fungus remains dormant until ripening begins (Crane, 2001). There are four mechanisms that have been thought to explain the resistance of unripe fruit to Anthracnose attack. These include the lack of nutritional requirements for the pathogen, the presence of preformed and inducible antifungal compounds and the lack of the activation of fungal pathogenicity factors (Beno-Moualem and Prusky, 2000).

Of these, antifungal compounds may be of particular importance. The compounds are concentrated in the outer layers of the fruit and in the exocarp, and act as the first line of defence and inhibit the further development (after initial infection) of the fungus (Prusky *et al*., 1991). The concentration of antifungal compounds tends to decline as fruit softens postharvest (Prusky *et al*., 1991). This reduction in antifungal concentrations has been found to correspond with an increase in the fruit's susceptibility to disease. Once the concentration of antifungal compounds has declined past a certain level, Anthracnose is able to resume development and produce symptoms (Prusky *et al*., 1991).

The antifungal compounds, 1,2,4-trihydroxyheptadec-16-yne, 1,2,4 trihydroxyheptadec-16-ene and 1-acetoxy-2, 4-dihydroxyheptadec-16-ene, have been isolated from avocado fruit peel (Adikaram *et al*., 1993). More mature fruit had lower antifungal diene levels than physiologically younger fruit. Although the antifungal diene is a preformed compound, several treatments, both abiotic and biotic, have shown to induce higher levels of antifungal dienes, and as a result increase the resistance of ripening avocado fruits to fungal attack (Prusky *et al*., 1994).

Control of this disease is important as it is usually symptomless at the time of harvest and infected fruit can therefore not be removed in the packhouse, with the disease manifesting

itself as the fruit ripens. By this stage considerable transport and marketing costs have been incurred. Control of the disease can be attained through use of various pre-harvest copper fungicides (McMillan, 1970), which although effective in control of the disease, do have certain disadvantages such as fruit residues and environmental concerns. Being contact fungicides, application timing and the need for frequent applications is also problematic. It is therefore necessary to find new methods of control. It would be particularly useful if disease resistance of the fruit could be enhanced through an increase in endogenous antifungal compounds pre- or postharvest or slower decline as fruit ripen.

Phosphoric acid has been used to achieve control against Phytophthora root rot in avocados. It does this in part by inducing plant defences (Guest *et al*., 1995) which would be in accordance with the concept of enhancing antifungal compounds for increased resistance to disease. Similarly, silicon has been found to offer protection against fungal infections in various crops by strengthening cell walls and thus making it difficult for the fungal penetration and colonisation within the plant (Fawe *et al*., 2001). It is also thought that silicon plays an important role in enhancing host resistance to plant diseases by stimulating defence reaction mechanisms (Kaluwa *et al*., 2010).

Before such compounds can be used for the purpose of Anthracnose control via the concept of altering fruit antifungal compounds, it is necessary to investigate whether they do indeed alter the concentration of such compounds in avocado fruit. The aim of this investigation was to use potassium silicate and phosphoric acid, known systemic resistance inducer compounds (Yakoby *et al*., 2002), as postharvest applications and the latter as a pre-harvest spray on ‘Fuerte’ avocado trees and fruit in order to modify exocarp antifungal concentrations.

2. Material and Methods

2.1. Fruit

Fruit were obtained from two locations in KwaZulu-Natal, Wartburg (29°27'S, 30°40'E) and Howick (29°45'S, 30°25'E). Pre-harvest applications were carried out on Wartburg fruit and postharvest treatments were applied to fruit obtained from Howick. The Howick site was a certified organic orchard, and the fruit had therefore not been exposed to the pre-harvest compounds through the use of phosphoric acid for Phytophthora root rot control. Postharvest dips of 500 mgL^{-1} Phosguard 400 SL and 1000 mgL^{-1} AgriSil™ K50 were applied to fruit after collection from the packhouse. Avoguard® 500 SL was also applied to trees (May/June) at the Wartburg site and fruit were harvested at the time of treatment as well as 7, 14 and 21 days thereafter. Fruit were then either stored at 5.5°C or 2°C for 28 days (to simulate a typical cold storage period of South African fruit exported to Europe) before ripening or left to ripen immediately at room temperature without storage.

