New strategies for the control of avocado fruit diseases

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PROJECT AV01004

Final Report

“New Strategies for the Control of Avocado Fruit Diseases”

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Purpose of report: The purpose of this report is to present the final results of all activities conducted under HAL Project AV01004 "New strategies for the control of avocado fruit diseases". The report also provides a summary of project findings, a description of technology transfer activities, and recommendations arising from the outcomes of the project. The overall objective of this project was to improve disease management practices for 'Hass' avocado based upon a number of approaches including rootstock selection, management of tree nutrition and application of new products such as plant defence activators and fungicides.

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MEDIA SUMMARY

Loss of avocado fruit in the marketplace due to anthracnose and stem-end rots is still a major concern for both growers and consumers. The ability of the rot causing fungi, to remain in a dormant or latent phase until after the fruit commences ripening makes disease control very difficult. In a previous project (AV97001), significant advances were made to control rots with the world first discovery that rootstock race affects the severity and incidence of fruit rots. This project aimed to advance these rootstock studies as well as investigate new products available for disease control, assess harvesting methods and evaluate other strategies such as cross-protection using non-pathogenic strains of the fungus (*Colletotrichum gloeosporioides*) that cause anthracnose rots. This project also included a PhD study into the preharvest disease, pepper spot. This research was also based on a discovery made in the previous project (AV97001) that pepper spot and anthracnose are caused by the same fungus, *Colletotrichum gloeosporioides*. The pepper spot investigations aimed to answer the questions of how and why the same fungus was able to cause two different disease symptoms and how we can control it.

A series of field experiments were conducted to broaden the rootstock and nutrition research. One set of field trials examined if the form of nitrogen fertiliser (ammonia vs nitrate) affects disease in 'Hass' fruit grown on 'Velvick' or 'Duke 6' rootstocks. The West Indian 'Velvick' rootstock was found to have a less vegetative canopy, higher crop load, better balance of mineral accumulation and less anthracnose than the Mexican 'Duke 6' rootstock. However, this rootstock effect was lost in the second season, possibly due to very high disease incidences. The form of nitrogen fertiliser did not affect anthracnose rots. However, withholding nitrogen fertiliser resulted in trees with less vegetative canopies, a better balance of nutrients, lower fruit skin pH levels and a tendency to have fewer anthracnose rots.

Another series of rootstock experiments compared a broader range of rootstocks for disease susceptibility. The Guatemalan ('A8', 'A10', 'Nabal') rootstocks were also found to be superior for disease control compared with the Mexican ('Parida 1') rootstock. These rootstock effects however, were not apparent in the last two seasons, possibly due to heavier crop loads on 'Parida 1'. In both rootstock studies, strong relationships between disease levels and mineral nutrient concentrations (in particular nitrogen and calcium) were evident. This suggests the balance of nutrients in the tree has an important predictable impact on fruit rots.

No new products were discovered for superior disease control but harvesting method recommendations were devised. Cross-protection studies require further research but still provide potential for new disease control strategies in the future.

The pepper spot studies discovered the fungus causing pepper spot and anthracnose belong to the one population but this population is highly variable. However, the same fungus causing anthracnose on mango is less aggressive on avocado and forms its own distinct population. This means the cross-infectivity potential of the fungus from mango to avocado is low in mixed orchards. The severity of pepper spot in orchards can be minimised by reducing tree stress and using Guatemalan or West Indian instead of Mexican rootstocks. It appears the fungus infects the fruit in a similar manner to cause both pepper spot and anthracnose but different symptoms are observed primarily due to differences in the fruits response. A better understanding of this differing fruit response may reveal new disease control strategies for the future.

TECHNICAL SUMMARY

Anthracnose and stem-end rot diseases continue to cause major postharvest loss of avocado fruit. Studies undertaken in a previous project (AV97001) successfully identified rootstock and tree nutrition (nitrogen and calcium) as two major factors affecting fruit quality and disease susceptibility. This current project aimed to build upon the findings of the previous work to improve disease management practices for 'Hass' avocado. Rootstock studies were expanded to include a wider range of Guatemalan/West Indian rootstocks and differences between rootstocks were further quantified by examining skin pH, tree phenology and crop load differences. Studies were also conducted to evaluate new products (eg., new copper formulations, plant defence activators, anti-gibberellins), harvesting method (snap vs clip) and cross-protection using non-pathogenic strains of *Colletotrichum gloeosporioides* for disease control.

In association with a closely related project funded by the Cooperative Research Centre for Tropical Plant Protection, a PhD student, Fiona Giblin, undertook extensive studies into the preharvest disease, pepper spot. This study was based on the discovery (project AV97001) that pepper spot is caused by the same fungus that causes anthracnose, *Colletotrichum gloeosporioides*. This research determined the degree of pathogenic and genetic variation in the avocado *C.g* population, identified the major factors affecting the development and spread of pepper spot infections and examined the infection process of *C.g* in relation to pepper spot and latent anthracnose infections.

A series of field experiments were conducted to broaden the rootstock and nutrition research. One set of field trials examined if the form of nitrogen fertiliser (ammonia vs nitrate) affects disease in 'Hass' fruit grown on 'Velvick' or 'Duke 6' rootstocks. These studies revealed that rootstock influences tree phenology (vegetative flushing, canopy colour), crop load, mineral nutrient accumulation and postharvest anthracnose susceptibility. The West Indian 'Velvick' rootstock was found to have a less vegetative canopy, higher crop load, better balance of mineral accumulation (low N/Ca, high Ca+Mg/K) and less anthracnose than the Mexican 'Duke 6' rootstock. However, this rootstock effect was lost in the second season, possibly due to very high disease incidences (>75%). Nitrogen fertiliser form (ammonium vs nitrate) did not significantly affect tree phenology, crop load, mineral nutrient concentrations or disease levels. However, withholding nitrogen fertiliser resulted in less vegetative canopies, a better balance of nutrients, a lower fruit skin pH and showed a trend of less postharvest anthracnose (although not significant).

Another series of rootstock experiments compared a broader range of rootstocks for disease susceptibility. The Guatemalan ('A8', 'A10', 'Nabal') rootstocks were also found to be superior for disease control compared with the Mexican ('Parida 1') rootstock. These rootstock effects however, were not apparent in the last two seasons, possibly due to heavier crop loads on 'Parida 1'.

Correlations between disease levels and nutrient concentrations were strong across both rootstock studies with lower disease levels related to more favourable balances of N/Ca and Ca+Mg/K ratios.

The current industry standard copper products (copper oxychloride and copper hydroxide) were found to be as effective as the four other new formulations tested. The new soluble silicon defence promoting product, PhotoFinish™, was found to be ineffective to induce a large enough host response. Another silicon product has been sourced and has shown much better disease control in preliminary trials. Other new products tested for disease control,

included Biocoat® and the anti-gibberellin, Sunny®. Both of these did not influence disease susceptibility.

The method of harvesting 'Hass' avocado (snap vs clip) was found to significantly affect disease development. Based on our findings, it is recommended that fruit should be snap harvested only if 1) trees are healthy and not under stress, 2) fruit is fully mature but not over mature (ca. 23-29% dry matter), 3) fruit is not harvested in wet humid weather and 4) growth regulants (eg., Sunny®) have not been used.

The potential to use non-pathogenic strains of *Colletotrichum gloeosporioides* to cross-protect against anthracnose was also investigated to a limited extent in this project. Unfortunately, these studies revealed problems with transmission techniques which require further research before a complete assessment of cross-protection can be made.

DNA fingerprinting studies found that *C.g* isolates from avocado causing pepper spot and anthracnose belong to one heterogenous population. However, *C.g* isolates collected from mango anthracnose were found to be less aggressive than *C.g* isolates from avocado and formed a distinct homogenous population. This means the cross-infectivity potential of *C.g* from mango to avocado is low. Pepper spot severity in orchards can be minimised by reducing tree stress (eg., Phytophthora root rot, water deficit, sunburn) and using Guatemalan or West Indian ('Velvick', 'A8', 'A10', 'Nabal') instead of Mexican ('Duke 6', 'Parida 1') rootstocks. The infection process of *C.g* causing pepper spot was not observed to be significantly different from *C.g* causing latent anthracnose infections.

1. INTRODUCTION

Postharvest diseases, particularly anthracnose and stem-end rot, continue to be a major quality issue for the retail marketing of 'Hass' avocado fruit (Hofman and Ledger, 2001). Symptoms of these diseases develop as the fruit ripens, but the dark skin of ripe 'Hass' fruit make it difficult for the consumer to detect these diseases until fruit are cut open at home.

Anthracnose, the most serious disease of avocado fruit, is predominantly caused by the fungus *Colletotrichum gloeosporioides* in Australia, although *C. acutatum* can also cause the disease in a minority of cases. The initial stages of infection by the anthracnose pathogen occur in the orchard on developing fruit. The fungus penetrates the outer wax layer and cuticle of the fruit skin, where it ceases growth and enters into a dormant or "quiescent" state until fruit ripening commences. During fruit ripening the fungus resumes growth, presumably due to a decline in the concentration of antifungal compounds called "dienes" (Prusky, 1996). In unripe fruit, diene levels are high and prevent the fungus from invading cells. However during fruit ripening, diene concentrations fall, allowing the fungus to resume growth.

Stem-end rot can be caused by a number of different fungi including *Botryosphaeria* spp. (*Dothiorella* spp.), *Lasiodiplodia theobromae* and *Phomopsis perseae*. The anthracnose pathogen *C. gloeosporioides* can also cause a stem-end rot of avocado fruit. There are a number of possible ways that the stem-end rot fungi can infect avocado fruit, although no comprehensive studies have been done to clearly establish the stem-end rot disease cycle in this host. One proposed mode of infection is that the fungi occur as symptomless "endophytes" in the stem tissue of avocado, gradually colonising inflorescences, fruit pedicels and fruit stem-end tissue as they form. Other theories are that the stem-end rot fungi infect avocado at flowering, or invade at harvest time through the freshly cut surface of the fruit pedicel (Everett, 1999). It is possible that each of these infection modes may be involved depending on the circumstances. Regardless, symptoms of the disease remain quiescent until fruit ripening like anthracnose.

Field diseases such as pepper spot and sooty blotch can also impact on the quality of 'Hass' avocado fruit, resulting in either rejection of fruit or downgrading of fruit quality during packing. Pepper spot in particular has become more prevalent in 'Hass' fruit over recent years, and is especially severe on fruit affected by mild sunburn. Thus, the disease can be quite common in trees affected by *Phytophthora* root rot where the canopy density is reduced and sunlight exposure to fruit is increased. Pepper spot is caused by the same fungus which causes anthracnose, *Colletotrichum gloeosporioides* (Willingham *et al*., 2000). Symptoms of the disease appear as small, shiny, black raised lesions on the surface of fruit skin and pedicels as well as on twigs and leaf petioles.

Research conducted under a previous HAL project (AV97001 – Field management of avocado postharvest diseases) has formed the foundation for the studies conducted under this project. In Project AV97001, considerable progress was made towards improving current disease management practices for 'Hass' avocado fruit. Rootstock selection was shown to have a major influence on disease susceptibility, with higher levels of anthracnose occurring in fruit from 'Hass' trees grafted to the Mexican rootstock 'Duke 6' than in those grafted to the West Indian rootstock 'Velvick'. Furthermore, this was related to differences in mineral nutrient levels in fruit skins (particularly nitrogen and calcium) as well as levels of antifungal dienes in leaves. Field studies showed that limiting the application of ammonium-based nitrogenous fertilisers could significantly reduce anthracnose levels in ripe fruit.

One of the aims of the current project therefore was to expand studies of rootstock effects on fruit diseases. A wider range of Guatemalan and Mexican rootstocks were evaluated in this project for their influence on disease incidence and severity in 'Hass' fruit. Investigations were also carried out on the effects of applying ammonium-based versus nitrate-based nitrogenous fertilisers to 'Hass' trees grafted to either 'Duke 6' or 'Velvick' rootstocks. The relationship of rootstock and fertiliser regime to disease levels, fruit and leaf nutrient concentrations, fruit skin pH and antifungal diene levels were defined in these studies. The project also evaluated a range of new products for fruit disease control including new formulations of copper-based fungicides, host defence promoting compounds (eg. acibenzolar-S-methyl and soluble silicon), Biocoat® (a product from Westfalia Estate, South Africa) and Sunny® (an anti-gibberellin). A study of the effect of harvesting methods (ie. clip vs snap harvesting) on stem-end rot and stem-end anthracnose in 'Hass' fruit was conducted using fruit grown in three different regions. The genetic diversity of the pepper spot pathogen (*Colletotrichum gloeosporioides*) was investigated on the basis of molecular analysis and host pathogenicity, and epidemiological studies were conducted to identify the most important factors contributing to the development of this disease. Finally, the potential for using endophytic or non-pathogenic strains of *Colletotrichum gloeosporioides* for cross-protection against anthracnose was investigated to a limited extent.

2. MATERIALS AND METHODS

2.1 Rootstock and Nutrition Studies

A. Rootstock x fertiliser studies

2001/02 Season

The aim of this experiment was to evaluate the effect of nitrogen fertiliser concentration and formulation type on fruit diseases in 'Hass' avocado grafted to 2 different rootstocks.

The trial was conducted on a block of 6½ year old 'Hass' avocado trees on Graham Anderson's property at Duranbah. The trees were grown on seedling 'Velvick' (West Indian) and 'Duke 6' (Mexican) rootstocks. A completely randomised design with 5 nitrogen (N) fertiliser treatments and 6 single tree replications /treatment was used for each rootstock. The following treatments were applied:

1. Nil N - no nitrogen fertiliser applied
2. Control ammonium- standard rate @ 13.3% (3.8 g NH_4^+-N/m^2/month)
3. High ammonium - double rate @ 26.6% (7.6 g NH_4^+-N/ m^2/month)
4. Control nitrate - standard rate @ 13.3% (3.8 g NO_3^- -N/ m^2/month)
5. High nitrate- double rate @ 26.6% (7.6 g NO_3^- -N/ m^2/ month)

A total of 12 fertiliser applications were made in total during the trial commencing in September 2001. Applications were made at fortnightly intervals during the first 3 months of the trial and then monthly until May 2002 in order to concentrate nitrogen applications during times of peak calcium uptake into fruitlets.

All trees received the standard fungicide spray program as applied by the grower.

At monthly intervals commencing in December 2001 and finishing in April 2002, trees were assessed for crop load, leaf flushing and canopy colour. Crop load was rated on 4 quadrants within each tree using a 1-3 scale where 1 = light crop load, 2 = moderate crop load and 3 = heavy crop load. Leaf flushing was rated on 4 quadrants within each tree using a 1-5 scale where 1 = no shoots flushing, 2 = 25% of shoots flushing, 3 = 50% of shoots flushing, 4 = 75% of shoots flushing and 5 = 100% of shoots flushing. Canopy colour was rated for each tree on a 1-3 scale where 1 = yellow/green, 2 = light green and 3 = dark green.

In May and July 2002, 16 leaves (4 leaves per quadrant) were sampled from non-fruiting terminals on each tree for analysis of mineral nutrients. In July 2002, 4 leaves (1 leaf per quadrant) were also sampled from each tree for diene analysis, and 8 fruit per tree (2 fruit per quadrant) were sampled for dry matter and mineral nutrients (flesh) and for dienes and mineral nutrients (skins). The green mature fruit were peeled to obtain skin samples, and the skin and leaves were dried for five days at 60°C. Dried and ground samples were sent to CASCO Agritech for complete nutrient analysis.

The pH of avocado fruit skin is thought to be involved in the regulation of the pathogenicity of the anthracnose fungus *Colletotrichum gloeosporioides* (Yakoby *et al*., 2000). Some cultivars that are resistant to anthracnose maintain skin pH levels of 5.5 or less during

ripening, whilst in susceptible cultivars the skin pH is initially low but increases above pH 5.5 as ripening progresses. The effect of rootstock and fertiliser on the skin pH as the fruit ripened was examined by harvesting fruit from the nil nitrogen, high ammonium and high nitrate treatments from both 'Velvick' and 'Duke 6' rootstocks. The pH was measured at three stages during ripening (green mature, sprung and eating soft) by slicing a fine layer of skin off the side of the fruit and measuring the pH using a flat-end pH probe. Measurements were taken at three points around the circumference of the fruit and the value averaged.

In July 2002, 2 trays of fruit (1 tray from the eastern side, 1 tray from the western side) were harvested from each tree and brought to the laboratory where they were ripened at 22^{o}C and 65% RH. At eating ripe stage, fruit were cut open and assessed for severity of postharvest diseases (% surface area affected by anthracnose and stem-end rot). The causal agents of stem-end rot were determined by isolation of diseased tissue onto streptomycin-amended potato dextrose agar.

Statistical analyses were conducted with Genstat® 5 release 4.21 data analysis software (Lawes Agricultural Trust, Rothamsted Experimental Station) using a completely randomised split-plot analysis of variance with treatments and rootstocks applied to trees as the whole plots and side (east and west) of the tree as the sub-plots. Arcsine angular transformations were applied to percentage data. However, if examination of residual plots indicated transformation did not improve the distribution of residuals, untransformed data is presented. Pair-wise testing between means was done using the protected least significant difference (LSD) procedure at $P = 0.05$.

2002/03 Season

During 2002/03 the previous year's fertiliser trial was repeated at the same site so that we could evaluate the effects of the different nitrogen formulations and concentrations on postharvest disease susceptibility of 'Hass' fruit grown on 2 different rootstocks over 2 consecutive seasons.

The same 5 fertiliser treatments were applied as previously described to 6 single tree replications for each of 2 rootstocks ('Velvick' and 'Duke 6') in a completely randomised design.

A total of 10 fertiliser applications were made in total during the trial commencing in November 2002. Applications were made at fortnightly intervals during the first 3 months of the trial and then monthly until May 2003 in order to concentrate nitrogen applications during times of peak calcium uptake into fruitlets.

All trees received the standard fungicide spray program as applied by the grower.

In May and July 2003, 16 leaves (4 leaves per quadrant) were sampled from non-fruiting terminals on each tree for analysis of mineral nutrients. In July 2003, 4 leaves (1 leaf per quadrant) were also sampled from each tree for diene analysis, and 8 fruit per tree (2 fruit per quadrant) were sampled for dry matter and mineral nutrients (flesh) and for dienes and mineral nutrients (skins). The green mature fruit were peeled to obtain skin samples, and the skin and leaves were dried for five days at 60°C. Dried and ground samples were sent to CASCO Agritech for complete nutrient analysis.

The effect of rootstock and fertiliser on the skin pH as the fruit ripened was again examined by harvesting fruit from the nil nitrogen, high ammonium and high nitrate treatments from

both 'Velvick' and 'Duke 6' rootstocks. The pH was measured at three stages during ripening (green mature, sprung and eating soft) by slicing a fine layer of skin off the side of the fruit and measuring the pH using a flat-end pH probe. Measurements were taken at three points around the circumference of the fruit and the value averaged.

In August 2003, 2 trays of fruit (1 tray from the eastern side, 1 tray from the western side) were harvested from each tree and brought to the laboratory where they were ripened at 22^{o}C and 65% RH. At eating ripe stage, fruit were cut open and assessed for severity of postharvest diseases (% surface area affected by anthracnose and stem-end rot). The causal agents of stem-end rot were determined by isolation of diseased tissue onto streptomycin-amended potato dextrose agar.

Crop load was assessed at harvest time in August on a rating scale of 1 to 3, where 1 = light, 2 = moderate and 3 = heavy.

Statistical analyses were conducted with Genstat® 5 release 4.21 data analysis software (Lawes Agricultural Trust, Rothamsted Experimental Station) using a completely randomised split-plot analysis of variance with treatments and rootstocks applied to trees as the whole plots and side (east and west) of the tree as the sub-plots. Arcsine angular transformations were applied to percentage data. However, if examination of residual plots indicated transformation did not improve the distribution of residuals, untransformed data is presented. Pair-wise testing between means was done using the protected least significant difference (LSD) procedure at $P = 0.05$.

B. Rootstock only studies

2001/02 Studies

The aim of this experiment was to determine the influence of 4 different rootstocks on the incidence and severity of postharvest disease in 'Hass' avocado fruit.

The trial was conducted on a block of 3½ year old 'Hass' avocado trees on Graham Anderson's property at Duranbah. The trees were grown on seedling 'Anderson 8' (Guatemalan), 'Anderson 10' (Guatemalan x Mexican), 'Nabal' (Guatemalan) and 'Parida 1' (Mexican) rootstocks. A completely randomised design with 10 single tree replications for each rootstock was used.

In July 2002 eight fruit per tree (2 fruit per quadrant) were sampled for dry matter and mineral nutrients (skins). In July 2002, trees were strip picked and all fruit from each tree were counted and weighed for determination of crop load. One tray of fruit was retained from each tree and brought to the laboratory where it was ripened at 22^{o}C and 65% RH. At eating ripe stage, fruit were cut open and assessed for severity of postharvest diseases (% surface area affected by anthracnose and stem-end rot). The causal agents of stem-end rot were determined by isolation of diseased tissue onto streptomycin-amended potato dextrose agar (SPDA).

2002/03 Studies

The rootstock only trial was repeated in the 2002/2003 season to further examine the effect of rootstock on the development of anthracnose. The trial was conducted using the same trees as the previous season.

In May 2003, 16 leaves (4 leaves per quadrant) were sampled from non-fruiting terminals on each tree for analysis of mineral nutrients. In June 2003, 8 fruit per tree (2 fruit per quadrant) were sampled for dry matter and mineral nutrients (skin).

In June 2003, trees were strip picked and all fruit from each tree were counted and weighed for determination of crop load. One tray of fruit was retained from each tree and brought to the laboratory where it was ripened at 22^{o}C and 65% RH. At eating ripe stage, fruit were cut open and assessed for severity of postharvest diseases (% surface area affected by anthracnose and stem-end rot). The causal agents of stem-end rot were determined by isolation of diseased tissue onto SPDA.