2.2. Fruit softness

Fruit softness was determined using a hand-held densimeter (Bareiss, Oberdischingen, Germany) with a 5 mm tip (Eaks, 1966). Fruit softness was measured on a scale of 0 to 100 whereby fruit was deemed hard and unripe at 90-100, medium at 68-75 and soft (eating ripe) at 50-55. Three equally spaced readings were taken around the circumference of each fruit and the average reading recorded.

2.3. Fruit sampling

Exocarp was sampled by removal of strips (taken from the top to the bottom of the avocado (lengthways) and approximately three to four cm in width) at three stages of fruit softness readings using a densimeter (>85, 70-75, 52-54). Three strips were taken at each softness level. Mesocarp was then protected from desiccation by Vaseline and fruit allowed to continue ripening (Blakey, 2011). After removal, strips of exocarp were immediately snap-frozen in liquid nitrogen, freeze-dried and finely ground before analysis.

2.4. Extraction and analysis of antifungal dienes

A 0.1 g sample of avocado exocarp was homogenized in 5 mL 95% ethanol in an Omni-Mixer (Sorvall, DuPont Company, Newtown, CT, USA) at full speed for 1 min. The ethanol extract was dried in a Savant Vacuum Concentrator (SpeedVac, Savant, NY, USA), redissolved in 5 mL of distilled water, and the organic phase was extracted by fractionation with dichloromethane (Yakoby *et al*., 2001). Following two extractions, the lower organic phases were pooled, dried over anhydrous $MgSO_4$, and evaporated to dryness. Samples were dissolved in 2 mL of 100% ethanol and filtered through a 0.45 µm nylon filter before HPLC analysis. Samples were quantified by HPLC on a C-18 RP column eluted with 90% methanol and recorded at 260 nm after 30 min by an L-4200, UV–Vis Detector, by comparing peak areas with that of the antifungal diene standard (Wang *et al*., 2004).

2.5. Ethylene production rate

In order to check ripening physiology of treated and untreated fruit, individual fruit were incubated in 1 L containers for 30 min, into which a 20 mL GC vial was placed. Ethylene was measured using a GC-FID (DANI 1000, DANI Instruments, Monzese, Italy). A stainless steel alumina G1 column was used to determine ethylene by comparison with an ethylene standard curve. The mobile phase was instrument grade N_2 at 35 kPa. The ethylene production rate was calculated taking into account the mass and volume of fruit as well as the free space in the jar using the formula:

$$\text{Ethylene} = \frac{\text{x}\mu\text{L}CO_2}{1\ \text{L}_{\text{air}}}\text{x}\ \frac{\text{V}_{\text{headspace}}}{1}\text{x}\frac{1}{\text{m}_{\text{fruit}}}\text{x}\frac{1}{\text{t}}$$

2.6. Respiration rate

In order to check ripening physiology, treated and untreated fruit respiration rate was determined by measuring the rate of carbon dioxide evolution using an infrared gas analyser (EGM-1, PP Systems, Hitchin, UK). Individual fruit were incubated in 1 L containers for 12 min. The headspace carbon dioxide concentration was converted to respiration rate taking into account the volume and mass of fruit, as well as the free space in the jar and the ambient

carbon dioxide concentration. The following formula was used to determine the respiration rate:

$$\text{Net } CO_2(\mu L) = \frac{x\mu L CO_2}{1\ L_{air}} x\ \frac{V_{headspace}}{1} x \frac{1}{m_{fruit}} x \frac{1}{t} - \text{ambient } CO_2$$

2.7. Statistical analysis

Data were analysed as a factorial design with each treatment consisting of 20 replicates. A general analysis of variance was performed using Genstat 14th edition (VSN International, Hemel Hempstead, UK).Least significant difference (LSD) at the 5% level was used to separate treatment means.

3. Results

Ethylene production and respiration rates indicated a normal ripening pattern for the fruit. However, actual values of CO_2 and ethylene between control and sampled (exocarp removal) fruit differed. The reason for this was that the exocarp had been removed from the avocado and therefore the control of gas movement and diffusion from the avocado fruit appeared to have been changed.