Tree diameters and heights were measured and the canopy volume of each tree was determined. This data and tree yields were used to determine the number and weight of fruit per cubic metre of canopy.

2003/04 Studies

The rootstock only trial was repeated in the 2003/2004 season to further examine the effect of rootstock on the development of anthracnose. Due to the size of the trees and the crop loads, the trial was conducted using the first six replicate trees for each rootstock used in previous seasons. The trees selected were growing on a uniform soil type.

In May 2004, 16 leaves (4 leaves per quadrant) were sampled from non-fruiting terminals on each tree for analysis of mineral nutrients. In August 2004, 8 fruit per tree (2 fruit per quadrant) were sampled for dry matter and mineral nutrients (skin). Ten fruit were sampled from around the tree for three replicates of each of the rootstocks for skin pH measurement at the green mature and the eating ripe stages.

In August 2004, trees were strip picked and all fruit from each tree were counted and weighed for determination of crop load. One tray of fruit was retained from each tree and brought to the laboratory where it was ripened at 22^{o}C and 65% RH. At eating ripe stage, fruit were cut open and assessed for severity of postharvest diseases (% surface area affected by anthracnose and stem-end rot). The causal agents of stem-end rot were determined by isolation of diseased tissue onto SPDA.

Tree diameters and heights were measured and the canopy volume of each tree was determined. This data and tree yields were used to determine the number and weight of fruit per cubic metre of canopy.

2.2 New Product Studies

A. New copper formulations

1. Efficacy experiment

The aim of this experiment was to evaluate new copper fungicides for the control of preharvest and postharvest fruit diseases in 'Hass' avocado. This experiment was conducted on Graham Anderson's property at Duranbah on 'Hass' avocado on 'Edranol' rootstock during the 2000/2001 cropping season.

A completely randomised design with 7 treatments and 5 single tree replications/treatment were used. The following treatments were applied as monthly foliar sprays (or fortnightly during wet weather) commencing late 2000:

1. Untreated
2. Kocide (ai. 500g/kg copper hydroxide) at 2g/L
3. Kocide® Blue (ai. 350g/kg copper hydroxide) at 1.5g/L
4. Liquicop® (ai. 80g Cu/L as copper ammonium carbonate) at 5ml/L
5. Copper oxychloride at 4 g/L
6. Kocide® Liquid Blue SC (ai. 360g/L copper hydroxide) at 1.5mL/L
7. Norshield® at 2mL/L

Approximately 10 L of fungicide suspension was applied to each tree or until runoff. Once fruit reached commercial maturity they were harvested, examined for field disease (pepper spot, sooty blotch) and visible fungicide residues, ripened at 22°C (65% RH) and assessed for postharvest diseases (anthracnose, stem-end rot). Any symptoms of foliar and fruit phytotoxicity or loss of tree vigour were also monitored throughout the experiment.

2. Phytotoxicity experiments

Two experiments were carried out to evaluate any phytotoxic effects of the new copper fungicides when used in combination with phosphonate foliar sprays for the control of avocado diseases. They were conducted on Graham Anderson's property at Duranbah on 'Hass' avocado on 'Edranol' rootstock during the 2000/2001 cropping season.

Experiment 1: A completely randomised design with 8 treatments and 2 single tree replications/treatment was used. The following treatments were applied as monthly foliar sprays commencing early 2001:

1. Control – potassium phosphonate (0.5% at pH 7.8)
2. Kocide (ai. 500g/kg copper hydroxide) at 2g/L + potassium phosphonate (0.5% at pH 7.8)
3. Kocide® Blue (ai. 350g/kg copper hydroxide) at 1.5g/L + potassium phosphonate (0.5% at pH 7.8)
4. Liquicop® (ai. 80g Cu/L as copper ammonium carbonate) at 5ml/L + potassium phosphonate (0.5% at pH 7.8)
5. Copper oxychloride at 4 g/L + potassium phosphonate (0.5% at pH 7.8)
6. Kocide® Liquid Blue SC (ai. 360g/L copper hydroxide) at 1.5mL/L + potassium phosphonate (0.5% at pH 7.8)
7. Norshield® at 2mL/L + potassium phosphonate (0.5% at pH 7.8)
8. Amistar® (ai. 500g/kg azoxystrobin) at 0.4g/L + potassium phosphonate (0.5% at pH 7.8)

Approximately 10 L of fungicide suspension was applied to each tree or until run-off. The development of any symptoms of foliar and fruit phytotoxicity or loss of tree vigour was monitored throughout the experiment.

Experiment 2: An additional phytotoxicity experiment was conducted where the phosphonate sprays are applied 7 days after copper fungicide applications rather than at the same time. Once again a completely randomised design with 8 treatments and 2 single tree replications/treatment was used. The following treatments were applied as foliar sprays:

1. Control – potassium phosphonate (0.5% at pH 7.8)
2. Kocide (ai. 500g/kg copper hydroxide) at 2g/L followed by potassium phosphonate (0.5% at pH 7.8) 7 days later
3. Kocide® Blue (ai. 350g/kg copper hydroxide) at 1.5g/L followed by potassium phosphonate (0.5% at pH 7.8) 7 days later
4. Liquicop® (ai. 80g Cu/L as copper ammonium carbonate) at 5ml/L followed by potassium phosphonate (0.5% at pH 7.8) 7 days later
5. Copper oxychloride at 4 g/L followed by potassium phosphonate (0.5% at pH 7.8) 7 days later
6. Kocide® Liquid Blue SC (ai. 360g/L copper hydroxide) at 1.5mL/L followed by potassium phosphonate (0.5% at pH 7.8) 7 days later
7. Norshield® at 2mL/L followed by potassium phosphonate (0.5% at pH 7.8) 7 days later
8. Amistar® (ai. 500g/kg azoxystrobin) at 0.4g/L + potassium phosphonate (0.5% at pH 7.8)

Approximately 10 L of fungicide suspension was applied to each tree or until run-off. The development of any symptoms of foliar and fruit phytotoxicity or loss of tree vigour was monitored throughout the experiment.

B. Field and glasshouse applications of Bion®

1. Field experiment

The aim of this experiment was to study the effect of the host defence promoter, Bion®, applied as a field fruit dip on 'Hass' avocado fruit susceptibility to postharvest disease.

The experiment was conducted at G. Anderson's property at Duranbah on commercially treated 'Hass' trees on seedling 'Velvick'. A completely randomised design with 2 treatments and 4 single tree replications/treatment was used. Approximately 50 fruitlets were tagged on each tree and then treated monthly from October 2000 until July 2001 (total of 10 treatments applied) with the following treatments;

1. Control - untreated
2. Bion® (CGA 245704, Novartis), 0.05g/l as a fruit spray

Treatment sprays will be applied until run-off. Once fruit reached commercial maturity they were harvested on 10/7/01 and ripened at 22°C (65% RH) before being assessed for anthracnose and stem-end rot.

2. Glasshouse experiment

This experiment was carried out to investigate the effects of soluble silicon and Bion® (Syngenta Crop Protection) on the antifungal compounds in young 'Hass' trees on 'Velvick' rootstocks.

Twenty trees were each sprayed with water, Bion® (0.05g/L) or soluble silicon (Photo-finish™ Nutri-tech Solutions) (10mL/L) until run off. Four days later 10 trees from each of the three treatments were sprayed with spore suspensions of *C. gloeosporioides* (1 x 10^6 spores/mL) and plastic bags secured over the plants for 48 hours. Newly mature leaves were collected prior to treatment as well as 2 and 7 days after inoculation.

The dichloromethane partitioning process was used to obtain crude extracts that were analysed using thin-layer chromatography (TLC). The TLC plates were examined under UV light to identify possible antifungal compounds and then a *C. gloeosporioides* spore suspension (1 x 10^6 spores/mL of potato dextrose broth) was sprayed onto the plate, plates incubated and then examined to identify zones of fungal growth inhibition.

C. Field and postharvest applications of new strobilurin fungicides and host defence promoters

No experiments were conducted on new strobilurin fungicides, as none were available for field evaluation.

To test the efficacy of defence promoting compounds against anthracnose and stem-end rot (SER), a field trial was carried out on 7-year-old 'Hass' avocado trees grafted to 'Velvick' seedling rootstocks. Fruit were dipped in either the control treatment (water + 0.1% Tween 80), Bion® (Syngenta Crop Protection 0.05g/L + 0.1 % Tween 80) or soluble silicon (Photo Finish ™, Nutri-Tech Solutions – 10mL/L + 0.1 % Tween 80) once a month until harvest.

After harvest, fruit were ripened at 22°C and 65% relative humidity (RH) to the "eating soft" stage and assessed for disease development.

D. Biocoat

Westfalia ™ Biocoat is a chemical product composed of fatty acids, alcohols and antioxidants which has been accepted for use on organic fruit in South Africa to prevent stem-end rots. To test it under Australian conditions we snap harvested 'Hass' avocado fruit and then dipped them for 30s after harvest in either:
1. Control – water
2. Sportak® (ai 450g/L prochloraz) 0.55mL/L
3. Westfalia ™ Biocoat – recommended rate, 34.5mL/L
4. Westfalia ™ Biocoat – double recommended rate, 69mL/L

Fruit were then ripened (22°C /65% RH) and assessed for disease development.

E. Antigibberellins

This field experiment was conducted to examine if the use of an antigibberellin (Sunny®) or pruning had an effect on postharvest disease susceptibility of avocado. The following treatments were applied by John Leonardi (QDPI) at Bundaberg:

1. Unpruned control (No Sunny®)
2. Unpruned + 1% Sunny® at flowering
3. Pruned after harvest (No Sunny®)
4. Pruned after harvest + 1% Sunny® at flowering
5. Pruned after harvest and Pruned in December (No Sunny®)
6. Pruned after harvest, pruned in December and 1% Sunny® on regrowth.

After harvest, fruit were ripened at 22°C (65%RH) and assessed for disease development.

2.3 Harvesting Method Studies

A. Clip vs. snap harvesting

The aim of this experiment was to determine if the method of snap harvesting fruit, as opposed to clip harvesting, increased the incidence and severity of postharvest diseases especially stem-end rot (SER).

This trial was carried on 'Hass' fruit from three locations (Bundaberg, Duranbah and Mt Tamborine) and at two different times of each harvest season (Table 1).

Table 1. Location, stage of season and date of harvest for the harvesting methods trials.

Location	Stage of season	Date
Bundaberg	Early season	30th April 2002
Bundaberg	Mid season	1st July 2002
Duranbah (Northern NSW)	Early season	28th May 2002
Duranbah (Northern NSW)	Later season	29th July 2002
Mt Tamborine (SE Qld)	Mid season	23rd August 2002
Mt Tamborine (SE Qld)	Late season	1st July 2002

Fruit were either harvested by clipping using secateurs or snap harvested by holding the fruit firmly and rotating the wrist quickly snapping the fruit off at the abscission point. The stems of the clipped fruit were trimmed to 5mm long. Matched pairs of fruit on the tree were selected and then one fruit was snap harvested whilst the other fruit was clip harvested. In the trials at Bundaberg and Duranbah the effect of fruit aspect was investigated by harvesting a tray of clipped and a tray of snapped fruit from each side of the tree (east vs. west). A replicate consisted of a tray of count 18 fruit. At Bundaberg and Duranbah there were six replicates, eight replicates were harvested from each trial at Mt Tamborine. Fruit were not picked from 'blind terminals' (where there was only fruit and no leaf cover).

Fruit were harvested directly into commercial trays and brought back to the laboratory at Indooroopilly for ripening at 22°C and 65% RH to the eating ripe stage and assessed for disease development. Where stem-end rot developed, the causal organism was determined by isolation from margins of diseased tissue onto SPDA.

Due to the ongoing drought, the trees at Bundaberg were showing some signs of water stress. The mid season harvest at Mt Tamborine was undertaken after showery weather.

B. Effect of copper spray, harvesting method and harvesting time on development postharvest diseases

The aim of this experiment was to determine the effect of the application of copper fungicide five days prior to harvest on stem-end rot of clip and snap harvested 'Hass' fruit.

On the 19th September 2002 five 'Hass'/'Velvick' trees were sprayed with Kocide (2g/L, 1g a.i./L) at Mt Tamborine. Five unsprayed control 'Hass'/'Velvick' trees were selected and tagged.
On the 24th September 2002 fruit were harvested whilst there was still dew on the ground (8am to 9am). One carton of each clip and snap fruit were harvested from each tree. At midday, when the ground and the lower limbs of the trees had dried, a second harvest was done. Fruit were packed into commercial cartons and taken back to Indooroopilly for

ripening at 22°C and 65% RH. Fruit were assessed for the development of stem-end rot and anthracnose. Where stem-end rot developed, the causal organism was determined by isolation from margins of diseased tissue onto SPDA.

2.4 Pepper Spot Studies

A. DNA fingerprinting

The aim of the work described in this section was to make comparisons of *Colletotrichum gloeosporioides* populations isolated from avocado and mango crops grown in relatively close proximity. It was anticipated that the study would achieve a picture of pathogen evolution and genetic diversity between strains causing different symptoms (anthracnose or pepper spot on avocado and anthracnose or tear stain on mango) on the same fruit. Are particular symptoms associated with different pathogen genotypes? It was also important to compare host specificity and pathogenicity between different fruits (avocado/mango). Was the preharvest avocado pepper spot symptom caused by distinct strains of *C. gloeosporioides* to those causing postharvest avocado anthracnose? Similarly, was the preharvest mango tear stain symptom caused by distinct strains of *C. gloeosporioides* to those causing postharvest mango anthracnose? Were there more aggressive strains present in the pathogen population? Using DNA fingerprints of isolates collected from a range of sites, it will be possible to ascertain the patterns of relationships that might exist and, therefore, the potential for spread of the diseases between avocado and mango. In addition, a further relationship may be shown between these results and the ability of the isolates to produce the sexual stage readily in culture. To avoid confusion, mango tear stain isolates were referred to as mango pepper spot isolates.

A. Collection of isolates

Five sites in northern New South Wales and south-east Queensland (Bangalow, Cudgen, Duranbah, Green Pigeon, Mt Tamborine) were identified for the collection of *Colletotrichum gloeosporioides* isolates from cv. Hass avocado fruit. Fifty isolates were obtained from each site: 25 anthracnose and 25 pepper spot isolates. Similarly, three sites were located in northern NSW and northern Queensland (Ayr, Bangalow, Green Pigeon) for the collection of isolates from cv. Kensington Pride mango fruit. Fifty mango isolates were obtained from each site: 25 anthracnose and 25 tear stain isolates.

For anthracnose isolates, about 20 fruit were selected randomly from each of 5 trees. Fruit were left to fully ripen and develop disease. Only 5 diseased fruit were required from each tree. After peeling the fruit, the fungus was isolated from a discrete lesion on the inner skin surface and grown on Potato Dextrose Agar (½ strength) + streptomycin (0.1%) (SPDA) at room temperature (~25^0C). Four samples of each lesion were grown on each plate with one eventual isolate being selected and subcultured for permanent retention.

For pepper spot (Plate 1) and tear stain (Plate 2) isolates, 5 fruit with visible symptoms were picked from trees corresponding to the trees from which anthracnose isolates were gathered. Fruit were surface sterilised with 70% ethanol and allowed to air dry. Using a sterile scalpel, tiny segments were cut from the raised lesions on the outer surface of the skin and plunged into SPDA and incubated at room temperature. Four samples were grown on each plate with one eventual isolate being selected and subcultured.

Plate 1: Typical pepper spot lesions on 'Hass' avocado fruit

Plate 2: Typical tear stain symptoms on mango fruit

Cultures were grown on SPDA for about 7 days at room temperature and an agar plug was taken from the margin of the fungus and subcultured onto Oatmeal Agar (OMA), which is a medium suited to inducing optimal sporulation of *C. gloeosporioides*. After approximately 7 days, when spores were prolific on the agar surface, a small amount of spore mass was scraped from the agar with a inoculating loop and transferred to 10.0mL of sterile distilled water in a McCartneys bottle and shaken vigorously. This suspension was then poured onto a Water Agar (WA) (1%) plate and immediately poured off. The WA plate was incubated at a 45^0 angle for up to 24 hours at room temperature. Using the microscope, a single spore was located. This was cut from the agar with a scalpel and transferred to SPDA. Just before each culture reached the edge of the plate, squares of agar were cut from within the margin. Five squares were transferred to a small glass bottle containing 3.0mL sterile distilled water and sealed with Parafilm. This was repeated 4 times for each isolate. Bottles were stored at room temperature (25^0C).

An agar plug from each single spore culture was also subcultured onto OMA and grown for about 7 days at room temperature. Spores were scraped generously from the surface using an inoculation loop and transferred into a Bijoux bottle containing 4.0mL sterile distilled water and shaken. 1.0mL of this suspension was transferred to a 1.8mL Nunc Cryotube containing 0.42mL of sterile 50% glycerol solution, resulting in a 15% glycerol suspension. This was repeated for each isolate. Cryotubes were stored at -70^0C.

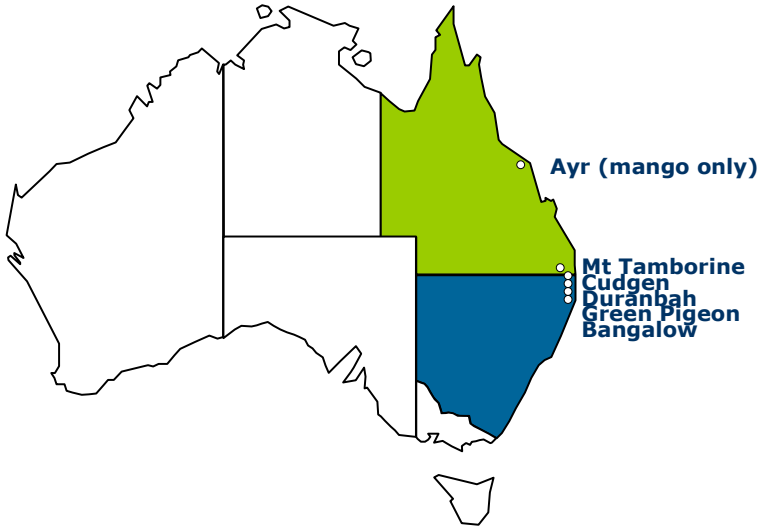

Figure 1: Map of Australia showing avocado and mango orchard sites

The collection listed below contains 250 *Colletotrichum gloeosporioides* isolates from avocado fruit and 150 *Colletotrichum gloeosporioides* isolates from mango fruit.

The nomenclature of each isolate represents the type of fruit, the infection type, the geographic source, the tree number, and the fruit number from that tree. For example, isolate AAD21 is from Avocado Anthracnose from Duranbah, tree 2, fruit 1. Tree number for anthracnose isolates corresponds to tree number for pepper spot isolates (ie. isolates are from the same tree). Fruit numbers are independent (i.e. anthracnose isolates 1-5 are from different fruit than pepper spot isolates 1-5). The collection has been submitted to the DPI&F Herbarium and BRIP numbers have been allocated to each isolate.

The following tables provide details of *Colletotrichum gloeosporioides* isolates from avocado fruit (BRIP numbers in brackets):

Avocado isolates from Bangalow (lat $28^0$40'S, long $153^0$31'E)

Tree 1	Anthracnose	AAB11 (45430)	AAB12 (45431)	AAB13 (45432)	AAB14 (45433)	AAB15 (45434)
	Pepper Spot	APB11 (45455)	APB12 (45456)	APB13 (45457)	APB14 (45458)	APB15 (45459)
Tree 2	Anthracnose	AAB21 (45435)	AAB22 (45436)	AAB23 (45437)	AAB24 (45438)	AAB25 (45439)
	Pepper Spot	APB21 (45460)	APB22 (45461)	APB23 (45462)	APB24 (45463)	APB25 (45464)
Tree 3	Anthracnose	AAB31 (45440)	AAB32 (45441)	AAB33 (45442)	AAB34 (45443)	AAB35 (45444)
	Pepper Spot	APB31 (45465)	APB32 (45466)	APB33 (45467)	APB34 (45468)	APB35 (45469)
Tree 4	Anthracnose	AAB41 (45445)	AAB42 (45446)	AAB43 (45447)	AAB44 (45448)	AAB45 (45449)
	Pepper Spot	APB41 (45470)	APB42 (45471)	APB43 (45472)	APB44 (45473)	APB45 (45474)
Tree 5	Anthracnose	AAB51 (45450)	AAB52 (45451)	AAB53 (45452)	AAB54 (45453)	AAB55 (45454)
	Pepper Spot	APB51 (45475)	APB52 (45476)	APB53 (45477)	APB54 (45478)	APB55 (45479)

Avocado isolates from Cudgen (lat $28^0$16'S, long $153^0$33'E)

Tree 1	Anthracnose	AAC11 (45480)	AAC12 (45481)	AAC13 (45482)	AAC14 (45483)	AAC15 (45484)
	Pepper Spot	APC11 (45505)	APC12 (45506)	APC13 (45507)	APC14 (45508)	APC15 (45509)
Tree 2	Anthracnose	AAC21 (45485)	AAC22 (45486)	AAC23 (45487)	AAC24 (45488)	AAC25 (45489)
	Pepper Spot	APC21 (45510)	APC22 (45511)	APC23 (45512)	APC24 (45513)	APC25 (45514)
Tree 3	Anthracnose	AAC31 (45490)	AAC32 (45491)	AAC33 (45492)	AAC34 (45493)	AAC35 (45494)
	Pepper Spot	APC31 (45515)	APC32 (45516)	APC33 (45517)	APC34 (45518)	APC35 (45519)
Tree 4	Anthracnose	AAC41 (45495)	AAC42 (45496)	AAC43 (45497)	AAC44 (45498)	AAC45 (45499)
	Pepper Spot	APC41 (45520)	APC42 (45521)	APC43 (45522)	APC44 (45523)	APC45 (45524)
Tree 5	Anthracnose	AAC51 (45500)	AAC52 (45501)	AAC53 (45502)	AAC54 (45503)	AAC55 (45504)
	Pepper Spot	APC51 (45525)	APC52 (45526)	APC53 (45527)	APC54 (45528)	APC55 (45529)

Avocado isolates from Duranbah (lat 28^{0}18'S, long 153^{0}31'E)

Tree 1	Anthracnose	AAD11 (45530)	AAD12 (45531)	AAD13 (45532)	AAD14 (45533)	AAD15 (45534)
	Pepper Spot	APD11 (45555)	APD12 (45556)	APD13 (45557)	APD14 (45558)	APD15 (45559)
Tree 2	Anthracnose	AAD21 (45535)	AAD22 (45536)	AAD23 (45537)	AAD24 (45538)	AAD25 (45539)
	Pepper Spot	APD21 (45560)	APD22 (45561)	APD23 (45562)	APD24 (45563)	APD25 (45564)
Tree 3	Anthracnose	AAD31 (45540)	AAD32 (45541)	AAD33 (45542)	AAD34 (45543)	AAD35 (45544)
	Pepper Spot	APD31 (45565)	APD32 (45566)	APD33 (45567)	APD34 (45568)	APD35 (45569)
Tree 4	Anthracnose	AAD41 (45545)	AAD42 (45546)	AAD43 (45547)	AAD44 (45548)	AAD45 (45549)
	Pepper Spot	APD41 (45570)	APD42 (45571)	APD43 (45572)	APD44 (45573)	APD45 (45574)
Tree 5	Anthracnose	AAD51 (45550)	AAD52 (45551)	AAD53 (45552)	AAD54 (45553)	AAD55 (45554)
	Pepper Spot	APD51 (45575)	APD52 (45576)	APD53 (45577)	APD54 (45578)	APD55 (45579)

Avocado isolates from Green Pigeon (lat 28^{0}29'S, long 153^{0}04'E)

Tree 1	Anthracnose	AAG11 (45580)	AAG12 (45581)	AAG13 (45582)	AAG14 (45583)	AAG15 (45584)
	Pepper Spot	APG11 (45605)	APG12 (45606)	APG13 (45607)	APG14 (45608)	APG15 (45609)
Tree 2	Anthracnose	AAG21 (45585)	AAG22 (45586)	AAG23 (45587)	AAG24 (45588)	AAG25 (45589)
	Pepper Spot	APG21 (45610)	APG22 (45611)	APG23 (45612)	APG24 (45613)	APG25 (45614)
Tree 3	Anthracnose	AAG31 (45590)	AAG32 (45591)	AAG33 (45592)	AAG34 (45593)	AAG35 (45594)
	Pepper Spot	APG31 (45615)	APG32 (45616)	APG33 (45617)	APG34 (45618)	APG35 (45619)
Tree 4	Anthracnose	AAG41 (45595)	AAG42 (45596)	AAG43 (45597)	AAG44 (45598)	AAG45 (45599)
	Pepper Spot	APG41 (45620)	APG42 (45621)	APG43 (45622)	APG44 (45623)	APG45 (45624)
Tree 5	Anthracnose	AAG51 (45600)	AAG52 (45601)	AAG53 (45602)	AAG54 (45603)	AAG55 (45604)
	Pepper Spot	APG51 (45625)	APG52 (45626)	APG53 (45627)	APG54 (45628)	APG55 (45629)

Avocado isolates from Mt Tamborine (lat 27^{0}58'S, long 153^{0}12'E)

Tree 1	Anthracnose	AAT11 (45630)	AAT12 (45631)	AAT13 (45632)	AAT14 (45633)	AAT15 (45634)
	Pepper Spot	APT11 (45655)	APT12 (45656)	APT13 (45657)	APT14 (45658)	APT15 (45659)
Tree 2	Anthracnose	AAT21 (45635)	AAT22 (45636)	AAT23 (45637)	AAT24 (45638)	AAT25 (45639)
	Pepper Spot	APT21 (45660)	APT22 (45661)	APT23 (45662)	APT24 (45663)	APT25 (45664)
Tree 3	Anthracnose	AAT31 (45640)	AAT32 (45641)	AAT33 (45642)	AAT34 (45643)	AAT35 (45644)
	Pepper Spot	APT31 (45665)	APT32 (45666)	APT33 (45667)	APT34 (45668)	APT35 (45669)
Tree 4	Anthracnose	AAT41 (45645)	AAT42 (45646)	AAT43 (45647)	AAT44 (45648)	AAT45 (45649)
	Pepper Spot	APT41 (45670)	APT42 (45671)	APT43 (45672)	APT44 (45673)	APT45 (45674)
Tree 5	Anthracnose	AAT51 (45650)	AAT52 (45651)	AAT53 (45652)	AAT54 (45653)	AAT55 (45654)
	Pepper Spot	APT51 (45675)	APT52 (45676)	APT53 (45677)	APT54 (45678)	APT55 (45679)

The following tables provide details of *Colletotrichum gloeosporioides* isolates from mango fruit (BRIP numbers in brackets):

NB. In this report, mango tear stain is referred to as pepper spot. Hence, MPA is the name for Mango Pepper spot isolates from Ayr.

Mango isolates from Ayr (lat 19^{0}34'S, long 147^{0}24'E)

Tree 1	Anthracnose	MAA11 (45680)	MAA12 (45681)	MAA13 (45682)	MAA14 (45683)	MAA15 (45684)
	Pepper Spot	MPA11 (45705)	MPA12 (45706)	MPA13 (45707)	MPA14 (45708)	MPA15 (45709)
Tree 2	Anthracnose	MAA21 (45685)	MAA22 (45686)	MAA23 (45687)	MAA24 (45688)	MAA25 (45689)
	Pepper Spot	MPA21 (45710)	MPA22 (45711)	MPA23 (45712)	MPA24 (45713)	MPA25 (45714)
Tree 3	Anthracnose	MAA31 (45690)	MAA32 (45691)	MAA33 (45692)	MAA34 (45693)	MAA35 (45694)
	Pepper Spot	MPA31 (45715)	MPA32 (45716)	MPA33 (45717)	MPA34 (45718)	MPA35 (45719)
Tree 4	Anthracnose	MAA41 (45695)	MAA42 (45696)	MAA43 (45697)	MAA44 (45698)	MAA45 (45699)
	Pepper Spot	MPA41 (45720)	MPA42 (45721)	MPA43 (45722)	MPA44 (45723)	MPA45 (45724)
Tree 5	Anthracnose	MAA51 (45700)	MAA52 (45701)	MAA53 (45702)	MAA54 (45703)	MAA55 (45704)
	Pepper Spot	MPA51 (45725)	MPA52 (45726)	MPA53 (45727)	MPA54 (45728)	MPA55 (45729)

Mango isolates from Bangalow (lat 28^{0}40'S, long 153^{0}31'E)

Tree 1	Anthracnose	MAB11 (45730)	MAB12 (45731)	MAB13 (45732)	MAB14 (45733)	MAB15 (45734)
	Pepper Spot	MPB11 (45755)	MPB12 (45756)	MPB13 (45757)	MPB14 (45758)	MPB15 (45759)
Tree 2	Anthracnose	MAB21 (45735)	MAB22 (45736)	MAB23 (45737)	MAB24 (45738)	MAB25 (45739)
	Pepper Spot	MPB21 (45760)	MPB22 (45761)	MPB23 (45762)	MPB24 (45763)	MPB25 (45764)
Tree 3	Anthracnose	MAB31 (45740)	MAB32 (45741)	MAB33 (45742)	MAB34 (45743)	MAB35 (45744)
	Pepper Spot	MPB31 (45765)	MPB32 (45766)	MPB33 (45767)	MPB34 (45768)	MPB35 (45769)
Tree 4	Anthracnose	MAB41 (45745)	MAB42 (45746)	MAB43 (45747)	MAB44 (45748)	MAB45 (45749)
	Pepper Spot	MPB41 (45770)	MPB42 (45771)	MPB43 (45772)	MPB44 (45773)	MPB45 (45774)
Tree 5	Anthracnose	MAB51 (45750)	MAB52 (45751)	MAB53 (45752)	MAB54 (45753)	MAB55 (45754)
	Pepper Spot	MPB51 (45775)	MPB52 (45776)	MPB53 (45777)	MPB54 (45778)	MPB55 (45779)

Mango isolates from Green Pigeon (lat $28^0$29'S, long $153^0$04'E)

Tree 1	Anthracnose	MAG11 (45780)	MAG12 (45781)	MAG13 (45782)	MAG14 (45783)	MAG15 (45784)
	Pepper Spot	MPG11 (45805)	MPG12 (45806)	MPG13 (45807)	MPG14 (45808)	MPG15 (45809)
Tree 2	Anthracnose	MAG21 (45785)	MAG22 (45786)	MAG23 (45787)	MAG24 (45788)	MAG25 (45789)
	Pepper Spot	MPG21 (45810)	MPG22 (45811)	MPG23 (45812)	MPG24 (45813)	MPG25 (45814)
Tree 3	Anthracnose	MAG31 (45790)	MAG32 (45791)	MAG33 (45792)	MAG34 (45793)	MAG35 (45794)
	Pepper Spot	MPG31 (45815)	MPG32 (45816)	MPG33 (45817)	MPG34 (45818)	MPG35 (45819)
Tree 4	Anthracnose	MAG41 (45795)	MAG42 (45796)	MAG43 (45797)	MAG44 (45798)	MAG45 (45799)
	Pepper Spot	MPG41 (45820)	MPG42 (45821)	MPG43 (45822)	MPG44 (45823)	MPG45 (45824)
Tree 5	Anthracnose	MAG51 (45800)	MAG52 (45801)	MAG53 (45802)	MAG54 (45803)	MAG55 (45804)
	Pepper Spot	MPG51 (45825)	MPG52 (45826)	MPG53 (45827)	MPG54 (45828)	MPG55 (45829)

B. Morphological characterisation of Colletotrichum gloeosporioides *isolates*

All isolates were grown on SPDA at 25^0C for at least 10 days in order to verify the development of the sexual stage of the fungus (*Glomerella cingulata*). Black perithecia were picked off the surface of the agar and transferred to a droplet of Lacto Fuchsin on a slide and covered with a cover slip and examined under the microscope. The black spheres were crushed under the cover slip and the presence or absence of ascospores in asci was determined. Conidia were transferred from 7 day old sporulating cultures to a droplet of stain on a slide and lengths were measured.

C. Genetic diversity of Colletotrichum gloeosporioides *isolates*

1. Extraction of DNA from cultures of *C. gloeosporioides*

350 isolates of *C. gloeosporioides* were analysed. All 150 mango isolates from Ayr, Bangalow and Green Pigeon were included along with 200 avocado isolates from Bangalow, Duranbah, Green Pigeon and Mt Tamborine. Details of these isolates are outlined above. Each isolate was grown on several V8 juice agar plates amended with streptomycin. After 8-10 days, mycelium was scraped off the plates into a 1.5µL tube. The sample was washed by adding ~1mL of ethanol to the mycelium in the tube and left for up to 24 hours. The tube was then centrifuged for 2 minutes and the ethanol then pipetted off.

Extraction of DNA from the mycelium was achieved using the NucleoSpin Plant support protocol for fungi (CTAB method) kit (slightly modified for optimal performance) (Macherey-Nagel-04/2000 GmbH & Co. Düren, Germany). 200µL of C1 buffer was added to the tube and the sample was homogenised using a micropestle and vortexing. An additional 100µL C1 buffer was added and homogenisation continued. 100µL of chloroform was added and the tube vortexed for 10 seconds. Phases were separated by centrifugation for 5 minutes in a microcentrifuge. The supernatant was transferred to a new 1.5mL reaction tube. After incubation at 65^0C in a water bath for 30 minutes, tubes were centrifuged for 10 minutes. The supernatant was transferred to another fresh tube and 300µL of buffer C4 and 200µL ethanol were added. This mixture was vortexed for 30 seconds.

A NucleoSpin Plant column was placed in a 2mL centrifuge tube and the mixture was pipetted into the top. This was then centrifuged for 60 seconds at maximum speed and the flowthrough was discarded. 400µL of buffer CW was pipetted onto the spin column and the tube was centrifuged for 30 seconds at 10 000 rpm and the flowthrough discarded. 700µL of buffer C5 was pipetted onto the column and the tube was centrifuged for 60 seconds at 10 000 rpm. The flowthrough was discarded and another 200µL buffer C5 was added to the column. This was centrifuged again at 10 000 rpm for 2 minutes in order to remove buffer C5 quantitatively. The spin column was the placed in a new 1.5µL centrifuge tube and 100µL of

elution buffer CE (incubated to 70^0C) was pipetted into the column. After 5 minutes the DNA was eluted by centrifugation for 60 seconds at 10 000 rpm.

DNA concentration was subsequently estimated spectrophotometrically using a DNA/RNA calculator, GeneQuant™ (Pharmacia Biotech, .Amersham Biosciences UK Limited Buckinghamshire England).

2. DNA Amplification Fingerprinting (DAF)

PCR reactions were carried out initially using 5 arbitrarily selected oligonucleotide primers and then two primers (HIRH and RKMI) were selected which could best distinguish between isolates.
Primers used:
EHKJ: 5'- GCT CAC GA –3'
HIRH: 5'- ACG TCC AC –3'
RKMI: 5'- CCC GTC GT –3'
IMBE: 5'- GAA ACG CC –3'
ILOE: 5'- GAT GAG CC –3'

The modified DAF system described by Bentley and Bassam (1996) was used. DNA amplification reactions contained 25ng of DNA, 15µM primer (GeneWorks Pty Ltd.), buffer (5mM $MgCl_2$, 10mM KCl, 10mM Tris (pH 8.3)), 3 units of AmpliTaq Stoffel Fragment DNA polymerase (Perkin Elmer Corporation, California, USA), 200µM of each dNTP (dCTP, dATP, dGTP, dTTP) (Biotech International), sterile distilled water to 20µL, with a 20µL paraffin oil overlay. Reactions were thermocylced using a MJ Research Inc. PTC-100 thermocycler programmed for 1 cycle at 94^0C for 5 minutes followed by 35 cycles at 94^0C for 30 seconds and 52^0C, 51^0C, 50^0C, 49^0C, 48^0C each for 1 minute and then one cycle at 72^0C for 5 minutes. PCR reactions were performed in duplicate and were reproducible.

3. Electrophoresis and detection of PCR products

DNA amplification products (1.4uL) were separated by polyacrylamide gel electrophoresis (PAGE) using a Mini-Protean II apparatus (Bio Rad Laboratories, California, USA). Each gel was made up of 4.0mL acrylamide solution [8%w/v acrylamide, 2% w/v bis-acrylamide, 10% w/v urea (Bio Rad Laboratories, USA), 5% v/v glycerol (BDH Chemicals), 10% v/v 10 x TBE], 50µL APS (10% ammonium persulphate), 4µL TEMED (N, N, N'N'-tetramethylethylenediamine). Gels were 0.5mm thick and were cast onto Gel Bond PAG backing film (FMC BioProducts, USA). This provided a solid support for the gels, allowing them to be preserved for subsequent analysis and future reference. Gels were electrophoresed in 1 x TBE buffer at 250V for 40 minutes. Fingerprints were detected by silver staining (Caetano-Anolles *et al.* 1991).

4. Analysis of genotypic variation

Gels were scored visually for each *Colletotrichum gloeosporioides* isolate. Scores of '1' for presence and '0' for absence were assigned to each band according to their patterns. This information was stored in a data matrix. Individual bands over 1500 and under 150 base pairs (bp) were difficult to differentiate on some gels. Therefore, bands from 150 to 1500 bp in size were chosen for analysis. The Numerical Taxonomy System (NTSYS) pc version 2.1 was used to analyse the data. The data matrix was converted to a similarity matrix for each of the primers by the SIMQUAL (similarity for qualitative data) programme of NTSYS using the Jacquard coefficient (which scores the number of common bands divided by the total

number of bands in the two samples being compared). A dendogram was generated from this similarity matrix by cluster analysis with the unweighted pair group arithmetic mean method (UPGMA) in the SAHN (Sequential agglomerative hierarchical nested cluster analysis) programme of NTSYSpc version 2.1.

B. Pathogenicity of pepper spot versus anthracnose isolates

This study was initiated due to the recent appearance and perceived spread of localised necrotic lesions (pepper spot) on avocado fruit in 'Hass' avocado orchards. Pepper spot is caused by *Colletotrichum gloeosporioides* (Willingham *et al.* 2000), the same fungus responsible for anthracnose. The emergence of the pepper spot symptom is different as the response is occurring either regardless of quiescence or before quiescence can be established.

The objectives of the study reported in this chapter were to screen avocado and mango isolates of *Colletotrichum gloeosporioides* for pathogenicity (ability to cause disease on a given host) as well as comparative aggressiveness (relative capacity to cause disease on a given host genotype) using 'Hass' avocado. The term 'aggressiveness' rather than 'virulence' was used as these laboratory, glasshouse and field experiments focused largely on the degree of damage that a particular isolate caused. Referring to virulence of a pathogen implies that the avocado cultivar has particular resistance genes which cannot be determined in this work.

Comparisons were made of fungal isolates from avocado and mango crops grown in relatively close proximity. The aim was to compare host specificity, pathogenicity and aggressiveness of isolates from different collection locations, from different hosts (avocado/mango) and from different symptom types (anthracnose or pepper spot of avocado and anthracnose or tear stain (referred to as pepper spot in this chapter) of mango).

A. Inoculation tests on detached avocado fruit in the laboratory

1. Preliminary inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado on detached mature 'Hass' avocado fruit

Mature 'Hass' avocado fruit were harvested in November 2000 from Eden's property at Mt Tamborine, QLD, ensuring that there were no visible fruit blemishes. The following day, fruit were rinsed in lukewarm water to remove any chemical residues, surface sterilised with 70% alcohol and air-dried. Three sites for inoculation were identified on each fruit and marked as circles with a pen.

The following isolate and water control treatments were used for each replicate:

APD11	24605 (avocado *C.g.* pepper spot)	AAT21
APC11	AAD11	23691 (avocado *C.g.* anthracnose)
APT11	AAC11	Water control
APT21	AAT11	

Fruit were inoculated by placing 3 filter paper discs saturated in the spore suspension (5 x 10^6 conidia/mL) of each isolate onto the surface of the fruit on the 3 circled areas. This was replicated 5 times i.e. 5 fruit per isolate. Control fruit were inoculated with water. Fruit were then placed in plastic crates (with all fruit from each replicate in a single crate) lined with moist paper and sealed to maintain high humidity. Crates were incubated at 25^0C. After 48 h, the discs were removed and fruit were transferred to avocado packing cartons and kept at 23^0C (65% RH). Fruit were assessed for disease incidence and the diameter of lesions measured at eating-ripe stage. An average was taken of the 3 measurements per fruit.

2. Inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado and mango on detached seedless "cocktail" 'Fuerte' avocado fruit

"Cocktail" 'Fuerte' (Mexican x Guatemalan) avocado fruit were harvested in May 2003 from Anderson's property at Duranbah, NSW, ensuring that fruit were free of any visible blemishes. Fruit were about 5-6cm in length and ca.2cm wide. Fruit were prepared as

described above in the previous experiment. The isolates used were from both pepper spot and anthracnose lesions of avocado, and pepper spot and anthracnose lesions of mango. In total, eighty isolates were screened for pathogenicity. Fifty isolates were from avocado fruit and thirty from mango fruit. Half the isolates from each host were from pepper spot lesions and the other half from anthracnose lesions. All geographic regions from where the isolates were collected were included.

The following isolate and water control treatments were used for each replicate:

Fruit 1: AAB11	Fruit 22: AAD21	Fruit 43: AAT31	Fruit 64: MAB41
Fruit 2: AAB21	Fruit 23: AAD31	Fruit 44: AAT41	Fruit 65: MAB51
Fruit 3: AAB31	Fruit 24: AAD41	Fruit 45: AAT51	Fruit 66: MPB11
Fruit 4: AAB41	Fruit 25: AAD51	Fruit 46: APT11	Fruit 67: MPB21
Fruit 5: AAB51	Fruit 26: APD11	Fruit 47: APT21	Fruit 68: MPB31
Fruit 6: APB11	Fruit 27: APD21	Fruit 48: APT31	Fruit 69: MPB41
Fruit 7: APB21	Fruit 28: APD31	Fruit 49: APT41	Fruit 70: MPB51
Fruit 8: APB31	Fruit 29: APD41	Fruit 50: APT51	Fruit 71: MAG11
Fruit 9: APB41	Fruit 30: APD51	Fruit 51: MAA11	Fruit 72: MAG21
Fruit 10: APB51	Fruit 31: AAG11	Fruit 52: MAA21	Fruit 73: MAG31
Fruit 11: AAC11	Fruit 32: AAG21	Fruit 53: MAA31	Fruit 74: MAG41
Fruit 12: AAC21	Fruit 33: AAG31	Fruit 54: MAA41	Fruit 75: MAG51
Fruit 13: AAC31	Fruit 34: AAG41	Fruit 55: MAA51	Fruit 76: MPG11
Fruit 14: AAC41	Fruit 35: AAG51	Fruit 56: MPA11	Fruit 77: MPG21
Fruit 15: AAC51	Fruit 36: APG11	Fruit 57: MPA21	Fruit 78: MPG31
Fruit 16: APC11	Fruit 37: APG21	Fruit 58: MPA31	Fruit 79: MPG41
Fruit 17: APC21	Fruit 38: APG31	Fruit 59: MPA41	Fruit 80: MPG51
Fruit 18: APC31	Fruit 39: APG41	Fruit 60: MPA51	Fruit 81: Water control
Fruit 19: APC41	Fruit 40: APG51	Fruit 61: MAB11	
Fruit 20: APC51	Fruit 41: AAT11	Fruit 62: MAB21	
Fruit 21: AAD11	Fruit 42: AAT21	Fruit 63: MAB31	

Fruit were inoculated by pipetting three single droplets (25µL) of spore suspension (5 x 10^6 conidia/mL) of an isolate onto the surface of a fruit on the 3 allocated areas. This was replicated 4 times i.e. 4 fruit per isolate. Control fruit were inoculated with water. Fruit were incubated as described above in the previous experiment. Fruit were assessed for presence or absence of a visible lesion and the diameter of each lesion was measured at eating-ripe stage.