The methods used to determine the presence of antifungal diene compounds in the fruit did not show any correlation between the HPLC peaks obtained from the injected samples derived from the fruit and the injected external standards. As a result antifungal compound concentrations could not be determined. It is thought that either the antifungal compound was unstable and degraded, or extraction was not successful. The external standard may also have degraded. A different external standard, methyl linoleate, was then used. However this also created difficulties. An attempt to analyse the samples with an internal standard to take into account the possible degradation failed due to failure of the HPLC during the analysis.

4. Discussion

Colletotrichum gloeosporioides attacks unripe avocado fruits in the orchard but becomes quiescent and does not develop further until fruit is harvested and ripens (Prusky, 1996). Adikarem *et al*. (1993) found that the antifungal diene (l-acetoxy-2-hydroxy-4-oxo-heneicosa-12, 15 diene) was the most suppressing of *C. gloeosporioides* in comparison with the other four antifungal compounds that were purified and analysed. They also found that there was a gradual increase in antifungal compound concentrations in the peel tissue of fruit with increasing maturity until harvest. When fruit were harvested and stored, the antifungal levels declined. Adikarem *et al*. (1993) found that it was not only the antifungal diene that contributed to disease control as had been suggested by Prusky *et al*.(1982), but the other four antifungal compounds contributed to disease control as well.

It is thought that one of the factors that may affectthe induction of resistance in unripe fruit is the abilityto react to the presence of naturally high ROS (reactive oxygen species) levels when compared with the reduced ability to react in ripe fruits (Prusky *et al*., 1996). It was also seen that avocado fruit pericarp might recognise the pathogen or the cell wall elicitors at an early stage in the infection process and, therefore react by generating ROS rapidly. This could imply that the plasma membranes of the avocado pericarp may act as a site for initial binding of elicitor molecules that could trigger a defence response (Prusky and Keen, 1993).

Beno-Moualem and Prusky (2000) hypothesized that the infection point of Anthracnose in the avocado exocarp, might subsequently become the local source for ROS production. It is thought that this will then activate the phenylpropanoid pathway which may preserve or increase the levels of the antifungal diene which would result in an inhibition of fungal development.

Anderson *et al*. (2005) found that by injecting 'Hass' trees with soluble silicon in the 2003/2004 avocado season, such fruit developed significantly less Anthracnose than untreated fruit. These authors concluded that injections of soluble silicon into avocado trees are able to reduce the severity and incidence of Anthracnose. Anderson *et al*. (2004) also showed that injecting soluble silicon into avocado trees proved successful in reducing Anthracnose incidence. This was, however, only possible if it was applied separately to phosphoric acid. Anderson *et al*. (2005) later confirmed these findings.

Phosetyl-Al has been used to control downy mildew and root rots such as Phytophthora in crops for numerous years (Hanrahan and Paviot, 1987). It has also been used extensively in the control of *Phytophthora cinnamomi*. The basipetal systemicity of phosetyl-Al allows for the translocation of the active ingredient from leaves to the root system, regardless of root depth or distance from the point of application. This natural distribution of phosetyl-Al provides protection from *Phytophthora cinnamomi* (Bezuidenhout *et al*., 1987).

From the literature cited, as well as the results of this study (Chapters 2 and 3) which indicated changes in total phenolics and PAL enzyme activity, it is suggested that despite the lack of analysis results, it is likely that the antifungal diene concentrations could have increased due to the treatments applied, and as a result could have prolonged the quiescent nature of Anthracnose in avocados.

5. Conclusions

Despite the lack of analysis results the high potential for the treatments to have altered the diene status of the fruit as indicated by the literature and other analyses in this study, makes it imperative that further work be conducted on the extraction and analysis of the compounds. This will create a better understanding of the manner in which phosphoric acid and potassium silicate may work, and clarify further the potential for their use as part of an integrated fungicide programme for prevention of Anthracnose caused by *C. gloeosporioides.*

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CHAPTER 5

Thesis Overview

1. General Discussion

Fruit diseases are a large concern for avocado farmers and with new regulatory rules as well as the increase in critical consumers, these diseases need to be controlled efficiently and effectively. The most common method of chemical control is the use of copper sprays (Manicom and Schoeman, 2008). As these are contact fungicides, adequate coverage is essential, and as such they are usually applied as high volume sprays. This results in high cost and chemical wastage which can result in the build-up of copper in soils (Duvenhage, 2002).