3. Inoculation tests on avocado nursery plants in the glasshouse

The experiment was carried out using 'Hass' avocado leaves and their petioles on immature (6 month old) grafted nursery trees ('Velvick' West Indian rootstock) in pots in the glasshouse in April, 2003 (early autumn) (Plate 3).

Plate 3: 'Hass' avocado nursery trees in the glasshouse. Branches have been tagged and plastic bags enclose the inoculated petioles and leaves

Five branches were selected at random on each plant and tagged. Isolates from both pepper spot and anthracnose lesions of avocado and mango were used. In total, eighty isolates were screened for pathogenicity. Fifty isolates were from avocado fruit and thirty from mango fruit. Half the isolates were from pepper spot and the other half from anthracnose. All geographic regions from where the isolates were collected were included.

The following isolate and water control treatments were used for each replicate:

Tree 1:AAB11, AAB21, AAB31, AAB41, AAB51
Tree 2: APB11, APB21, APB31, APB41, APB51
Tree 3: AAC11, AAC21, AAC31, AAC41, AAC51
Tree 4: APC11, APC21, APC31, APC41, APC51
Tree 5: AAD11, AAD21, AAD31, AAD41, AAD51
Tree 6: APD11, APD21, APD31, APD41, APD51
Tree 7: AAG11, AAG21, AAG31, AAG41, AAG51
Tree 8: APG11, APG21, APG31, APG41, APG51
Tree 9: AAT11, AAT21, AAT31, AAT41, AAT51
Tree 10: APT11, APT21, APT31, APT41, APT51
Tree 11: MAA11, MAA21, MAA31, MAA41, MAA51
Tree 12: MPA11, MPA21, MPA31, MPA41, MPA51
Tree 13: MAB11, MAB21, MAB31, MAB41, MAB51
Tree 14: MPB11, MPB21, MPB31, MPB41, MPB51
Tree 15: MAG11, MAG21, MAG31, MAG41, MAG51
Tree 16: MPG11, MPG21, MPG31, MPG41, MPG51
Tree 17: AAB11, AAB21, AAB31, AAB41, AAB51
Tree 18: APB11, APB21, APB31, APB41, APB51
Tree 19: AAC11, AAC21, AAC31, AAC41, AAC51
Tree 20: APC11, APC21, APC31, APC41, APC51
Tree 21: AAD11, AAD21, AAD31, AAD41, AAD51
Tree 22: APD11, APD21, APD31, APD41, APD51
Tree 23: AAG11, AAG21, AAG31, AAG41, AAG51
Tree 24: APG11, APG21, APG31, APG41, APG51
Tree 25: AAT11, AAT21, AAT31, AAT41, AAT51
Tree 26: APT11, APT21, APT31, APT41, APT51
Tree 27: MAA11, MAA21, MAA31, MAA41, MAA51
Tree 28: MPA11, MPA21, MPA31, MPA41, MPA51
Tree 29: MAB11, MAB21, MAB31, MAB41, MAB51
Tree 30: MPB11, MPB21, MPB31, MPB41, MPB51
Tree 31: MAG11, MAG21, MAG31, MAG41, MAG51
Tree 32: MPG11, MPG21, MPG31, MPG41, MPG51
Tree 33: AAB11, AAB21, AAB31, AAB41, AAB51
Tree 34: APB11, APB21, APB31, APB41, APB51
Tree 35: AAC11, AAC21, AAC31, AAC41, AAC51
Tree 36: APC11, APC21, APC31, APC41, APC51
Tree 37: AAD11, AAD21, AAD31, AAD41, AAD51
Tree 38: APD11, APD21, APD31, APD41, APD51
Tree 39: AAG11, AAG21, AAG31, AAG41, AAG51
Tree 40: APG11, APG21, APG31, APG41, APG51
Tree 41: AAT11, AAT21, AAT31, AAT41, AAT51
Tree 42: APT11, APT21, APT31, APT41, APT51
Tree 43: MAA11, MAA21, MAA31, MAA41, MAA51
Tree 44: MPA11, MPA21, MPA31, MPA41, MPA51
Tree 45: MAB11, MAB21, MAB31, MAB41, MAB51
Tree 46: MPB11, MPB21, MPB31, MPB41, MPB51
Tree 47: MAG11, MAG21, MAG31, MAG41, MAG51
Tree 48: MPG11, MPG21, MPG31, MPG41, MPG51
Tree 49: AAB11, AAB21, AAB31, AAB41, AAB51
Tree 50: APB11, APB21, APB31, APB41, APB51
Tree 51: AAC11, AAC21, AAC31, AAC41, AAC51
Tree 52: APC11, APC21, APC31, APC41, APC51
Tree 53: AAD11, AAD21, AAD31, AAD41, AAD51
Tree 54: APD11, APD21, APD31, APD41, APD51
Tree 55: AAG11, AAG21, AAG31, AAG41, AAG51
Tree 56: APG11, APG21, APG31, APG41, APG51
Tree 57: AAT11, AAT21, AAT31, AAT41, AAT51
Tree 58: APT11, APT21, APT31, APT41, APT51
Tree 59: MAA11, MAA21, MAA31, MAA41, MAA51
Tree 60: MPA11, MPA21, MPA31, MPA41, MPA51
Tree 61: MAB11, MAB21, MAB31, MAB41, MAB51
Tree 62: MPB11, MPB21, MPB31, MPB41, MPB51

Tree 63: MAG11, MAG21, MAG31, MAG41, MAG51
Tree 64: MPG11, MPG21, MPG31, MPG41, MPG51
Tree 65: AAB11, AAB21, AAB31, AAB41, AAB51
Tree 66: APB11, APB21, APB31, APB41, APB51
Tree 67: AAC11, AAC21, AAC31, AAC41, AAC51
Tree 68: APC11, APC21, APC31, APC41, APC51
Tree 69: AAD11, AAD21, AAD31, AAD41, AAD51
Tree 70: APD11, APD21, APD31, APD41, APD51
Tree 71: AAG11, AAG21, AAG31, AAG41, AAG51
Tree 72: APG11, APG21, APG31, APG41, APG51
Tree 73: AAT11, AAT21, AAT31, AAT41, AAT51
Tree 74: APT11, APT21, APT31, APT41, APT51
Tree 75: MAA11, MAA21, MAA31, MAA41, MAA51
Tree 76: MPA11, MPA21, MPA31, MPA41, MPA51
Tree 77: MAB11, MAB21, MAB31, MAB41, MAB51
Tree 78: MPB11, MPB21, MPB31, MPB41, MPB51
Tree 79: MAG11, MAG21, MAG31, MAG41, MAG51
Tree 80: MPG11, MPG21, MPG31, MPG41, MPG51
Tree 81: Control, Control, Control, Control, Control
Tree 82: Control, Control, Control, Control, Control
Tree 83: Control, Control, Control, Control, Control
Tree 84: Control, Control, Control, Control, Control
Tree 85: Control, Control, Control, Control, Control

Using an artist's airbrush, leaves and petioles were sprayed with spore suspensions ($5x10^6$ conidia/mL) containing 0.01% v/v Tween 80 ensuring that the entire surface was saturated. Five isolates were inoculated onto each tree (1 isolate per leaf) and this was replicated 5 times. Control leaves were sprayed with water containing Tween 80. Leaves were enclosed in a plastic bag containing a water-soaked cotton wool ball and held in place with staples to maintain high humidity. After 48 h the bags were removed. Leaves were assessed for disease after 2 weeks and then at weekly intervals to assess pepper spot development. Samples of lesions which developed within the treated area were excised and returned to the laboratory for isolation and culturing.

B. Inoculation tests on avocado fruit in the field

1. Preliminary inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado on 'Hass' avocado fruit in the field (Mt Tamborine)

This trial was carried out in November 2000 on small attached fruit (6 weeks old and 2-3cm in length) on a block of 9 year old 'Hass' trees grown on 'Velvick' rootstock at Eden's property at Mt Tamborine, QLD. Fruit were selected at random and tagged, ensuring that there were no obvious fruit blemishes. A total of 11 isolate and control treatments were tested on 2 fruit per tree, on 3 trees giving 6 replicates per isolate tested.

The following isolate and water control treatments were used for each replicate:

APD11	24605 (avocado *C.g.* pepper spot)	AAT21
APC11	AAD11	23691 (avocado *C.g.* anthracnose)
APT11	AAC11	Water control
APT21	AAT11	

Spore suspensions were prepared for each isolate (5 x 10^6 conidia/mL). Approximately 1 mL of each spore suspension containing 0.01% v/v Tween 80 was sprayed onto the surface of the fruit. Fruit was enclosed in a plastic bag containing a water-soaked cotton wool ball to maintain high humidity (Plate 4). Paper bags were placed over the plastic bag and stapled into place to prevent sunburn. After 48 h, both bags were removed. Fruit were assessed for disease at regular intervals over 1-2 months.

Plate 4: Avocado fruit enclosed within a plastic bag containing a moist cotton wool ball to maintain humidity for the first 48 h

2. Further inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado on 'Hass' avocado fruit in the field (Mt Tamborine)

This trial was carried out in March 2001 and repeated in July 2001 at Eden's property at Mt Tamborine, QLD on 4 month old fruit (approximately 10cm long and 6cm wide) on a block of 10 year old 'Hass' trees grown on 'Velvick' rootstock. Fruit were selected at random and tagged, ensuring that there were no obvious fruit blemishes or pepper spot symptoms. A total of 15 isolate and control treatments were used on 2 fruit per tree on 3 trees (6 replicates).

The following isolate and water control treatments were used for each replicate:

APD11
APC11
APT11
APT21
APB11
APG11
24605 (avocado *C.g.* pepper spot)
AAD11
AAC11
AAT11
AAT21
AAB11
AAG11
23691a (avocado *C.g.* anthracnose)*
Water control

* Isolate has been regenerated on fruit and, therefore, has a modified number.

A spore suspension of about 400mL was prepared for each isolate (5 x 10^6 conidia/mL). Fruit were dipped into the spore suspension containing 0.01% v/v Tween 80 ensuring that the entire fruit and pedicel surfaces were saturated. Dipping was used from this trial onwards, to avoid the possibility of spray drift making contact with neighbouring fruit. Control fruit were dipped into water containing 0.01% v/v Tween 80. Fruit were enclosed for 48 h in a plastic

bag and paper bag as previously described. Fruit were assessed for disease after 2 weeks and then fortnightly.

3. Further inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado on 'Hass' avocado fruit in the field (Mt Tamborine)

The previously described trial was repeated in July 2001 to compare inoculation in different seasons at different fruit maturity.

4. Inoculation of *Colletotrichum gloeosporioides* isolates from avocado and mango on 'Hass' avocado fruit in the field (Duranbah)

The trial was carried out in November 2003 at Anderson's property at Duranbah, NSW on a block of 3 year 'Hass' avocado fruit on clonal 'Velvick' rootstock (Plate 5). Fruit were on average 2cm in length at this stage (4-8 weeks after fruit set).

Plate 5: Tagged avocado fruit on trees with plastic and paper bags enclosing the inoculated fruit for increased humidity and protection from the sun for the first 48 h

Isolates were obtained from both pepper spot and anthracnose lesions of avocado and tear stain and anthracnose lesions of mango. In total, eighty isolates were evaluated for pathogenicity. Fifty isolates were from avocado fruit and thirty from mango fruit. Half the isolates were from pepper spot and the other half from anthracnose. All geographic regions from where the isolates were collected were included. A control was included on each tree, giving a total of 20 control fruit.

The following isolate and water control treatments were used for each replicate:

Tree 1: AAB41, APB21, AAC31, APC11, AAD21, AAD41, APD21, AAG11, AAG31, AAG51, APG31, APG51, AAT11, AAT41, APT41, MPA11, MPA51, MAB41, MPB11, MPG11, Water control

Tree 2: APB51, AAC31, AAC41, APC11, APC31, APC41, APD21, APD31, AAG11, AAG41, APG11, APG21, APG41, AAT51, APT21, APT31, MPA21, MPB21, MAG31, Water control

Tree 3: AAB11, APB11, APB41, AAC11, AAC31, APC41, AAD21, AAD31, AAD51, AAG31, APG51, AAT41, APT21, APT31, AA21, MAB21, MPB41, MAG41, MPG41, MPG51, Water control

Tree 4: AAB41, AAC11, APC11, APC21, AAD11, AAD21, AAD41, APD41, AAG41, AAT21, AAT31, AAT51, APT11, MAA11, MAA21, MPA21, MAB11, MAB31, MAB41, MPB21, MPG41, Water control

Tree 5: AAB21, AAB31, AAC31, AAC41, APC31, APC51, AAD11, AAD21, APD41, APD51, AAG31, APT41, MPA41, MAB21, MAB31, MAB51, MAG21, MAG31, MAG51, MPG21, Water control

Tree 6: AAB51, APB51, AAC11, AAC21, APC11, APC41, AAD51, APD41, AAG11, AAG21, APG21, AAT11, APT11, MAA41, MAA51, MPA11, MAB11, MAB21, MAB51, MAG21, Water control

Tree 7: AAB41, APB21, AAD11, AAD51, APD31, AAG31, AAT11, AAT21, AAT31, AAT51, APT21, MAA51, MPA31, MPB31, MPB51, MAG11, MAG21, MAG31, MPG11, MPG51, Water control

Tree 8: AAB21, APB21, APB41, APB51, AAC21, APC11, AAD41, APD11, APG11, APG41, AAT21, APT51, MAA21, MAA41, MPA41, MAB51, MAG51, MPG11, MPG41, MPG51, Water control

Tree 9: AAB31, APB21, AAC41, AAC51, APD11, AAG51, APG11, APG21, APG51, APT11, MAA31, MPA11, MAB21, MAB31, MPB21, MPB31, MPB51, MPG31, MPG41, MPG51, Water control

Tree 10: AAB11, AAB51, APB11, APB41, AAC51, APC21, APC41, AAD41, APD11, AAG11, APG31, AAT31, APT41, MAA51, MPA31, MPA41, MAB31, MPB51, MAG31, MPG31, Water control

Tree 11: AAB11, AAB41, AAB51, APB31, AAD31, APD31, APD41, APD51, APG21, APG31, AAT41, APT51, MAA21, MAA51, MPB21, MPB31, MAG41, MAG51, MPG11, Water control

Tree 12: AAB21, AAB31, AAB41, APB41, AAC31, APC31, APC41, APT11, APT41, APT51, MAA11, MAA31, MAA41, MAA51, MPA21, MPA31, MPA51, MPB11, MPB31, MPB41, Water control

Tree 13: AAB11, AAB21, AAB51, APC11, APC21, APC51, APD51, APG11, APG41, AAT11, APT11, APT21, MPA31, MPA51, MAB31, MAB41, MAG11, MAG41, MPG21, MPG41, Water control

Tree 14: AAB51, APB11, APB21, APB51, APC21, APC51, AAD11, AAD31, APD21, AAG21, APG51, AAT11, APT31, APT51, MAA11, MAA31, MPA41, MPB21, MPB41, MAG21, Water control

Tree 15: AAB21, APB31, AAC21, AAD31, AAG11, AAG31, AAG41, APG51, AAT31, MPA11, MPA21, MPA41, MAB11, MAB41, MPB31, MPB41, MAG11, MAG51, MPG21, MPG31, Water control

Tree 16: AAB31, APB31, APB51, AAC11, AAC21, AAC41, AAC51, APC21, APC51, AAD41, APD31, APD51, AAG51, AAT31, AAT41, AAT51, APT21, MAA41, MPB11, MPB41, Water control

Tree 17: AAB11, APB11, APB31, AAC51, APD21, AAG21, AAG41, APG11, AAT21, AAT51, APT41, MAA21, MAA31, MPA51, MAB21, MAB51, MPB11, MAG11, MAG21, MAG51, Water control

Tree 18: AAC21, AAC51, APC31, APC51, AAD11, AAD51, APD11, AAG21, APG21, APG31, AAT21, AAT41, APT31, MAA11, MPA21, MPA51, MAG41, MPG11, MPG21, MPG31, Water control

Tree 19: AAB31, APB11, APB41, AAC11, APC31, AAD31, APD21, APD31, APD41, AAG21, AAG51, APG31, APG41, MAA41, MAB11, MAB41, MPB51, MAG11, MPG21, MPG51, Water control

Tree 20: APB31, AAD21, AAD51, APD11, APD51, AAG41, AAG51, APG41, APT31, APT51, MAA11, MAA31, MPA11, MPA31, MAB11, MAB51, MPB11, MPB51, MAG31, MAG41, Water control

Twenty trees were used and 20 fruit per tree were selected at random and tagged, ensuring that fruit were free of any obvious blemishes or disease. Each isolate was inoculated onto 5 fruit randomly chosen throughout the 20 trees. The experimental design was generated by a program called CycDesigN (Whitaker, Williams and John 2001) and was a non-resolvable block design, where a block was represented by a tree.

Spore suspensions were prepared for each isolate (5 x 10^6 conidia/mL). Fruit were dipped with spore suspension containing 0.01% v/v Tween 80 ensuring that the entire fruit surface was saturated. Control fruit were dipped with water containing Tween 80. Fruit were enclosed for 48 h in a plastic bag and paper bag as previously described. Fruit and pedicels were assessed for disease after 2 weeks, 4 weeks and 6 weeks.

C. Factors affecting pepper spot development

The infection process by *Colletotrichum gloeosporioides* in avocado is well documented (Coates and Gowanlock 1993). Spores germinate on unripe avocado fruit to produce germ tubes from which appressoria form after 5-6 h. Within 48 h, the appressoria germinate to produce infection pegs that penetrate about 1.5 µm into the wax layer and cuticle of the fruit and remain quiescent until after harvest. Quiescence has been defined as the arrested development of the pathogen due to temporarily imposed physiological conditions. The possible role of antifungal dienes in *C. gloeosporioides* has been discussed in the previous section. Fruit are susceptible to infection at all stages from fruit set to harvest in the cultivars Fuerte (Peterson 1978, Coates *et al*. 1993a) and Hass (Willingham *et al.* 2001). The development of pepper spot on the fruit surface presents a different scenario. Histopathology studies were included in this section to examine the infection structures of *Colletotrichum gloeosporioides* involved in pepper spot development. It was necessary to make comparisons with work done on latent infections of avocado fruit cv. Fuerte (Coates 1991). It was supposed that the likely findings would be that pepper spot isolates are penetrating further into the fruit cells, sufficient to trigger a host response.

The biology of the interaction between the pathogen and the host raises numerous issues. It is believed that the pepper spot symptom is the result of a hypersensitive-like response by the avocado fruit as a reaction to the presence of certain *C. gloeosporioides* isolates. Obviously this response is not occurring in the interaction between the host and the isolates which become quiescent. Therefore, it is reasoned that the relatively recent appearance of the pepper spot symptom is a co-evolution of host-pathogen recognition. Whether or not the host alone has developed this resistance response, or the fungus has advanced its capabilities to bypass the quiescent phase, or both, remains unknown. The plant does not appear to kill the pathogen, just contain it. Signalling pathways controlling active cell death during plant-microbe interactions remain to be elucidated (Sasabe *et al.* 2000). Defence responses in plants can be separated into 3 steps: recognition of the pathogen; signal transduction; and execution of defence programmes such as HR cell death, oxidative burst, transcriptional activation of defence genes, and subsequent induction of systemic acquired resistance (SAR) (Sasabe *et al.* 2000). It is only possible to speculate about such defence responses in avocados.

The previous two sections showed that there is limited distinction between pepper spot and anthracnose isolates of avocado which seems to confirm that the pepper spot symptom is occurring as a result of host and environmental factors. In the absence of detailed resistance gene (*avr/R*) studies, it is presumptuous to discuss gene-for-gene systems, however, general defence mechanisms can be discussed.

The experiments in this section aimed to investigate the effects of several factors on infection by *Colletotrichum gloeosporioides* and the development of pepper spot. A main study involved the inoculation of avocado fruit with pepper spot isolates of *Colletotrichum gloeosporioides* throughout the growing season from soon after fruit set until full fruit maturity in an assessment of disease incidence during changing annual seasons (spring through to winter). All fruit inoculations carried out in field experiments were done on the warmer, sunnier side of the trees. This inoculation study also incorporated a comparison of inoculum concentration and the resulting pepper spot incidence and severity. High concentrations of conidia may be a factor in the development of preharvest disease symptoms (Pegg *et al.* 2002). Inoculation of 'Fuerte' fruit (Coates 1991, Coates and Gowanlock 1993) with *Colletotrichum gloeosporioides* resulted in limited lesions not dissimilar to pepper spot. The production of these symptoms were dependent on inoculum density. Inoculation with *C.*

gloeosporioides with a density of 1 x 10^6 conidia/mL sometimes resulted in the production of limited lesions on the peel but these symptoms were not produced when fruit were inoculated at 1 x 10^4 conidia/mL. Coates (1991) speculated that this was an incompatible host response where pathogen growth was inhibited and hypersensitive cell death triggered. In cross infection studies of *C. gloeosporioides* isolates by Alahakoon *et al.* (1994a) it was noted that infection appeared to be dependent on inoculum density. Their trials were carried out at 5 x 10^6 conidia/mL, an inoculum level unlikely to occur in nature.

Pepper spot inoculations were also carried out on various avocado rootstocks in the field to compare disease incidence and it was also possible to inoculate trees undergoing different nitrogen fertiliser programmes. pH measurements were included in a number of experiments in this chapter and mineral analyses were incorporated into one of the rootstock studies to detect any measurable trends.

Another objective of this section was to determine if the presence of pepper spots offered cross protection against postharvest anthracnose (i.e. do isolates trigger a systemic acquired resistance?). If the pepper spot symptom is due to a host response, it would be anticipated that fruit with a high incidence of pepper spot would tend to be less affected by anthracnose during ripening. However, if the fungus remains viable until harvest and ripening, it may then be able to infect and produce typical anthracnose lesions. If environmental factors such as heat stress and root rot pressure are significant influences, and inoculum levels are high, then anthracnose levels would be expected to be high also.

A. Effect of Colletotrichum gloeosporioides *inoculum concentration and fruit maturity on the development of pepper spot symptoms on avocado fruit and pedicels*

The trial commenced in October 2001 at Anderson's property at Duranbah, NSW on a block of 3 year old 'Hass' avocado fruit on cv. Edranol (Guatemalan) trees. Fruit were selected at random and tagged, ensuring that there were no visible fruit blemishes. Only fruit with a north-western aspect were used. Two aggressive pepper spot isolates (APT11 and APD11 selected from first year of trials) were used at 3 inoculum concentrations at 11 inoculation times (17.10.01, 30.10.01, 13.11.01, 27.11.01, 17.12.01, 15.01.02, 12.02.02, 12.03.02, 16.04.02, 14.05.02, 18.06.02). Fruit were inoculated at each of the 11 inoculation times with the selected isolate and spore concentration. At the first inoculation time in October, fruit were pea-sized. The next 4 inoculation times were at fortnightly intervals thereafter, during which time, fruit expanded to ca. 20mm. The 6^{th} to 11^{th} inoculation times were then at monthly intervals. Fruit were up to 130mm at the final inoculation time in June. At each inoculation time, 5 trees were selected and 35 fruit tagged on each tree. Each treatment and control were inoculated onto 5 fruit each. It would have been desirable to carry out this trial on the same 5 trees but due to the large number of fruit to be inoculated this was logistically impossible. Therefore, it was necessary to use additional trees as fruit numbers were insufficient on original trees. It was necessary to inoculate a high number of fruit to allow for fruit drop which is particularly prevalent in the early months.

The following isolate and water control treatments were used for each replicate:
APT11 5 x 10^4 conidia/mL
APT11 5 x 10^6 conidia/mL
APT11 5 x 10^8 conidia/mL
APD11 5 x 10^4 conidia/mL
APD11 5 x 10^6 conidia/mL
APD11 5 x 10^8 conidia/mL
Water control

Spore suspensions were prepared for each isolate (5 x 10^4, 5 x 10^6, 5 x 10^8 conidia/mL). Fruit and pedicels were dipped into spore suspension containing 0.01% v/v Tween 80 for about 5

secs ensuring that the entire area was saturated. Control fruit were dipped into water containing Tween 80. Fruit were enclosed in a plastic bag containing a water-soaked cotton wool ball and held in place with a staple. Paper bags were placed over the plastic bag and held on by a staple. After 48 h, both bags were removed. Fruit were assessed for pepper spot severity 2 weeks after inoculation for 12 weeks. Fruit pedicels were only assessed for pepper spot severity at the final 12 week assessment time. After this time, remaining fruit were harvested and taken to the laboratory to re-isolate inoculated fungi and to account for any naturally occurring *C. gloeosporioides* infections. Fruit were surface sterilised by spraying with 70% ethanol and left to air dry. Using a sterile scalpel, black spots resembling pepper spots were picked off the surface and plunged into potato dextrose agar amended with streptomycin (SPDA). Four samples were taken from each fruit with all samples placed on a single agar plate. Four samples were taken from the pedicel of each fruit. Four samples of green flesh were also taken by cutting a small piece (<1mm^2) out of an area of green flesh and placing on SPDA. These plates were incubated at room temp under black light for about a week and then assessed for the presence/absence of *C. gloeosporioides.* Cultures were compared morphologically with the original cultures.

1. Inoculation of petioles of 'Hass' on several nursery avocado rootstocks in the glasshouse with lower concentrations of *Colletotrichum gloeosporioides* spore suspensions, and treatment of petioles with spore germinating fluid (SGF)

Six rootstocks (5 plants each) were used in this experiment: 'A1' (Guatemalan), 'Duke 6' (Mexican), 'Duke 7' (Mexican), 'Reed' (Guatemalan), 'Velvick' (West Indian), and 'Zutano' (Mexican). Three pepper spot isolates which produced high ratings (>4) in field experiments were used at 4 inoculum concentrations.

The following isolate and water control treatments were used on each rootstock:
APT11 (avg field trial p. spot rating at 5x10^8 conidia/mL=4.39)
APD11 (avg field trial p. spot rating at 5x10^8 conidia/mL=4.08)
APC41 (avg field trial p. spot rating at 5x10^8 conidia/mL=4.32)
Water control

Each isolate was inoculated onto each rootstock at 3 suspension concentrations: 5 x 10^2 conidia/mL, 5 x 10^3 conidia/mL and 5 x 10^4 conidia/mL. One control was included for each rootstock. In addition, each rootstock was inoculated with a selected isolate at 5 x 10^8 conidia/mL suspension. 'Duke 7' and 'Zutano' were both inoculated with APD11 at 5 x 10^8 conidia/mL. 'Duke 6' and 'Velvick' were inoculated with APC41 at 5 x 10^8 conidia/mL and 'A1' and 'Reed' were inoculated with APT11 at 5 x 10^8 conidia/mL.

Spore suspensions were prepared for each isolate. Using an artist's airbrush, petioles were sprayed with spore suspensions containing 0.01% v/v Tween 80 ensuring that the entire surface was saturated. Control leaves were sprayed with water containing Tween 80. Leaves were enclosed in a plastic bag containing a water-soaked cotton wool ball and held in place with staples. After 48 h the bags were removed. Petioles were inspected for disease after 2 weeks to assess pepper spot development.

An aqueous spore suspension of isolate APC41 was prepared and adjusted to 5 x 10^4 conidia/mL. The suspension was incubated overnight to allow for spore germination. The fluid was filtered through Whatman No. 4 filter paper (Whatman, Inc., Clifton, NJ) and then ultrafiltrated using a 0.20 μm cellulose acetate filter (Sartorius AG, Goettingen, Germany). After checking for the absence of spores, the pH of the fluid was measured and then the fluid was sprayed onto a petiole of each plant (5) of each rootstock and treated as for other inoculations.

2. Measurements of pH of spore suspensions of *C. gloeosporioides*

Suspensions of *C. gloeosporioides* isolates of APC41, APD11 and APT11 were prepared at 5 x 10^2, 5 x 10^3, 5 x 10^4, 5 x 10^5, 5 x 10^6, 5 x 10^8. APC11 also included a suspension at 9 x 10^5, APD11 included a suspension at 2.5 x 10^6, and APT11 included a suspension at 7 x 10^5. pH measurements were recorded at the time of preparation.

B. Effect of nitrogen fertilisation and rootstock on avocado pepper spot disease susceptibility

1. Preliminary trial (2001-2002) to evaluate the effect of nitrogen fertiliser level on pepper spot development after *Colletotrichum gloeosporioides* inoculation on 'Hass' avocado fruit on two different race rootstocks

This trial commenced in December, 2001 on Anderson's property at Duranbah, NSW on a block of 6.5 year old trees. Trees used were 'Hass' grafted to 'Duke 6' Mexican seedling rootstock and to 'Velvick' Guatemalan seedling rootstock.

Application of the 3 nitrogen treatments commenced 2 months prior to fruit inoculations (October, 2001) and were as follows:

1. Control nitrogen (0% N)
2. Low nitrogen (N applied as ammonia 13.3%)
3. High nitrogen (N applied as ammonia 26.6%)

Approximately 11 applications of fertiliser were made in total. Applications were made fortnightly for the first 10 weeks after fruit set and then monthly until harvest. For each rootstock, 5 trees were selected for each of the three nitrogen treatments. Six fruit were then selected and tagged on each tree: 5 for *C. gloeosporioides* inoculation and one for water control.

A spore suspension was prepared for the isolate APD11 at 5 x 10^6 conidia/mL. Fruit were rinsed with water to remove copper residues and then treated following the method described in section A. Fruit were assessed for disease incidence and severity after 2 weeks and then rated every 6 weeks until harvest.

2. Trial to evaluate the effect of nitrogen fertiliser level on pepper spot on 'Hass' avocado fruit on two different race rootstocks (2002-2003)

The trial was carried out at Anderson's property at Duranbah, NSW on a block of 7.5 year old 'Hass' grafted onto 'Duke 6' (Mexican) and 'Velvick' (West Indian) rootstock trees. This is the same trial site used in Experiment 1.

Application of 2 nitrogen treatments commenced in November, 2002 and were as follows:

1. Control nitrogen (0% N)
2. High nitrogen (N applied as ammonia 26.6%)

For this trial, there were only 2 nitrogen levels and 5 treatment fruit plus 5 control fruit were included on each tree on each of the two rootstocks. Fruit were inoculated in January and March 2003 with isolate APD11 using the same methods as in experiment 1. A single assessment was made for disease severity after 4 weeks.

3. Effect of pepper spot infection following *C. gloeosporioides* inoculation on the subsequent development of postharvest anthracnose during fruit ripening

Fruit from inoculation trials described in experiment 2 were left on the trees until harvest in August 2003. Fruit were harvested and assessed for pepper spot severity. Fruit were ripened at 22^0C (65% RH) and peeled at eating ripe stage. Ripe fruit were assessed for anthracnose incidence. Anthracnose lesion severity was estimated as a percentage of fruit surface area affected by disease.

C. Effect of avocado rootstock cultivars Anderson 8 (Guatemalan), Anderson 10 (Guatemalan), Nabal (Guatemalan) and Parida 1 (Mexican) rootstocks on pepper spot disease susceptibility

1. Field inoculation

The trial was carried out in 2003 at Anderson's property at Duranbah, NSW on a block of 4 year old trees. For each rootstock, 5 trees were selected. Five fruit were treated and 5 fruit were controls on each tree. Fruit were inoculated with pepper spot isolate APD11 in January and March (2003) using the same methods as in experiment 1. A single assessment was made for disease severity after 4 weeks.

2. Effect of avocado rootstock on mineral concentrations of leaves and the relationship with pepper spot disease incidence and severity after inoculation with *C. gloeosporioides* on 'Hass' avocado fruit

The trees which were treated in the experiment above were used for this experiment. Leaves were taken from the same trees in May. Sixteen mature, clean leaves were taken from all sides of the tree. Leaves were oven dried at 60^0C for about a week and ground to a fine powder using an electric coffee grinder (Philips, Groningen, The Netherlands) and sent for sample analysis (CASCO Agritech, CASCO Australia Pty Ltd, Toowoomba QLD).

3. Effect of pepper spot infection following *C. gloeosporioides* inoculation on the subsequent development of postharvest anthracnose during fruit ripening

Fruit from inoculation trials described above were left on the trees until harvest in August 2003. Fruit were harvested and assessed for pepper spot severity. Fruit were ripened at 22^0C (65% RH) and peeled at eating ripe stage. Ripe fruit were assessed for anthracnose incidence. Anthracnose lesion severity was estimated as a percentage of fruit surface area affected by disease.

D. Effect of skin pH on the susceptibility of 'Hass' avocado to pepper spot disease after inoculation with Colletotrichum gloeosporioides

The trial commenced in November 2002 at Anderson's property at Duranbah, NSW on a block of 4 year old 'Hass' avocado fruit on cv. Edranol trees. Fruit were selected at random and tagged, ensuring that there were no visible fruit blemishes. Only fruit with a north-western aspect were used. Two aggressive pepper spot isolates (APT11 and APD11 selected from first year of trials) were used at an inoculum concentration of 5×10^6 conidia/mL at 7 monthly inoculation times from November through to May 2003. Inoculated fruit were harvested 2 weeks after inoculation and pH measurements of fruit tissue were recorded. From

the first to the final inoculation time, fruit increased from ca. 20mm to ca. 120mm. At each inoculation time, 5 trees were selected and 15 fruit tagged on each tree. Each treatment and control were inoculated onto 5 fruit each. It would have been desirable to carry out this trial on the same 5 trees but due to the large number of fruit to be inoculated and the low fruit yield of the trees for this season, this was logistically impossible. Therefore, it was necessary to use additional trees for inoculation.

Spore suspensions were prepared for isolates AAD11 and APD11 at 5 x 10^6 conidia/mL and fruit were treated as previously described. Once fruit were harvested and transported to the laboratory, pH was determined by slicing off a 1 mm-deep section of peel with a scalpel blade and then placing the flat electrode of a flat sensor pH meter (Yakoby *et al.* 2000b) directly against the exposed mesocarp tissue for up to 3 minutes. pH measurements were taken at 3 sites of one fruit from each treatment.

E. Histopathology of Colletotrichum gloeosporioides *causing pepper spot infection on avocado fruit*

1. Field inoculation of fruit

The trial was carried out in 2003 at Anderson's property at Duranbah, NSW on a 5 year old block of 'Hass' trees grafted onto rootstock 'Edranol'. Fruit were inoculated with 2 pepper spot isolates (APD11 and APT11). Fruit were harvested after 24 h, 48 h, 72 h and 7 days and transported to the laboratory.

Spore suspensions were prepared for the isolates at 1 x 10^8 conidia/mL. Fruit were inoculated using 3 methods:

1. Sprayed with spore suspension
2. Dipped into spore suspension
3. blotting paper (1cm x 1cm) saturated in spore suspension attached to the fruit with waterproof tape (Plate 6).

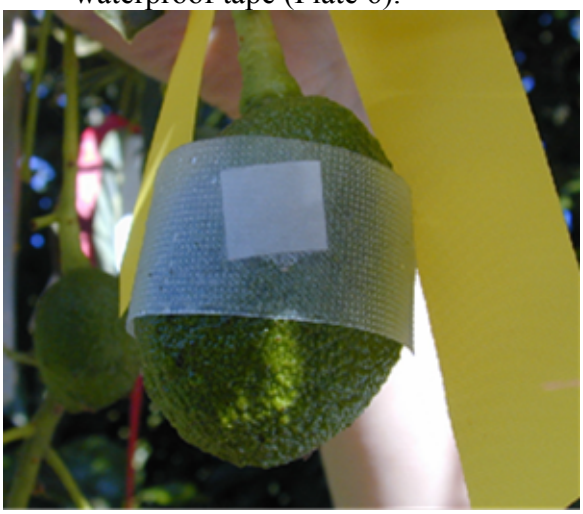

Plate 6: Inoculation of avocado fruit by the blotting paper method prior to bagging

Fruit were enclosed in a plastic bag containing water-soaked cotton wool and held in place with a staple. Paper bags were placed over the plastic bag and held on by a staple. For the first set of fruit (12 fruit), both bags were removed along with tape and filter paper after 24 h, and the fruit harvested for analysis. After 48 h, all bags were removed.

2. Tissue preparation for Light Microscopy (LM)

Fruit were transported to the lab at the stated intervals and tissue from the fruit was prepared and stored for LM.

Primary Fixation
Thin slices, approximately 200 μm thick, 5mm long and 5mm wide, were taken from the fruit skin. Each slice was placed on a "waxy sheet" and immersed in a drop of Primary Fixative (3% (v/v) glutaraldehyde + 1% (w/v) caffeine in 0.1M phosphate buffer, pH 7.0). Using a stainless steel razor blade, slices were further dissected into smaller pieces no larger than $1mm^2$. The droplet of Fixative plus peel was poured into a small labelled vial. The vial was left at room temperature for 2 – 4 h or stored at 4^0C. Fixative solution was removed carefully with a pipette, and the small peel pieces were washed 4 times for 10 mins each worth 0.1M phosphate buffer, pH 7.0. Samples were stored at room temperature overnight in buffer.

Post Fixation
Tissue pieces were post-fixed in 1% (w/v) osmium tetroxide in 0.1M phosphate buffer, pH 7.0 for 2 h at room temperature. Samples were then washed in buffer for 10 mins at room temperature, then washed twice in water for 10 mins each.

Tissue pieces were dehydrated at room temperature in a graded acetone series as follows:

10% Acetone	10 minutes
20% Acetone	10 mins
30% Acetone	10 mins
40% Acetone	10 mins
50% Acetone	10 mins
60% Acetone	10 mins
70% Acetone	10 mins
80% Acetone	10 mins
90% Acetone	10 mins
100% Acetone	15 mins
100% Acetone	15 mins
100% Acetone	15 mins
100% Acetone	15 mins

Tissue was then infiltrated with a 1:1 mixture of Spurr's resin (Spurr 1969) and absolute acetone at room temperature on a sample rotor followed by higher resin ratios as follows:

1:1 resin + 100% acetone (ie. 50:50)	12 – 24 hours
3:1 resin + 100% acetone (ie. 75:25)	12 – 24 hours
5:1 resin + 100% acetone (ie. 85:15)	12 – 24 hours
100% resin	Up to 3 days
100% resin	4 hours

Polymerisation
Plastic moulds (ca. 10mm x 4mm) were half filled with 100% Spurr's resin. Tissue pieces were removed from vials using a whittled wooden stick and positioned at each end of the mould. Moulds were topped up with resin and a printed label was placed on the surface using fine forceps. Moulds were incubated in a plastic box at 60^0C for 3 days.

Sectioning
Thick sections (~1μm) were cut with a glass knife onto water with a Reichert Ultracut E Ultramicrotome. Sections were collected onto a glass microscope slide in a drop of water. The slide was then heated on a hotplate until dry (~1 min). Sections were then stained with 1% (w/v) toluidine blue, dried on the hotplate, rinsed with water and then viewed under the microscope.

3. Tissue preparation for Transmission Electron Microscopy (TEM)

Fruit tissue was fixed, dehydrated, infiltrated and embedded in Spurr's resin as for LM. Thin sections (60-90nm) were cut with a glass knife and mounted onto coated copper grids (200 mesh hexagonal, ProSciTech, QLD). Each grid was stained with uranyl acetate (5% UAT in 50% ethanol) for 5 minutes, followed by 3 water washes of 10 secs each. The grid was blotted dry and soaked in a droplet of Reynolds lead citrate (Reynolds 1963?) for 2 minutes followed by 3 rinses in water. Sections were viewed using a JEM-1010 Transmission Electron Microscope.

2.5 Cross-Protection Studies

One of the aims of this project was to evaluate endophytic, weakly virulent and non-pathogenic isolates of *Colletotrichum* for their ability to enhance antifungal activity and suppress anthracnose development in 'Hass' avocado fruit.

A total of 80 isolates of *Colletotrichum gloeosporioides*, including 50 isolates from avocado and 30 isolates from mango, have been screened for their pathogenicity on avocado fruit. Many of the mango isolates were found to be only weakly pathogenic on avocado, and as such may be suitable candidates for cross-protection studies. However, due to ongoing difficulties in analysing antifungal (ie. diene) levels in avocado, we were not able to screen these isolates for their ability to induce antifungals in 'Hass' fruit.

One of the other aims of this research was to determine if it is possible to introduce *C. gloeosporioides* as an endophyte (which is an organism which internally colonises plant tissue without causing disease) into the stem tissue of young avocado plants. Previous research (Freeman and Rodriguez, 1993) has shown that introduction of an endophytic, non-pathogenic isolate of *C. magna* into watermelon seedlings resulted in cross-protection against pathogenic strains of the fungus. The rationale behind the avocado studies conducted in this project was essentially the same.

A preliminary experiment was conducted to test a method for isolating endophytic *C. gloeosporioides* from young avocado plants. Young avocado trees cv. Hass growing in the glasshouse were inoculated with an isolate of *C. gloeosporioides* ($5x10^6$ spores/mL + 0.01% Tween 80, BRIP 19778). The isolate used was selected for this trial because it is resistant to the fungicide benomyl – which means that it can be easily distinguished from other isolates of *C. gloeosporioides* by its ability to grow on media containing benomyl. Inoculated trees were sprayed with a spore suspension of the isolate and then covered with a plastic bag for 48 h containing a moistened cotton ball to maintain humidity. Control trees were sprayed with water + Tween 80 only. One week after inoculation, leaves, petioles and stem pieces were taken for isolations using a triple sterilisation technique. The following technique has been used for isolating endophytic fungi from mango stem tissue:

1. immerse tissue in ethanol 70% for 1 min
2. immerse tissue in 10% sodium hypochlorite solution for 2 mins
3. immerse tissue in ethanol 70% for 30 secs
4. dry segments on blotting paper
5. cut a 1 mm thick disk from stems and petioles and a 2x2mm square from leaf and then cut these pieces into quarters
6. place each quarter on 4 quarters of same plate

Isolates obtained from cultures were then subcultured onto media containing 1 ppm benomyl to check for the presence of the benomyl-resistant isolate.

The benomyl-resistant isolate was readily recovered from leaves, stem pieces and petioles of all inoculated avocado trees, indicating that the triple sterilisation technique was unable to kill latent infections of *C. gloeosporioides* present in the surface tissue layers. The conclusion drawn was that a new sterilisation technique would have to be developed for isolating endophytes from avocado, since the one used here was not sufficient to kill non-endophytic latent infections.

The following two experiments were conducted to determine if *C. gloeosporioides* can be introduced as an endophyte into avocado seedlings by A) dipping wounded root systems in a spore suspension of the fungus or by B) inoculating fruit.

A. Root dip experiment

Thirty-two 'Zutano' avocado seedlings were obtained and removed from their pots. Their root systems were rinsed in distilled water, trimmed to provide points of entry for the fungus, and then dipped in a spore suspension ($5x10^6$ spores/mL + 0.01% Tween 80, BRIP 19778) of the fungus. Once again the fungus used was the benomyl-resistant isolate of *C. gloeosporioides*. Control seedlings were dipped in water + Tween 80 only. Seedlings were then re-potted and then transferred to a growth cabinet at 27°C. Seedlings were then destructively sampled at weekly intervals for isolation of the fungus from surface sterilised stem pieces. The following surface sterilising method was used:

1. immerse tissue in ethanol 70% for 1 min
2. immerse tissue in 10% sodium hypochlorite solution for 3 mins
3. immerse tissue in ethanol 70% for 30 secs
4. dry segments on blotting paper
5. cut a 1 mm thick disk from stems and then cut these pieces into quarters
6. place each quarter on 4 quarters of same plate

Isolates obtained from cultures were then subcultured onto media containing 1 ppm benomyl to check for the presence of the benomyl-resistant isolate.