Anthracnose, caused by *Colletotrichum gloeosporioides*, infects avocado fruit pre-harvest and remains dormant until fruit have been picked and ripens off the tree (Nelson, 2008). The activation of fungal growth is considered to be a result of a decrease in antifungal compounds in the fruit during ripening (Prusky, 1996).

Avocados have been seen to improve in quality with the addition of silicon and phosphoric acid. Silicon has been used in various crop species, mainly members of the Poaceae, and has been found to infer host resistance to crops. Silicon has also been seen to offer protection against fungal infections in horticultural crops such as tomatoes, beans and peas. It does this by strengthening the cell walls and as a result, makes it more difficult for the fungus to penetrate the plant (Fawe *et al*., 2001). Silicon may also play an active role in enhancing host resistance to plant diseases by stimulating defence reaction mechanisms. Ma and Takahashi (2002), however, reported that the effect of silicon on plants is much more evident when a plant is under stress. Silicon has been used to increase the resistance of rice to various diseases, leaf neck blast, sheath blast, brown spot and stem rot (Datnoff and Rodrigues, 2005) and Dann and Muir (2002) found that if pea plants were grown in silicon amended potting mix, an increase in the production of defence proteins was seen.

Phosphoric acid based fungicides are systemic and are therefore able to move both upward and downward in the tree. The ability of phosphoric acid to move in this way makes the product easy to apply as either trunk paint, foliar spray or trunk injection (Menge, 1999). A phosphoric acid based fungicide, Avoguard 500 SL, contains high levels of phosphoric acid and has been found effective in controlling Phytophthora root rot.

Vidhyasekaran (1997) reported that in avocados, some phenolics may act as antioxidants and induce resistance. This increase in phenolic concentrations can therefore result in an increase in disease resistance due to the induced resistance brought about by the plants in response to the addition of phosphoric acid and potassium silicate.

The results of this study have shown that the addition of potassium silicate as well as phosphoric acid applied either pre- or postharvest resulted in an increase in phenolic concentrations as well as PAL enzyme activity. This effect was seen for all three storage temperatures tested, although, storage temperatures did have an effect on the increases recorded. This is considered an important finding. Most previous work relating to the use of these products for fruit disease control has been conducted at ambient temperature and without long storage periods. This is not realistic if the products are to be used as part of a disease control strategy on fruit exported using low temperatures for long periods. An understanding of how they work at low storage temperatures is essential, especially if there is a temperature effect.

When fruit were not stored, phenolic concentrations and PAL enzyme activity appeared to be the highest when fruit were harvested 14 days after treatment. This finding is also of importance. It means that the physiological reaction to the inducer compounds takes some time, and this needs to be taken into account. Consequently, if fruit are going to local markets, and not to the far European markets, the best or optimum time to harvest the avocados where trees have been sprayed with phosphoric acid, is 14 days after treatment. This increase in phenolics could mean that the antifungal activities within the avocados are remaining higher and therefore disease can remain at bay for a longer period of time.

Fruit stored at 5.5°C for 28 days, which replicated how avocados are stored for their long distance shipping overseas, showed similar results for enzyme activity but not for phenolic concentrations. Although, the greatest phenolic concentrations were seen for fruit harvested 7

days after treatment, treated fruit harvested 14 days after treatment showed to have an increase in phenolic concentrations during softening. This shows that fruit harvested 14 days after treatment with phosphoric acid would have an increase in phenolic concentrations while it is being shipped to Europe. On arriving in Europe, although fruit may be softer as they were stored at 5.5°C, they would have increased levels of phenolics and therefore the latent Anthracnose could remain dormant for longer due to the increased phenolic concentrations and enzyme activity which was significantly higher for fruit harvested 14 days after treatment.