B. Seedling transmission experiment

In mango, it has been shown that the stem-end rot fungi can be transmitted endophytically from infected mango fruit to their seeds, and then to the seedlings which grow from these seeds (Johnson *et al*., 1992). To test if this could be done in avocado, we obtained twenty 'Hass' avocado fruit and inoculated half of these with mycelial plugs of the benomyl-resistant *C. gloeosporioides*. The other 10 fruit (controls) were inoculated with an agar plug containing no fungus. Fruit were incubated at 23-25°C until symptoms of stem-end rot appeared on inoculated fruit (when fruit showed approximately 10-20% decay). Seeds were then extracted from diseased fruit, the seed coat removed and then planted in commercial potting mix. Seeds were also extracted from control fruit. Pots were held in a growth cabinet at 27°C for germination. Seedlings were then destructively sampled at approximately 4-6 weeks after germination, for isolation of the fungus from various points along the stem from surface sterilised pieces. The following surface sterilising method was used:

1. immerse tissue in ethanol 70% for 1 min
2. immerse tissue in 10% sodium hypochlorite solution for 3 mins
3. immerse tissue in ethanol 70% for 30 secs
4. dry segments on blotting paper
5. cut a 1 mm thick disk from stems and then cut these pieces into quarters
6. place each quarter on 4 quarters of same plate

Isolates obtained from cultures were then subcultured onto media containing 1 ppm benomyl to check for the presence of the benomyl-resistant isolate.

3. RESULTS

3.1 Rootstock and Nutrition Studies

A. Rootstock x fertiliser studies

2001/02 Season

Rootstock and fertiliser treatments had significant impacts on vegetative flushing when trees were assessed in December (Table 2). Trees receiving no nitrogen fertiliser (nil N treatment) had significantly fewer vegetative flushing terminals than trees receiving NH_4 and NO_3 nitrogen fertilisers when averaged across both rootstocks (Table 2). Rootstock also had an effect, with 'Duke 6' trees producing significantly more new vegetative terminals than 'Velvick' (Table 2). Canopy colour was also significantly influenced by rootstock and nitrogen treatments. 'Velvick' trees had canopies that were significantly darker green in colour than 'Duke 6' trees when assessed in December (Table 2). Trees receiving no nitrogen fertiliser (nil N) also had canopies that were significantly paler green in colour than trees receiving NH_4 and NO_3 fertilisers when assessed in December (Table 2).

When flushing was assessed in January, smaller treatment effects were observed as the trees had undergone a recent flush (Table 3). The nil N treatment trees had significantly more vegetative flush than the control and high NH_4 treatments but not the NO_3 treatment trees (Table 3). No significant differences in crop load or canopy colour were observed in January (Table 3).

In February, a significant rootstock effect on flushing and crop load, but not canopy colour was observed (Table 4). 'Velvick' trees had significantly fewer flushing terminals and a significantly higher crop load than 'Duke 6' trees (Table 4). Whilst there was not a significant overall nitrogen effect, there was a significant rootstock x nitrogen effect. 'Velvick' trees treated with control rates of NO_3 had significantly fewer flushing terminals than 'Duke 6' trees treated with control rates of NO_3 (Table 4).

In March, the 'Velvick' trees had significantly more vegetative flushing terminals than the 'Duke 6' trees, but also still had a significantly higher crop load (Table 5). Again, this strong rootstock effect was reflected in the only other significant difference between treatments where 'Velvick' trees receiving nil N had significantly more vegetative flushing terminals than 'Duke 6' trees treated with nil N (Table 5). No significant effects on canopy colour were evident in March.

In April, the strong rootstock effect on flushing that was previously evident, was not significant but nitrogen fertiliser treatment did have a significant impact (Table 6). Trees treated with control rates of NO_3 had significantly fewer flushing terminals than nil N trees and trees treated with NH_4 (Table 6). Trees treated with the higher rate of NO_3 had slightly but not significantly lower flushing ratings than the control NO_3 rate. The effect of rootstock on crop load was still evident in April, with 'Velvick' trees carrying a larger crop than 'Duke 6' trees (Table 6). Canopy colour was not significantly influenced by rootstock in the April observations, but nitrogen fertiliser treatment did have a significant impact. Trees receiving no nitrogen (nil N) had a significantly paler green canopy colour than trees receiving nitrogen treatments across both rootstocks (Table 6).

To summarise, rootstock had a significant impact on vegetative flushing and crop load. Throughout the observed growing season (December – April), 'Velvick' appeared to have

slightly fewer vegetative terminals flushing than 'Duke 6'. 'Velvick' also maintained a larger crop load than 'Duke 6'. Nitrogen fertiliser effects on flushing were also observed. Trees receiving no nitrogen (nil N) had significantly fewer flushing terminals in December than the nitrogen fertiliser treatment trees, but by April, trees receiving NH_4 had similar vegetative growth as the nil N trees. Rootstock and fertiliser treatments also had a significant impact on canopy colour. 'Velvick' trees had significantly darker green canopies than 'Duke 6' trees. However, this effect was only significant in December. Withholding nitrogen (nil N) also resulted in trees having significantly paler green canopy colours.

As shown in previous years, the severity and incidence of anthracnose were significantly greater in fruit from 'Hass' grafted to 'Duke 6' than 'Hass' grafted to 'Velvick' rootstock (Table 7). None of the fertiliser treatments were significantly different in their effects on fruit diseases. However, there was a trend for the fruit from nil nitrogen trees to have less disease. There were no significant interactions between any of the treatments (data not shown). Fruit position also did not influence shelf life or disease levels (Table 7).

Leaf samples taken in May from 'Hass' trees grafted to 'Velvick' rootstock had significantly lower N concentrations, significantly lower N/Ca ratios (more favourable), significantly higher Ca concentrations and significantly higher Ca+Mg/K ratios (more favourable) than leaves from 'Hass' trees grafted to 'Duke 6' rootstock. None of the fertiliser treatments had a significant effect on the nutrient levels in the leaves in May, with the exception of an interaction effect of fertiliser treatment on Ca concentrations and Ca+Mg/K ratios (however this data was inconsistent and is not presented).

Nitrogen and Ca concentrations and the N/Ca ratios in fruit skins for July were not significantly different between fruit from the 'Hass'/'Velvick' and 'Hass'/'Duke 6' combinations (Table 8). There was however, a significantly higher Ca+Mg/K ratio (more favourable) in the skin of fruit from 'Hass'/'Velvick' than in fruit skin from 'Hass'/'Duke 6'. The N concentration and N/Ca ratios in fruit skin was significantly lower (more favourable) in fruit from the nil nitrogen treatment compared with fruit from the other treatments. No interaction effects between the rootstock and fertiliser regimes were observed in nutrient concentrations for the skins from fruit harvested in July.

July leaf samples from 'Hass' trees grafted to 'Velvick' rootstock had a significantly lower N concentration, a significantly lower N/Ca ratio (more favourable) and a significantly higher Ca+Mg/K ratio (more favourable) than leaves from 'Hass' trees grafted to 'Duke 6' rootstock (Table 9). Nutrient concentrations in the leaf samples were also affected by the fertiliser treatment. Leaves sampled from the nil nitrogen treatment had a significantly lower concentration of Ca than all of the other treatments, except for the high nitrate treatment. All of the fertiliser treatments, except for the control ammonium treatment, significantly increased the leaf nitrogen concentrations in July. There were however, no significant fertiliser x rootstock effects on mineral concentration for the leaf sample taken in July.

Table 2. The effects of rootstock and fertiliser on vegetative flushing, crop load and canopy colour of 'Hass' avocado trees assessed 18th December 2001. Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 6). LSD indicates least significant difference value.

Treatment	Vegetative flush (1-5)	Crop load (1-3)	Colour (1-3)
Rootstock			
'Velvick'	2.7b	2.2	2.8a
'Duke 6'	3.1a	2.1	2.5b
LSD	0.3	-	0.2
Fertiliser			
Nil N	1.8b	2.0	2.2b
Control NH_4	2.9a	2.2	2.9a
Control NO_3	3.2a	2.3	2.8a
High NH_4	3.3a	2.1	2.8a
High NO_3	3.1a	2.3	2.7a
LSD	0.5	-	0.4
Rootstock x Fertiliser			
'Velvick'			
Nil N	2.0c	2.2	2.3
Control NH_4	2.8b	2.3	3.0
Control NO_3	2.8b	2.2	3.0
High NH_4	2.7b	2.3	3.0
High NO_3	3.0ab	2.2	2.8
'Duke 6'			
Nil N	1.5c	1.8	2.0
Control NH_4	3.0ab	2.2	2.8
Control NO_3	3.6a	2.4	2.7
High NH_4	3.5a	2.0	2.7
High NO_3	3.7a	2.4	2.5
LSD	0.7	-	-

Table 3. The effects of rootstock and fertiliser on vegetative flushing, crop load and canopy colour of 'Hass' avocado trees assessed 15th January 2002. Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 6). LSD indicates least significant difference value.

Treatment	Vegetative flush (1-5)	Crop load (1-3)	Colour (1-3)
Rootstock			
'Velvick'	3.3	1.8	3.0
'Duke 6'	3.3	1.8	2.9
LSD	-	-	-
Fertiliser			
Nil N	3.6a	1.7	2.8
Control NH_4	3.3b	1.9	3.0
Control NO_3	3.3ab	1.8	3.1
High NH_4	3.1b	1.8	2.9
High NO_3	3.3ab	1.8	2.9
LSD	0.4	-	-
Rootstock x Fertiliser			
'Velvick'			
Nil N	3.6	1.6	2.8
Control NH_4	3.2	2.0	3.0
Control NO_3	3.3	1.8	3.2
High NH_4	3.3	2.0	3.0
High NO_3	3.3	1.8	3.0
'Duke 6'			
Nil N	3.7	1.7	2.7
Control NH_4	3.3	1.9	3.0
Control NO_3	3.4	1.9	3.0
High NH_4	2.9	1.6	2.8
High NO_3	3.3	1.8	2.8
LSD	-	-	-

Table 4. The effects of rootstock and fertiliser on vegetative flushing, crop load and canopy colour of 'Hass' avocado trees assessed 18th February 2002. Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 6). LSD indicates least significant difference value.

Treatment	Vegetative flush (1-5)	Crop load (1-3)	Colour (1-3)
Rootstock			
'Velvick'	3.5a	1.9b	2.9
'Duke 6'	3.8b	1.6a	2.9
LSD	0.2	0.2	-
Fertiliser			
Nil N	3.5	1.6	2.6a
Control NH_4	3.6	1.8	2.9b
Control NO_3	3.8	1.7	3.0b
High NH_4	3.6	1.7	3.0b
High NO_3	3.8	1.9	3.0b
LSD	-	-	0.2
Rootstock x Fertiliser			
'Velvick'			
Nil N	3.5ab	1.5	2.5
Control NH_4	3.6ab	2.0	3.0
Control NO_3	3.3a	1.9	3.0
High NH_4	3.4ab	2.0	3.0
High NO_3	3.7ab	1.9	3.0
'Duke 6'			
Nil N	3.6ab	1.6	2.7
Control NH_4	3.7ab	1.7	2.8
Control NO_3	4.3c	1.4	3.0
High NH_4	3.8bc	1.3	3.0
High NO_3	3.8bc	1.8	3.0
LSD	0.5	-	-

Table 5. The effects of rootstock and fertiliser on vegetative flushing, crop load and canopy colour of 'Hass' avocado trees assessed 19th March 2002. Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 6). LSD indicates least significant difference value.

Treatment	Vegetative flush (1-5)	Crop load (1-3)	Colour (1-3)
Rootstock			
'Velvick'	2.3b	1.8b	3.0
'Duke 6'	2.2a	1.4a	3.0
LSD	0.1	0.2	-
Fertiliser			
Nil N	2.2	1.6	2.9
Control NH_4	2.2	1.7	3.0
Control NO_3	2.3	1.6	2.9
High NH_4	2.2	1.7	3.0
High NO_3	2.3	1.7	3.0
LSD	-	-	-
Rootstock x Fertiliser			
'Velvick'			
Nil N	2.5c	1.8	3.0
Control NH_4	2.3bc	1.8	3.0
Control NO_3	2.3bc	1.8	2.8
High NH_4	2.3bc	2.0	3.0
High NO_3	2.2abc	1.8	3.0
'Duke 6'			
Nil N	2.0a	1.4	2.8
Control NH_4	2.1ab	1.7	3.0
Control NO_3	2.3bc	1.4	3.0
High NH_4	2.2ab	1.3	3.0
High NO_3	2.3bc	1.5	3.0
LSD	0.3	-	-

Table 6. The effects of rootstock and fertiliser on vegetative flushing, crop load and canopy colour of 'Hass' avocado trees assessed 16th April 2002. Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 6). LSD indicates least significant difference value.

Treatment	Vegetative flush (1-5)	Crop load (1-3)	Colour (1-3)
Rootstock			
'Velvick'	1.3	1.7b	2.8
'Duke 6'	1.5	1.4a	2.9
LSD	-	0.2	-
Fertiliser			
Nil N	1.3a	1.5	2.4a
Control NH_4	1.3a	1.7	3.0b
Control NO_3	1.7b	1.5	2.9b
High NH_4	1.3a	1.7	3.0b
High NO_3	1.5ab	1.5	2.9b
LSD	0.3	-	0.3
Rootstock x Fertiliser			
'Velvick'			
Nil N	1.2	1.4	2.3
Control NH_4	1.3	1.8	3.0
Control NO_3	1.4	1.6	2.8
High NH_4	1.3	2.0	3.0
High NO_3	1.3	1.5	3.0
'Duke 6'			
Nil N	1.3	1.6	2.5
Control NH_4	1.3	1.5	3.0
Control NO_3	1.9	1.4	3.0
High NH_4	1.2	1.3	3.0
High NO_3	1.6	1.4	2.8
LSD	-	-	-

Table 7. The effects of rootstock and fertiliser on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in July 2002 and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 6). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose severity	% Anthracnose Incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Rootstock						
'Velvick'	8.2	14.7a	38.3a	0.19	1.4	70.1a
'Duke 6'	8.2	23.4b	51.1b	0.39	2.4	55.4b
LSD	-	8.5	12.1	-	-	12.1
Fertiliser						
Nil N	8.4	10.6	35.1	0.19	1.6	72.2
Control NH_4	8.2	13.9	36.0	0.30	1.3	70.7
Control NO_3	8.2	27.3	54.0	0.47	2.1	52.3
High NH_4	8.1	21.9	51.4	0.26	1.3	57.4
High NO_3	8.1	21.5	47.1	0.30	3.3	61.1
LSD	-	-	-	-	-	-
Position						
East	8.2	19.9	46.7	0.20	2.0	61.4
West	8.1	18.2	42.7	0.39	1.8	64.1
LSD	-	-	-	-	-	-

Table 8. Mineral concentrations in the skin of fruit harvested in July 2002. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	N (% DW)	Ca (% DW)	N/Ca ratio	Ca+Mg/K ratio
Rootstock				
'Velvick'	0.98	0.05	18.45	0.105 a
'Duke 6'	1.11	0.07	20.44	0.073 b
LSD	-	-	-	0.03
Fertiliser				
Nil N	0.88 b	0.06	14.11 c	0.08
Control NH_4	0.99 a	0.05	20.74 ab	0.09
Control NO_3	1.04 a	0.05	20.51 ab	0.08
High NH_4	1.01 a	0.05	18.91 b	0.09
High NO_3	1.06 a	0.09	23.02 a	0.11
LSD	0.07	Ns	3.96	n.s

Table 9. Mineral concentrations in leaves harvested in July 2002. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	N (% DW)	Ca (% DW)	N/Ca ratio	Ca+Mg/K ratio
Rootstock				
'Velvick'	2.78b	1.66	1.69b	1.89a
'Duke 6'	2.99a	1.62	1.89a	1.34b
LSD	0.09	-	0.15	0.17
Fertiliser				
Nil N	2.71c	1.49b	1.86	1.48
Control NH_4	2.83bc	1.74a	1.66	1.84
Control NO_3	2.91ab	1.68a	1.77	1.66
High NH_4	2.96ab	1.67a	1.78	1.62
High NO_3	3.03a	1.63ab	1.88	1.50
LSD	0.15	0.16	-	-

Regressions between nutrient concentrations and disease data

The disease data obtained from fruit harvested in July was correlated against the mineral nutrient concentrations for the leaf samples taken in May and July and also for the fruit skin samples taken in July (Table 10). There were highly significant positive correlations between fruit anthracnose levels and nitrogen concentrations in both fruit skins and leaves (Table 10). Significant negative correlations were also found between fruit anthracnose levels and Ca concentrations in the fruit skin but not leaf tissues. As a result, significant, positive correlations were evident between fruit anthracnose levels and N/Ca ratios in both fruit skin and leaf tissues (Table 10). The ratios of Ca+Mg/K in fruit skin and leaf tissues were also negatively correlated with fruit anthracnose levels (Table 10).

Table 10. Correlation co-efficients (r) of nutrient concentrations from leaves harvested in May 2002 and leaves and skins harvested in July 2002 with disease data from fruit harvested in July 2002.

	N (% dry wgt)	Ca (% dry wgt)	N/Ca	Ca+Mg/K
July – fruit skins	n=60	N=59	N=59	n=59
Severity of anthracnose	0.4689***	-0.2567*	0.3813**	-0.4473***
Incidence of anthracnose	0.4140***	-0.2828*	0.3711**	-0.4497***
Severity of stem-end rot	Ns	n.s	0.2780*	n.s
Incidence of stem-end rot	0.2588*	n.s	0.2963*	n.s
Marketability	-0.4446***	0.2613*	-0.3677**	0.4540***
Days to eating soft	n.s	0.3396**	-0.3659**	0.2571*
July – leaves (n=60)				
Severity of anthracnose	0.4635***	n.s	0.3901**	-0.3367**
Incidence of anthracnose	0.4553***	-0.2581*	0.4329***	-0.3361**
Severity of stem-end rot	n.s	n.s	n.s	n.s
Incidence of stem-end rot	n.s	n.s	n.s	n.s
Marketability	-0.4471***	n.s	-0.4074***	0.3401**
Days to eating soft	n.s	n.s	n.s	n.s
May – leaves (n=60)				
Severity of anthracnose	0.4502***	n.s	0.3298*	-0.3254*
Incidence of anthracnose	0.3779**	n.s	0.3138*	-0.3023*
Severity of stem-end rot	n.s	n.s	n.s	n.s
Incidence of stem-end rot	n.s	n.s	n.s	n.s
Marketability	-0.4177***	n.s	-0.3329**	0.3167*
Days to eating soft	n.s	n.s	n.s	n.s

(* indicates significance at P<0.05, ** at P<0.01, *** at P<0.001)

Antifungal diene concentrations

While samples for diene analysis were taken from the nitrogen fertiliser x rootstock trials and extracts prepared from these samples, we have so far been unable to analyse levels of the compound in these samples. Difficulties in contracting specialist chemistry expertise (particularly for developing standards and methodology for diene analysis) has been the main reason for this problem. In November 2002 collaboration was initiated with Dr Craig Williams, Department of Chemistry, University of Queensland. A research assistant was appointed during December 2002-January 2003. A crude solvent extract was prepared from the skin of mature but unripe avocados, via soaking skin in ethanol, concentrating *in vacuo*, partitioning in dichloromethane:water and further concentrating. The crude extract was subjected to silica column chromatography (or "flash" chromatography), and several fractions collected, and pooled appropriately. Thin layer chromatography (TLC) followed by bioassay with *Cladosporium* sp. identified Fractions 8 and 9 that were strongly antifungal. Proton NMR (or 1HNMR) and mass spectroscopic analyses provided data consistent with published reports confirming the presence of 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (or

"diene") compound in Fractions 8 and 9. A sub-sample of Fraction 8 and a few other avocado skin and leaf extracts were sent for HPLC analyses to Prof Dov Prusky, Volcani Center, Israel. Their analyses provided further confirmation of the presence and relative abundance of the antifungal diene compound in our samples. We are currently interacting with Prof Prusky's laboratory in order to establish and optimise the procedures for routine HPLC analyses on avocado skin samples.

Fruit skin pH

For both rootstocks, the pH increased significantly over time during ripening but there was no significant effect due to rootstock or fertiliser regime (Table 11).

Table 11. Skin pH levels in 'Hass' avocado fruit harvested in July 2002. Means followed by the same letter are not significantly different at the $P < 0.05$ (LSD indicates least significant difference value).

Treatments	Average pH	pH - Mature	pH - Sprung	pH – Ripe
Rootstock				
'Velvick'	5.45	4.97	5.55	5.82
'Duke 6'	5.58	5.22	5.58	5.94
LSD	n.s			n.s
Fertiliser				
Nil N	5.48	5.20	5.56	5.68
High NH_4	5.64	5.16	5.61	6.15
High NO_3	5.42	4.93	5.52	5.81
LSD	n.s			n.s
Time				
1 – Mature	5.10a			
2 – Sprung	5.56b			
3 – Ripe	5.88c			
LSD	0.25			
Rootstock x Fertiliser				
'Velvick'				
Nil N	5.47	5.13	5.50	5.78
High NH_4	5.57	5.17	5.62	5.91
High NO_3	5.30	4.6	5.53	5.77
'Duke 6'				
Nil N	5.48	5.26	5.61	5.58
High NH_4	5.71	5.14	5.61	6.39
High NO_3	5.54	5.27	5.51	5.85
LSD	n.s			n.s

2002/03 Season

Unlike last season, there were no significant rootstock or fertiliser effects on disease in the 2002/03 season (Table 12). There was however, a significant fertiliser effect on mineral nutrient concentrations in the fruit skin and leaf tissues (Table 13). Fruit from trees receiving no nitrogen (nil N treatment) had a significantly lower skin N/Ca ratio (more favourable) than all of the other treatments, except the control ammonium treatment. Leaves sampled from trees receiving no nitrogen fertiliser (nil N treatment) also had a significantly lower concentration of N than the control nitrate, high ammonium and high nitrate treatments (Table 13).

Table 12. The effects of rootstock and fertiliser on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in August 2003 and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 6). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot Severity	% Stem-end rot incidence	Marketable fruit (%)
Rootstock						
'Velvick'	10.3	43.3	76.4	1.3	11.7	32.8
'Duke 6'	10.4	46.4	77.1	1.3	10.2	31.6
LSD	-	-	-	-	-	-
Fertiliser						
Nil N	10.7	35.0	38.5	1.4	9.5	44.4
Control NH_4	10.3	39.0	73.4	1.2	9.5	35.6
Control NO_3	10.2	48.7	78.1	1.2	11.9	29.0
High NH_4	10.2	52.4	84.7	1.3	13.2	25.7
High NO_3	10.2	49.1	79.1	1.5	10.7	26.5
LSD	-	-	-	-	-	-
Position						
East	10.3	47.2	78.0	1.5	11.6	30.6
West	10.3	42.5	75.5	1.1	10.3	33.9
LSD	-	-	-	-	-	-

Table 13. Mineral concentrations in the skin of fruit harvested in July 2003. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	N (% DW)	Ca (% DW)	N/Ca ratio	Ca+Mg/K ratio
Rootstock				
'Velvick'	0.9	0.055	17.18	0.090
'Duke 6'	0.9	0.055	18.88	0.082
LSD	-	-	-	-
Fertiliser				
Nil N	0.85	0.058	15.02b	0.091
Control NH_4	0.89	0.056	16.36ab	0.089
Control NO_3	0.87	0.048	19.21a	0.084
High NH_4	0.95	0.051	19.48a	0.080
High NO_3	0.93	0.049	20.09a	0.086
LSD	-	-	3.90	-

Significant correlations between disease and nutrient concentrations were again observed in the 2002/03 season (Table 15). Similar to last season, there were highly significant positive correlations between fruit anthracnose levels and N concentrations in both leaf and fruit skin tissues (Table 15). Significant negative correlations were also evident between anthracnose levels and Ca concentrations in both leaf and fruit skin tissues (Table 15). This resulted in strong positive correlations between fruit anthracnose levels and N/Ca ratios in both fruit skin and leaf tissue (Table 15). Again, similar to last season, the ratio of Ca+Mg/K in fruit skin and leaf tissues were negatively correlated with fruit anthracnose levels (Table 15).

Table 14. Mineral concentrations in leaves harvested in May 2003. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	N (% DW)	Ca (% DW)	N/Ca ratio	Ca+Mg/K ratio
Rootstock				
'Velvick'	2.98	1.51b	2.08a	2.94b
'Duke 6'	2.93	1.16a	2.59b	1.53a
LSD	-	0.173	0.222	0.477
Fertiliser				
Nil N	2.76a	1.48	2.13	2.56
Control NH_4	2.91ab	1.32	2.25	2.37
Control NO_3	3.00bc	1.36	2.29	2.23
High NH_4	2.99bc	1.21	2.51	2.14
High NO_3	3.11c	1.28	2.49	1.87
LSD	0.174	-	-	-

Table 15. Correlation co-efficients (r) of nutrient concentrations from leaves harvested in May 2003 and leaves and skins harvested in July 2003 with disease data from fruit harvested in July 2003.

	N (% dry wgt)	Ca (% dry wgt)	N/Ca	Ca+Mg/K
July – fruit skins (n=60)				
Severity of anthracnose	0.503***	-0.387**	0.511***	-0.566***
Incidence of anthracnose	0.410**	-0.353**	0.417***	-0.492***
Severity of stem-end rot	n.s	n.s	n.s	n.s
Incidence of stem-end rot	Ns	n.s	n.s	n.s
Marketability	-0.437***	0.386**	-0.464***	0.540***
Days to eating soft	n.s	0.397**	-0.372**	0.397**
May – leaves (n=60)				
Severity of anthracnose	0.527***	-0.380**	0.532***	-0.397**
Incidence of anthracnose	0.460***	-0.387**	0.509***	-0.343**
Severity of stem-end rot	0.272*	n.s	n.s	n.s
Incidence of stem-end rot	0.261*	n.s	n.s	-0.279*
Marketability	-0.489***	0.407**	-0.532***	0.391**
Days to eating soft	n.s	n.s	n.s	n.s

(* indicates significance at P<0.05, ** at P<0.01, *** at P<0.001)

Fruit skin pH results for the 2003 season were similar to the previous season, as pH increased significantly over time during ripening but again there were no significant differences between the two rootstocks (Table 16). However, unlike the previous season, there was a significant fertiliser effect on skin pH. Fruit from trees receiving no nitrogen fertiliser had a significantly lower skin pH than fruit from trees receiving double the normal rate of nitrate nitrogen fertiliser (High NO_3 treatment) (Table 16). The nil nitrogen treatment was also slightly but not significantly lower than the high ammonium treatment (Table 16).

Table 16. Skin pH levels in 'Hass' avocado fruit harvested in July 2003. Means followed by the same letter are not significantly different at the $P < 0.05$ (LSD indicates least significant difference value).

Treatment	Average pH	pH - Mature	pH - Sprung	pH – Ripe
Rootstock				
'Velvick'	6.01	5.57	5.84	6.62
'Duke 6'	6.05	5.62	6.02	6.52
LSD	n.s			n.s
Fertiliser				
Nil N	5.90b	5.51	5.86	6.32
High NH_4	6.06ab	5.64	6.00	6.76
High NO_3	6.14a	5.62	5.94	6.63
LSD	0.18			n.s
Time				
1 – Mature	5.60c			
2 – Sprung	5.93b			
3 – Ripe	6.57a			
LSD	0.14			
Rootstock x Fertiliser				
'Velvick'				
Nil N	5.88	5.49	5.76	6.39
High NH_4	6.06	5.58	5.85	6.67
High NO_3	6.10	5.63	5.92	6.82
'Duke 6'				
Nil N	5.91	5.54	5.96	6.26
High NH_4	6.07	5.65	5.97	6.59
High NO_3	6.17	5.65	6.15	6.71
LSD	n.s			n.s

B. Rootstock only studies

2001/02 Studies

Rootstock had no effect on maturity of the 'Hass' fruit, the dry matters of the fruit harvested in July 2002 were not significantly different between rootstocks (Table 17).

The severity of anthracnose was significantly greater in fruit from 'Parida 1' than fruit from 'Anderson 8' and 'Anderson 10' (Table 18). The fruit from the 'Nabal' rootstock did not have a significantly different level of severity of anthracnose to the fruit from 'Parida 1', 'Anderson 8' and 'Anderson 10' (Table 18). Shelf life, incidence of anthracnose, development of stem-end rot and the percentage of marketable fruit were not affected by rootstock (Table 18).

Table 17. Effect of rootstock on percent dry matter in 'Hass' fruit harvested from Duranbah in July 2002 (n=40). Mean values within columns followed by the same letter are not significantly different at *P* < 0.05. LSD indicates least significant difference value.

Rootstock	% Dry matter
'Anderson 8'	27.6
'Anderson 10'	27.3
'Nabal'	27.4
'Parida 1'	27.3
LSD	n.s

Table 18. The effect of rootstock on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in July 2002 from Duranbah and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at *P* < 0.05 (n = 40). LSD indicates least significant difference value.

	Shelf Life	% Anthracnose		% Stem-end rot		Marketable fruit
Treatment	(days)	severity	incidence	Severity	Incidence	(%)
Rootstock						
'Anderson 8'	8.4	53.4b	83.2	1.99	11.0	23.9
'Anderson 10'	8.4	57.5b	88.7	1.28	12.3	17.4
'Nabal'	8.1	64.4ab	90.7	0.44	6.0	16.0
'Parida 1'	8.0	78.0a	97.8	2.42	13.1	5.7
LSD	n.s	17.8	n.s	n.s	n.s	n.s

Rootstock had a significant effect on the mineral concentrations in the skins of fruit harvested in July 2002. 'Hass' fruit from 'Anderson 10' rootstocks had significantly less nitrogen than 'Hass' fruit from 'Nabal' and 'Parida 1' rootstocks. The nitrogen levels in the fruit from 'Anderson 8' were part way between the levels in fruit from 'Anderson 10' and 'Nabal' and 'Parida 1' (Table 19). There was no difference in the calcium levels of the fruit harvested from the four rootstocks (Table 19). The nitrogen to calcium ratios in fruit skins from 'Parida 1'was around 1.3 times the ratios in skins of fruit from the other three rootstocks (Table 19). The Ca+Mg/K ratios were similar in fruit from 'Anderson 8', 'Anderson 10' and 'Nabal'.

The Ca+Mg/K ratio in fruit skins from 'Parida 1' was significantly lower than in the fruit skins from 'Anderson 8' and 'Anderson 10' (Table 19).

Table 19. Mineral concentrations in fruit skins harvested in July 2002. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	N (% DW)	Ca (% DW)	N/Ca ratio	Ca+Mg/K ratio
Rootstock				
'Anderson 8'	1.190ab	0.047	26.92b	0.080a
'Anderson 10'	1.080b	0.049	23.85b	0.077a
'Nabal'	1.220a	0.044	28.39b	0.070ab
'Parida 1'	1.230a	0.036	35.01a	0.059b
LSD	0.116	n.s	6.28	0.013

The levels of nitrogen in the skins of fruit harvested in July 2002 strongly correlated with anthracnose levels in fruit harvested in July 2002 (Table 20). There was a significant positive relationship between nitrogen in the skin of the fruit and the incidence and severity of anthracnose. There was a strong negative correlation between the concentration of nitrogen in the fruit skins and the shelf life and the percentage of marketable fruit (Table 20).

There was a negative correlation between the levels of calcium in the fruit skins and the severity and incidence of anthracnose (Table 20). There was a significant positive relationship between calcium levels and the percentage of marketable fruit. The relationship between severity and incidence of anthracnose and marketable fruit with calcium levels was not as strong as the relationships to nitrogen levels (Table 20).

The higher the nitrogen to calcium ratio the greater the incidence and severity of anthracnose. Hence the higher the nitrogen to calcium ratio, the lower the percentage of marketable fruit (Table 20). A higher nitrogen to calcium level significantly correlated with a decease in shelf life (Table 20).

Higher Ca+Mg/K ratios correlated with a decrease in severity and incidence of anthracnose, and an increase in shelf life and the percentage of marketable fruit (Table 20). The development of stem-end rot did not correlate with levels of any of the mineral nutrients from the skins of the fruit harvested in July 2002(Table 20).

Rootstock had a significant effect on number of fruit per tree and total weight of fruit per tree. 'Parida 1' trees had significantly less fruit and weight of fruit than 'Anderson 8', 'Anderson 10' and 'Nabal' trees. Average fruit size was not affected by rootstock (Table 21).

Table 20. Correlation co-efficients (r) of nutrient concentrations from fruit skins harvested in July 2002 with disease data from fruit harvested in July 2002.

	N (% dry wgt)	Ca (% dry wgt)	N/Ca	Ca+Mg/K
July – fruit skins (n=40)				
Severity of anthracnose	0.608***	-0.330*	0.545***	-0.578***
Incidence of anthracnose	0.488**	-0.343*	0.536***	-0.510***
Severity of stem-end rot	-0.132ns	-0.110ns	0.051ns	-0.093ns
Incidence of stem-end rot	-0.131ns	-0.030ns	0.099ns	-0.046ns
Marketable fruit (%)	-0.468**	0.360*	-0.548***	0.563***
Shelf life	-0.504***	0.225ns	-0.468**	0.468**

(* indicates significance at P<0.05, ** at P<0.01, *** at P<0.001)

Table 21. Effect of rootstock on number and weight of fruit per tree and on average weight of individual fruit from trial harvested in July 2002. Means followed by the same letter are not significantly different at *P* < 0.05 (LSD indicates least significant difference value).

Treatment	Number of fruit Per tree	Total weight of fruit per tree (kg)	Average fruit size (g)
Rootstock			
'Anderson 8'	90a	19.7a	221.0a
'Anderson 10'	83a	17.8a	213.7a
'Nabal'	76a	17.1a	227.9a
'Parida 1'	51b	11.5b	226.6a
LSD	19.6	4.17	n.s

2002/03 Studies

As in 2001/2002, rootstock did not affect fruit maturity. There were no significant differences in the percentage of dry matter in fruit harvested from trees on 'Anderson 8', 'Anderson 10', 'Nabal' or 'Parida 1' rootstocks (Table 22).

Rootstock did not affect the shelf life or disease development in fruit harvested in June 2003 (Table 23).

Table 22. Effect of rootstock on percent dry matter in 'Hass' fruit harvested from Duranbah in June 2003 (n=40). Mean values within columns followed by the same letter are not significantly different at *P* < 0.05. LSD indicates least significant difference value.

Rootstock	% Dry Matter
'Anderson 8'	26.4
'Anderson 10'	26.1
'Nabal'	26.2
'Parida 1'	25.9
LSD	n.s

Table 23. The effect of rootstock on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in June 2003 from Duranbah and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 40). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot Severity	% Stem-end rot Incidence	Marketable fruit (%)
Rootstock						
'Anderson 8'	10.9	6.6	22.0	0.99	3.5	85.5
'Anderson 10'	11.1	14.2	36.0	3.01	20.5	65.0
'Nabal'	10.6	7.5	25.5	1.44	8.5	77.5
'Parida 1'	10.2	8.1	30.0	2.06	10.5	76.5
LSD	n.s	n.s	n.s	n.s	n.s	n.s

Nitrogen levels, nitrogen to calcium ratios and Ca+Mg/K ratios were not significantly different in leaves harvested in May 2003 from trees grafted to any of the four rootstocks (Table 24). Calcium levels were significantly less in leaves from 'Anderson 8' than in leaves from 'Nabal' and 'Parida 1'. Calcium levels in leaves from 'Anderson 10' were significantly less than calcium levels from 'Nabal' but not than less than leaves from 'Parida 1' (Table 24).

Table 24. Mineral concentrations in leaves harvested in May 2003. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	N (% DW)	Ca (% DW)	N/Ca ratio	Ca+Mg/K ratio
Rootstock				
'Anderson 8'	2.72	1.013c	2.87	1.76
'Anderson 10'	2.88	1.145bc	2.57	1.71
'Nabal'	2.80	1.350a	2.14	1.82
'Parida 1'	2.86	1.208ab	2.42	1.74
LSD	n.s	0.192	n.s	n.s

There were no significant differences in the mineral concentrations in the skins of the fruit harvested in June 2003 between rootstocks (Table 25). However, there were significant correlations between the mineral concentrations in the fruit skins harvested in June 2003 and the disease data from fruit harvested in June 2003 (Table 26).

The nitrogen levels in the fruit harvested in June 2003 showed a positive correlation with the severity and incidence of anthracnose, the incidence and severity of stem-end rot and a negative correlation with the percentage of marketable fruit (Table 26).

Decreasing severity and incidence of anthracnose was correlated with higher levels of calcium in fruit skins. Increased shelf life and percentage of marketable fruit was correlated with increased levels of calcium. Calcium levels appeared to have no relationship to the development of stem-end rot (Table 26).

Table 25. Mineral concentrations in fruit skins harvested in June 2003. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	N (% DW)	Ca (% DW)	N/Ca ratio	Ca+Mg/K ratio
Rootstock				
'Anderson 8'	0.99	0.050	22.3	0.102
'Anderson 10'	1.00	0.051	21.1	0.103
'Nabal'	0.85	0.053	17.4	0.100
'Parida 1'	0.87	0.051	18.0	0.099
LSD	n.s	n.s	n.s	n.s

An increased nitrogen to calcium ratio significantly correlated with an increase in the incidence and severity of anthracnose and an increase in the incidence of stem-end rot. Lower nitrogen to calcium ratios were significantly related to longer shelf life and higher numbers of marketable fruit (Table 26). High Ca+Mg/K ratios had a positive effect on fruit with lower levels of anthracnose (incidence and severity) and longer shelf life and more marketable fruit (Table 26).

Only the nitrogen levels in the leaves harvested in May 2003 correlated with any of the disease, shelf life or marketability data. The incidence and severity of anthracnose in fruit harvested in June 2003 showed a significant positive correlation with the nitrogen levels of the leaves harvested in May 2003. The incidence of stem-end rot also showed a positive correlation with the nitrogen concentrations in the leaves. Shelf life and the percentage of marketable fruit showed a strong negative correlation with the concentration of nitrogen in the leaves harvested in May 2003 (Table 26).

Rootstock did not have a significant effect on the number of fruit per tree, weight of fruit per tree or fruit size. Rootstock did not have a significant effect on the average number of fruit per cubic metre of canopy, despite 'Parida 1' having on average, one extra fruit per cubic metre of canopy than the other three rootstocks (Table 27).

Table 26. Correlation co-efficients (r) of nutrient concentrations from leaves harvested in May 2003 and leaves and skins harvested in June 2003 with disease data from fruit harvested in June 2003.

	N (% dry wgt)	Ca (% dry wgt)	N/Ca ratio	Ca+Mg/K ratio
June – fruit skins (n=40)				
Severity of anthracnose	0.679***	-0.387*	0.623***	-0.401*
Incidence of anthracnose	0.605***	-0.489**	0.635***	-0.516***
Severity of stem-end rot	0.357*	-0.077ns	0.304ns	-0.060ns
Incidence of stem-end rot	0.440**	-0.240ns	0.365*	-0.101ns
Marketable fruit (%)	-0.651***	0.421**	-0.611***	0.362*
Shelf life	-0.252ns	0.560***	-0.464**	0.652***
May – leaves (n=40)				
Severity of anthracnose	0.533***	-0.042ns	0.219ns	-0.202ns
Incidence of anthracnose	0.562***	-0.004ns	0.222ns	-0.171ns
Severity of stem-end rot	0.175ns	0.150ns	-0.031ns	0.089ns
Incidence of stem-end rot	0.323*	-0.004ns	0.103ns	-0.194ns
Marketable fruit (%)	-0.553***	-0.021ns	-0.173ns	0.201ns
Shelf life	-0.479**	0.020ns	-0.155ns	0.258ns

(* indicates significance at P<0.05, ** at P<0.01, *** at P<0.001)

Table 27. Effect of rootstock on number and weight of fruit per tree and on average weight of individual fruit and number of fruit per cubic metre of canopy from trial harvested in June 2003. Means followed by the same letter are not significantly different at *P* < 0.05 (LSD indicates least significant difference value).

Treatment	Number of fruit per tree	Total weight of fruit per tree (kg)	Average fruit size (g)	Average number of fruit/m^3 canopy
Rootstock				
'Anderson 8'	239	49.0	209.6	6.31
'Anderson 10'	298	58.9	204.8	6.36
'Nabal'	213	45.9	217.4	6.04
'Parida 1'	226	50.0	222.7	7.36
LSD	n.s	n.s	n.s	n.s

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Rootstock did not have an effect on the maturity of 'Hass' fruit harvested from the rootstock trial in August 2004 (Table 28). Rootstock also did not have an effect on shelf life, development of anthracnose, development of stem-end rot or the percentage of marketable fruit (Table 29).

Table 28. Effect of rootstock on percent dry matter in 'Hass' fruit harvested from Duranbah in August 2004 (n=24). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$. LSD indicates least significant difference value.

Rootstock	% Dry Matter
'Anderson 8'	29.2
'Anderson 10'	28.3
'Nabal'	28.4
'Parida 1'	28.8
LSD	n.s

Table 29. The effect of rootstock on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in August 2004 from Duranbah and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 24). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Rootstock						
'Anderson 8'	10.4	21.6	55.9	1.40	15.5	56.0
'Anderson 10'	10.7	21.7	51.4	1.38	14.5	58.4
'Nabal'	10.7	16.0	50.8	0.92	9.2	62.1
'Parida 1'	10.0	13.2	41.3	0.63	8.3	68.3
LSD	n.s	n.s	n.s	n.s	n.s	n.s

Similar to the previous season, rootstock did not have an effect on the nitrogen concentration in the leaves harvested from trees grafted to the four different rootstocks. Calcium levels and Ca+Mg/K ratios were lower in leaves from 'Nabal' and 'Parida 1' then in leaves from 'Anderson 8' and 'Anderson 10'. Nitrogen to calcium ratios were significantly higher in leaves from 'Nabal' and 'Parida 1' than in leaves from 'Anderson 8' and 'Anderson 10' (Table 30).

Table 30. Mineral concentrations in leaves harvested in May 2004. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	N (% DW)	Ca (% DW)	N/Ca ratio	Ca+Mg/K ratio
Rootstock				
'Anderson 8'	2.60	1.28ab	2.13b	1.90a
'Anderson 10'	2.63	1.42a	1.88b	1.78a
'Nabal'	2.67	0.62c	4.91a	0.93b
'Parida 1'	2.73	0.93bc	4.31a	1.04b
LSD	n.s	0.44	2.14	0.64

In the skins of the fruit harvested in August 2004, rootstock did not have any effect on the mineral concentrations or ratios (Table 31). There was however, across the whole trial, a relationship between mineral concentrations and disease, shelf life and marketability (Table 32). Higher nitrogen levels in skins of fruit harvested in 2004 correlated with higher incidence and severity of anthracnose, higher incidence and severity of stem-end rot and lower marketability. Calcium concentrations showed no relationship to incidence of stem-end rot, lower levels of calcium significantly correlated with higher stem-end rot severity and higher severity and incidence of anthracnose. Longer shelf life and more marketable fruit were significantly correlated with increasing concentrations of calcium (Table 32).