Due to the increase in softening during shipping when fruit are stored at 5.5°C, shipping at 2°C was researched. This low temperature caused some differences in phenolics and PAL enzyme activity as a result. Phenolic concentrations were higher for fruit harvested 7 days after treatment, and these concentrations were higher than those found in fruit stored at room temperature and 5.5°C. It also showed concentrations to increase during softening and as a result improved disease resistance could be possible. Enzyme activity was seen to be higher for fruit harvested either 7 or 14 days after treatment and as a result, harvesting at either of these days and shipping at 2°C would result in an increase in enzyme activity. This shows that if phosphoric acid is used to treat avocados before harvest, the optimum results would be seen when fruit is harvested 7 days after treatment and shipped at 2°C. This lower shipping temperature could provide great promise for the avocado industry as fruit would remain harder for longer as well as maintain increased levels of phenolics and PAL enzyme and as a result, an increase in induced antifungal defence compounds being produced and a longer protection period against the latent Anthracnose.

Postharvest dips did not show as much of an increase in phenolic concentrations as pre-harvest sprays. This was possibly due to the lag time in reaction after application for pre-harvest applications. Where fruit was ripened without storage, softening occurred faster than the phenolic synthesis, and where fruit were stored, lower temperatures may have influenced the reaction. PAL enzyme activity was lowest for fruit stored at 2°C, which could explain the effects of temperature on phenolics. The highest phenolic concentrations were seen for fruit remaining at room temperature, and as a result fruit being sent to local markets would benefit from this type of treatment more than those sent to European markets.

Potassium silicate proved to be slightly more beneficial in inducing phenolic concentrations than phosphoric acid. Overall, it is suggested that a combination of the two treatments of

phosphoric acid and potassium silicate could be used as postharvest dips in order to obtain increased phenolic and PAL enzyme levels and as a result increase disease protection. These postharvest dips seem to be much more beneficial for fruit destined to local markets, and appear to give little added effect at the lower temperatures and would therefore not be of much use.

The hypothesis that addition of postharvest dips of potassium silicate and phosphoric acid and pre-harvest sprays of phosphoric acid to 'Fuerte' avocado fruit will enhance the phenolic concentrations, the activity of phenylalanine ammonia lyase (PAL) and the production of antifungal compounds, thereby demonstrating the possibility of their use in a new approach to Anthracnose control, was found to be mostly correct. However, the judgement cannot be definitive as antifungal diene concentrations could not be determined.

Due to a lack of antifungal results, a true conclusion cannot be made.

2. Future Research

The use of potassium silicate and phosphoric acid applied pre- and postharvest to increase antifungal diene concentrations to lengthen the quiescent nature of Anthracnose needs to be researched further. The stability of the antifungal diene is questionable and new methods may need to be investigated in order to obtain adequate results. The investigations into phenolic concentrations and PAL enzyme activity do, however, show great promise and as a result, diene concentrations can be presumed to increase. The results obtained are sufficiently positive to warrant further research with different postharvest fruit treatments.

South African fruit is normally waxed for export, and the role of waxing in the absorption of postharvest applications needs further investigation, as possible interactions of waxing with potassium silicate or phosphorous acid are unknown. Different cultivars should also be considered, as it is unknown whether the physiology of cultivars other than 'Fuerte' will be similar. Considerable quantities of fruit shipped from South Africa are treated with 1-MCP (1-Methylcyclopropene), modifying ethylene metabolism and prolonging the ripening phase of fruit (Nelson, 2005); this may also have an effect on the development of Anthracnose and may influence phenolic production, and therefore a combination of 1-MCP and the treatments (potassium silicate and phosphorous acid) should also be investigated.

Ardi *et al*. (1998) found that an increase in PAL activity was highly correlated with an increase in epicatechin concentrations and as a result disease resistance. Prusky *et al*. (1994) researched the induction of PAL in order to determine whether disease reduction was affected by an increased epicatechin content of the fruit. It could be beneficial to therefore investigate specific phenolics such as epicatechin, with the addition of phosphoric acid and potassium silicate, both pre- and postharvest.

Prusky *et al*. (1992) found that the lipoxygenase enzyme might be involved in the decrease of the antifungal diene compound, which decreases as fruit ripens. These authors found that the activity of the enzyme in avocado extracts increased during fruit ripening. By inhibiting this enzyme, Prusky *et al*. (1992) found that decay of the avocado was delayed. Activation of the disease results from the decrease of the antifungal diene, which is catalyzed by lipoxygenase and regulated by the decline of its inhibitor epicatechin. As PAL is a critical enzyme in the enhancement of epicatechin, it would be reasonable to expect that, in the presence of high PAL activity, this phenolic will be enhanced, or at least a decline to be reduced. This would be critical for maintaining the presence of the antifungal diene, and therefore enhanced resistance to fungi such as those in the Anthracnose complex.

A further area of investigation is nutrigenomics. Nutrigenomics is the relationship between gene activity and cell function in response to environmental factors (Fogg-Johnson and Kaput, 2003) which could include the application of phosphoric acid and potassium silicate. The application of this in plants is, however, little researched, although it could enhance the knowledge of the mode of action considerably. Nutrigenomics has been researched for the control of soil-borne diseases on vegetable crops such as *Phytophthora capsici*, *Sclerotium rolfsii*, *Ralstonia solanacearum*. The efficacy of this management process is currently limited but the development of integrated programs could be highly desirable for the sustainable management of such diseases (Pingsheng, 2012).

3. Conclusions

Silicon and phosphoric acid have the potential to increase disease resistance as both a systemic resistance inducer as well as a fungicide. Silicon and phosphoric acid applied post-harvest increased phenolic concentrations as well as PAL enzyme activity which could lead to the potential control of Anthracnose in avocados. Phosphoric acid applied pre-harvest also appeared to increase phenolic concentrations and enzyme activities with no harm caused to the fruit. The addition of phosphoric acid as a pre-harvest spray and shipping at 2°C could result in less ripe fruit arriving in Europe with less disease incidence. Although results were not obtained for antifungal diene concentrations, it is suggested that they would increase due to the addition of the applied treatments from the results obtained for phenolics and enzyme activity. Therefore, sufficient results are now available to suggest that a disease management programme for Anthracnose which includes systemic resistance inducers is possible, although further research is needed.

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APPENDIX A

Publications and Conference Outputs

1. Publications

Bosse, R.J., Bower, J.P. and Bertling, I. 2011. Pre- and post-harvest treatments on 'Fuerte' avocados to control Anthracnose (*Colletotrichum gloeosporioides*) during ripening. South African Grower's Association Yearbook 34: 65-69.

Bosse, R.J., Bower, J.P. and Bertling, I. 2012. Systemic Resistance Inducers Applied Pre-Harvest for *Colletotrichum gloeosporioides* Control in Avocados. Acta Horticulturae (*in press*).

Bosse, R.J., Bower, J.P. and Bertling, I. 2012. Systemic Resistance Inducers Applied Pre-Harvest for Anthracnose Control in 'Fuerte' Avocados. South African Grower's Association Yearbook (*in press*).

2. Conferences

Bosse, R.J., Bower, J.P. and Bertling, I. 2011. Postharvest Treatments on 'Fuerte' Avocados to control Anthracnose (*Colletotrichum gloeosporioides*) and prolong fruit ripening. Combined Congress, Pretoria, South Africa.

Bosse, R.J., Bower, J.P. and Bertling, I. 2011. Pre- and post-harvest treatments on 'Fuerte' avocados to control Anthracnose(*Colletotrichum gloeosporioides*) during ripening. Annual SAAGA Conference, Tzaneen, South Africa.

Bosse, R.J., Bower, J.P. and Bertling, I. 2011. Systemic resistance inducers applied postharvest for potential control of Anthracnose (*Colletotrichum gloeosporoides*). World Avocado Congress, Cairns, Australia.

Bosse, R.J, Bower, J.P. and Bertling, I 2012. Systemic Resistance Inducers Applied Pre-Harvest for *Colletotrichum gloeosporioides* Control in Avocados. The 2nd All Africa Horticulture Congress, Skukuza, Kruger National Park, South Africa.

Bosse, R.J, Bower, J.P. and Bertling, I. 2012. Systemic Resistance Inducers Applied Pre-Harvest for Anthracnose Control in 'Fuerte' Avocados. Annual SAAGA Conference, Tzaneen, South Africa.