Table 31. Mineral concentrations in fruit skins harvested in August 2004. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	N (% DW)	Ca (% DW)	N/Ca ratio	Ca+Mg/K ratio
Rootstock				
'Anderson 8'	0.83	0.06	14.2	0.114
'Anderson 10'	0.90	0.06	18.3	0.114
'Nabal'	0.80	0.06	15.5	0.103
'Parida 1'	0.82	0.05	18.4	0.087
LSD	n.s	n.s	n.s	n.s

Increasing nitrogen to calcium ratios in fruit skins were significantly correlated with an increase in disease (severity and incidence of stem-end rot and anthracnose). Whilst increasing nitrogen to calcium ratios in the fruit skins correlated with a decrease in the percentage of marketable fruit (Table 32).

The Ca+Mg/K ratio in fruit skins did not show a significant relationship to disease development or marketability, however, a higher Ca+Mg/K ratio did show a strong correlation with longer fruit shelf life (Table 32).

The mineral concentrations in the leaves collected in May 2004 showed no significant correlation with disease and shelf life data from fruit harvested in August 2004 (Table 32).

Rootstock did not have any effect on number of fruit per tree, weight of fruit per tree or fruit size for fruit harvested in August 2004 (Table 33). Rootstock did however, have an effect on

the yield of fruit per cubic metre of canopy. 'Nabal' yielded a significantly higher number of fruit and greater weight of fruit than 'Anderson 8' and 'Anderson 10' per cubic metre of canopy. On a cubic metre of canopy basis, 'Parida 1' yielded mid-way between 'Nabal' and 'Anderson 8', 'Anderson 10' (Table 33).

Table 32. Correlation co-efficients (r) of nutrient concentrations from leaves harvested in May 2004 and leaves and skins harvested in August 2004with disease data from fruit harvested in August 2004.

	N (% dry wgt)	Ca (% dry wgt)	N/Ca ratio	Ca+Mg/K ratio
August – fruit skins (n=24)				
Severity of anthracnose	0.585**	-0.510*	0.606**	-0.245ns
Incidence of anthracnose	0.552**	-0.456*	0.551**	-0.272ns
Severity of stem-end rot	0.672***	-0.470*	0.645***	-0.050ns
Incidence of stem-end rot	0.580**	-0.331ns	0.524**	-0.060ns
Marketable fruit (%)	-0.576**	0.505*	-0.599**	0.284ns
Shelf life	-0.311ns	0.430*	-0.378ns	0.565**
May – leaves (n=24)				
Severity of anthracnose	0.078ns	-0.034ns	-0.017ns	0.024ns
Incidence of anthracnose	0.114ns	-0.023ns	-0.036ns	0.059ns
Severity of stem-end rot	0.176ns	0.180ns	-0.163ns	0.293ns
Incidence of stem-end rot	0.114ns	0.193ns	-0.112ns	0.293ns
Marketable fruit (%)	-0.092ns	0.055ns	-0.027ns	-0.007ns
Shelf life	0.269ns	0.040ns	0.021ns	0.050ns

(* indicates significance at P<0.05, ** at P<0.01, *** at P<0.001)

Table 33. Effect of rootstock on number and weight of fruit per tree, average weight of individual fruit and yield/ m^3 of canopy from trial harvested in August 2004. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

Treatment	Number of fruit per tree	Total weight of fruit per tree (kg)	Average fruit size (g)	Average number of fruit/m^3 canopy	Weight of fruit/m^3 canopy
Rootstock					
'Anderson 8'	229	55.1	245.6	4.09b	0.97b
'Anderson 10'	228	57.7	253.7	3.83b	0.96b
'Nabal'	333	80.5	241.2	7.71a	1.85a
'Parida 1'	265	64.8	242.8	6.89ab	1.64ab
LSD	n.s	n.s	n.s	3.16	0.72

Rootstock did not have an effect on the skin pH of fruit at the green mature stage. 'Anderson 8' had a significantly higher skin pH than 'Anderson 10' and 'Nabal' at the eating ripe stage. The skin pH of ripe fruit from 'Parida 1' was not significantly different to the eating ripe pH of the fruit from other rootstocks (Table 34).

Table 34. The effect of rootstock on skin pH of skin at green mature and eating ripe stages. The fruit were harvested from Duranbah in August 2004. Means followed by the same letter are not significantly different at $P < 0.05$ (LSD indicates least significant difference value).

	pH	
Rootstock	**Green mature**	**Eating ripe**
'Anderson 8'	5.40	6.30a
'Anderson 10'	5.50	6.17b
'Nabal'	5.40	6.16b
'Parida 1'	5.39	6.24ab
LSD	n.s	0.096

3.2 New Product Studies

A. New copper formulations

1. Efficacy experiment

The copper oxychloride and Kocide® Blue copper fungicide formulations were the only treatments which significantly reduced the severity of anthracnose in ripe 'Hass' fruits compared with the untreated control (Table 35). The incidence of anthracnose was significantly reduced by the copper oxychloride, Kocide®, Kocide® Blue and Norshield® formulations compared with the untreated control (Table 35). The Liquicop® and Kocide® Liquid Blue formulations were found to be the least effective in reducing the severity and incidence of anthracnose, and in fact the Kocide® Liquid Blue had approximately the same levels of anthracnose as the untreated control fruits and a significantly higher incidence of anthracnose than all of the other tested copper formulations (Table 35). The incidence of stem-end rot was significantly reduced by all of the copper formulations tested compared with the untreated control fruits (Table 35). Overall, the percentage of marketable fruit was significantly improved by all of the copper formulations except for the Kocide® Liquid Blue fungicide (Table 35).

Table 35. The effectiveness of new copper fungicide formulations as applied as monthly field sprays to control postharvest disease and improve fruit marketability in ripe 'Hass' avocado fruits ripened at 22°C (65% RH). Mean values within columns are not significantly different at $P < 0.05$.

Treatment	Shelf life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-End Rot severity	% Stem-End Rot incidence	Marketable fruit[#] (%)
Control untreated	8.7a	42.2ab	81.9ab	0.8a	7.5a	29.4c
Cu Oxychloride	7.5a	25.8c	57.9c	0.0a	0.0b	58.5a
Kocide	7.0a	23.1bc	56.9c	0.0a	0.0b	63.1a
Kocide Blue	7.8a	17.0c	58.1c	0.0a	0.0b	61.9a
Kocide Liquid Blue	8.6a	46.6a	83.1a	0.8a	1.3b	31.3bc
Liquicop	7.3a	28.1abc	63.7bc	0.0a	0.0b	51.6ab
Norshield	7.5a	25.0bc	56.9c	0.1a	1.3b	55.6a

[#] Marketable fruit calculated as the proportion of fruits with 5% or less anthracnose severity and no stem-end rot.

2. Phytotoxicity experiments

For both experiments no visible phytotoxicity symptoms were observed on the leaves or fruit of the treated trees (data not shown).

B. Field and glasshouse applications of Bion®

1. Field experiment

The results showed that field treating 'Hass' avocado fruits with the host-defence promoter compound, Bion®, did not significantly reduce the development of postharvest diseases or improve fruit marketability (data not shown). One problem encountered in this experiment was the high degree of tree-to-tree variability in disease susceptibility.

2. Glasshouse experiment

Results from the thin-layer chromatography show that antifungal compounds were already present in the leaves of some of the trees prior to treatment and inoculation. The compound, which we believe to be diene, may be produced constitutively in the leaves or may have been produced due to the trees already having been exposed to *C. gloeosporioides* in the nursery and their defences challenged. Two and 7 days after inoculation (6 and 11 days after treatment) all of the extracts exhibited antifungal activity. Production of other antifungal compounds varied across each of the treatments and no clear trend was evident.

C. Field and postharvest applications of new strobilurin fungicides and host defence promoters

We found no significant differences in disease incidence or severity between the field treated (Bion and Soluble silicon) and the control fruit.

D. Biocoat

The severity of stem-end rot (SER) was not significantly different between treatments. However, Sportak® had a significantly lower incidence of SER (0.0 %) than the control (10.0 %). The recommended rate of Biocoat (3.8%) was no better than the control, whilst the incidence of SER in the fruit receiving double Biocoat (2.5 %) was not significantly different to either Sportak® or control fruit. Only the Sportak® treatment significantly reduced the incidence and severity of anthracnose compared with the control.

E. Antigibberellins

Applying the antigibberellin, Sunny®, or pruning avocado trees, did not significantly affect fruit ripening times or postharvest disease levels (data not shown).

3.3 Harvesting Method Studies

A. Clip vs. snap harvesting

Bundaberg – early season fruit

Fruit were just mature at 22% dry matter. Fruit which were snap harvested had a significantly greater incidence of stem-end rot (Table 36). Harvesting method had no effect on shelf life, anthracnose development (incidence or severity), severity of stem-end rot or percentage marketable fruit (Table 36). The side of the tree from which the fruit was picked did not affect any of the parameters measured (Table 36), nor was there a significant interaction between harvesting method and aspect (data not shown).

Table 36. The effect of harvesting method on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in April 2002 from Bundaberg and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 24). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Harvesting method						
Clip	17.6	47.8	69.0	2.91	8.8b	33.9
Snap	17.3	45.9	74.5	5.08	19.4a	26.4
LSD	n.s	n.s	n.s	n.s	8.77	n.s
Aspect						
East	17.5	43.6	72.2	4.25	14.4	31.0
West	17.4	50.2	71.3	3.75	13.9	29.2
LSD	n.s	n.s	n.s	n.s	n.s	n.s

Bundaberg – mid season fruit

The fruit which were harvested from Bundaberg in July 2002 were mature (23.8% dry matter). There was no significant effect of harvesting method or aspect from which the fruit was picked on the shelf life of the fruit, anthracnose or stem-end rot development (incidence or severity) or on the percentage of marketable fruit harvested (Table 37). There was no significant interaction between harvesting method and aspect (data not shown).

Table 37. The effect of harvesting method on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in July 2002 from Bundaberg and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at *P* < 0.05 (n = 24). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Harvesting method						
Clip	13.1	16.1	46.8	2.74	12.0	61.6
Snap	12.8	17.0	43.5	4.26	16.2	57.9
LSD	n.s	n.s	n.s	n.s	n.s	n.s
Aspect						
East	13.1	16.3	42.1	3.45	13.4	61.6
West	12.8	16.9	48.1	3.55	14.8	57.9
LSD	n.s	n.s	n.s	n.s	n.s	n.s

Duranbah – early season fruit

The fruit harvested from Duranbah in May 2002 were just mature at 22.2% dry matter. Fruit which were clip harvested had a significantly longer shelf life of 13 days than fruit which were snap harvested (average of 12.4 days) (Table 38). There was no effect of aspect on shelf life.

The side of the tree from which the fruit were picked had a significant effect on the development of stem-end rot. The incidence of stem-end rot was almost five times higher in fruit which were from the western side of the tree compared with fruit from the eastern side of the tree (Table 38). The severity of stem-end rot was also significantly greater in fruit from the western side of the tree (Table 38).

There was no significant interaction between harvesting method and aspect (data not shown).

Table 38. The effect of harvesting method on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in May 2002 from Duranbah and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 24). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Harvesting method						
Clip	13.0a	18.7	42.6	3.75	11.7	65.5
Snap	12.4b	11.2	35.5	2.35	8.1	71.1
LSD	0.52	n.s	n.s	n.s	n.s	n.s
Aspect						
East	12.7	14.3	38.6	0.83b	3.40b	73.7
West	12.7	15.6	39.5	5.26a	16.40a	63.4
LSD	n.s	n.s	n.s	3.46	7.77	n.s

Duranbah – later season fruit

The later season fruit harvested from Duranbah in July 2002 were 28.2% dry matter. The harvesting method had a significant effect on the severity of anthracnose, fruit which were clip harvested had significantly more severe side anthracnose than fruit which were snap harvested (Table 39). There was no effect of harvesting method or aspect on stem-end rot, shelf-life or percentage of marketable fruit (Table 39).

There was no significant interaction between harvesting method and aspect (data not shown).

Table 39. The effect of harvesting method on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in July 2002 from Duranbah and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 24). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Harvesting method						
Clip	7.1	14.6a	50.5	0.00	0.46	64.7
Snap	7.1	11.3b	45.2	0.36	2.04	64.6
LSD	n.s	3.25	n.s	n.s	n.s	n.s
Aspect						
East	7.1	12.8	50.1	0.06	0.93	62.0
West	7.1	13.1	45.6	0.32	1.57	67.3
LSD	n.s	n.s	n.s	n.s	n.s	n.s

Mt Tamborine – mid season fruit

The fruit harvested from Mt Tamborine mid season were on average 28.2% dry matter. There was no effect of harvesting method on the shelf life or development of stem-end rot (Table 40). Snap harvested fruit had significantly greater severity and incidence of anthracnose and hence a significantly lower percentage of marketable fruit (Table 40). The severity and incidence of anthracnose at the stem-end of the fruit was significantly higher in snap harvested fruit than in clip harvested fruit (Table 41).

There was no significant interaction between harvesting method and aspect (data not shown).

Table 40. The effect of harvesting method on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in August 2002 from Mt Tamborine and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 16). LSD indicates least significant difference value.

Harvesting Method	Shelf Life (days)	% Anthracnose		% Stem-end rot		Marketable fruit (%)
		severity	incidence	severity	incidence	
Clip	11.1	0.7b	8.8b	0.19	0.63	96.3a
Snap	10.6	8.3a	26.3a	0.05	0.63	79.4b
LSD	n.s	3.13	11.4	n.s	n.s	7.9

Table 41. The effect of harvesting method on anthracnose severity (% surface area affected) and incidence (% fruit affected) at the side and stem-end of the fruit of 'Hass' avocado fruit harvested in August 2002 from Mt Tamborine and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at $P < 0.05$ (n = 16). LSD indicates least significant difference value.

Harvesting Method	Severity of anthracnose (%)		Incidence anthracnose (%)	
	side	Stem	side	stem
Clip	0.19	0.53b	3.13	5.62b
Snap	0.58	7.72a	4.38	24.37a
LSD	n.s	2.83	n.s	8.81

Tamborine – late season fruit

The fruit harvested from Mt Tamborine late in the growing season were approximately 32% dry matter. There was a significant effect of harvesting method on shelf life; fruit which were clip harvested on average had a shelf life of 1.7 days longer than snap harvested fruit (Table 42).

There was no effect of harvesting method on disease development or percentage of marketable fruit (Table 42). There was no significant interaction between harvesting method and aspect (data not shown).

Table 42. The effect of harvesting method on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in November 2002 from Mt Tamborine and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at *P* < 0.05 (n = 16). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Harvesting method						
Clip	13.3a	6.15	45.6	2.9	10.0	71.3
Snap	11.6b	6.59	41.9	2.9	13.1	61.9
LSD	1.19	n.s	n.s	n.s	n.s	n.s

B. Effect of copper spray and harvesting method on development postharvest diseases

Snap harvesting fruit in the morning significantly increased the incidence of stem-end rot. Harvesting method did not affect shelf life, severity or incidence of anthracnose, severity of stem-end rot, or percentage of marketable fruit (Table 43).

Of the fruit harvested in the morning, spraying with a copper fungicide 5 days prior to harvest significantly increased the incidence of anthracnose (Table 43). The overall increase in anthracnose was due to an increase in side anthracnose (Table 44). There were no significant differences between shelf life, severity of anthracnose, incidence or severity of stem-end rot, or percentage of marketable fruit (Table 43). For the morning harvest there was no significant interaction between harvesting method and copper treatment (data not shown).

Table 43. The effect of harvesting method and a copper spray five days prior to harvest on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested in the morning in September 2002 from Mt Tamborine and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at *P* < 0.05 (n = 20). LSD indicates least significant difference value.

Treatment	Shelf Life (days)	% Anthracnose		% Stem-end rot		Marketable fruit (%)
		Severity	incidence	severity	incidence	
Harvesting method						
Clip	11.7	1.18	12.7	0.03	0.7b	96.0
Snap	11.5	0.93	6.7	1.29	8.0a	90.0
LSD	n.s	n.s	n.s	n.s	6.3	n.s
Spray treatment						
Copper spray	12.0	1.46	15.3a	0.35	2.0	94.0
Control	11.2	0.65	4.0b	0.97	6.7	92.0
LSD	n.s	n.s	7.07	n.s	n.s	n.s

Table 44. The effect of a copper spray on anthracnose severity (% surface area affected) and incidence (% fruit affected) at the side and stem-end of the fruit of 'Hass' avocado fruit harvested in September 2002 from Mt Tamborine and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at *P* < 0.05 (n = 16). LSD indicates least significant difference value.

Harvesting Method	Severity of anthracnose (%)		Incidence of anthracnose (%)	
	side	Stem	Side	stem
Copper spray	0.48	0.98	9.3a	6.0
Control	0.15	0.50	2.7b	1.3
LSD	n.s	n.s	5.19	n.s

Of the fruit harvested at midday, there was no effect of harvesting method or copper spray on the shelf life, severity or incidence of stem-end rot or the percentage of marketable fruit. The

copper spray increased the incidence and severity of anthracnose although not significantly (Table 45). For the afternoon harvest there was no significant interaction between harvesting method and copper treatment (data not shown).

Table 45. The effect of harvesting method and a copper spray five days prior to harvest on fruit shelf life, anthracnose and stem-end rot severity (% surface area affected) and incidence (% fruit affected) and fruit marketability (% of fruit with ≤ 5% anthracnose severity and no stem-end rot) of 'Hass' avocado fruit harvested at midday in September 2002 from Mt Tamborine and ripened at 22°C (65% RH). Mean values within columns followed by the same letter are not significantly different at *P* < 0.05 (n = 20). LSD indicates least significant difference value.

Treatment	Shelf life (days)	% Anthracnose severity	% Anthracnose incidence	% Stem-end rot severity	% Stem-end rot incidence	Marketable fruit (%)
Harvesting method						
Clip	11.6	1.05	10.0	0.20	2.0	93.3
Snap	11.0	0.82	7.3	0.65	5.3	92.7
LSD	n.s	n.s	n.s	n.s	n.s	n.s
Spray treatment						
Copper spray	11.4	1.54	11.3	0.57	4.0	90.7
Control	11.1	0.33	6.0	0.28	3.3	95.3
LSD	n.s	n.s	n.s	n.s	n.s	n.s

3.4 Pepper Spot Studies

A. DNA fingerprinting

A. Morphological characterisation of isolates

Isolates which didn't produce perithecia were cultured again from the collection. A lack of ascospore production in this study does not suggest that the sexual stage is non-existent. The assessment was based on ready production of perithecia under one set of laboratory conditions. Tables 46 and 47 show that production of ascospores is limited to *Colletotrichum gloeosporioides* isolates from avocado. None of the mango isolates produced perithecia under our conditions.

No ascospores were detected in isolates from Bangalow. Ascospores were detected in 16 anthracnose isolates from Cudgen but no pepper spot isolates from Cudgen. 15 anthracnose isolates and one pepper spot isolate from Duranbah produced ascospores. No anthracnose isolates from Green Pigeon produced ascospores but 18 pepper spot isolates did. Only a few isolates from Mt Tamborine produced the sexual stage all of which were anthracnose isolates.

Table 46 Screening of *Colletotrichum gloeosporioides* isolates from **avocado** fruit for the production of *Glomerella cingulata*

Avcaodo Anthracnose Bangalow		Avocado Pepper spot Bangalow		Avocado Anthracnose Cudgen		Avocado Pepper spot Cudgen		Avocado Anthracnose Duranbah		Avocado Pepper spot Duranbah		Avocado Anthracnose Green Pigeon		Avocado Pepper spot Green Pigeon		Avocado Anthracnose Mt Tamborine		Avocado Pepper spot Mt Tamborine	
AAB 11	-	**APB** 11	-	**AAC** 11	+	**APC** 11	-	**AAD** 11	+	**APD** 11	-	**AAG** 11	-	**APG** 11	+	**AAT** 11	-	**APT** 11	-
AAB 12	-	**APB** 12	-	**AAC** 12	+	**APC** 12	-	**AAD** 12	+	**APD** 12	-	**AAG** 12	-	**APG** 12	-	**AAT** 12	-	**APT** 12	-
AAB 13	-	**APB** 13	-	**AAC** 13	+	**APC** 13	-	**AAD** 13	-	**APD** 13	-	**AAG** 13	-	**APG** 13	+	**AAT** 13	-	**APT** 13	-
AAB 14	-	**APB** 14	-	**AAC** 14	+	**APC** 14	-	**AAD** 14	-	**APD** 14	-	**AAG** 14	-	**APG** 14	+	**AAT** 14	-	**APT** 14	-
AAB 15	-	**APB** 15	-	**AAC** 15	+	**APC** 15	-	**AAD** 15	-	**APD** 15	-	**AAG** 15	-	**APG** 15	+	**AAT** 15	-	**APT** 15	-
AAB 21	-	**APB** 21	-	**AAC** 21	-	**APC** 21	-	**AAD** 21	+	**APD** 21	-	**AAG** 21	-	**APG** 21	+	**AAT** 21	-	**APT** 21	-
AAB 22	-	**APB** 22	-	**AAC** 22	+	**APC** 22	-	**AAD** 22	+	**APD** 22	-	**AAG** 22	-	**APG** 22	+	**AAT** 22	-	**APT** 22	-
AAB 23	-	**APB** 23	-	**AAC** 23	+	**APC** 23	-	**AAD** 23	+	**APD** 23	-	**AAG** 23	-	**APG** 23	+	**AAT** 23	-	**APT** 23	-
AAB 24	-	**APB** 24	-	**AAC** 24	-	**APC** 24	-	**AAD** 24	+	**APD** 24	-	**AAG** 24	-	**APG** 24	+	**AAT** 24	-	**APT** 24	-
AAB 25	-	**APB** 25	-	**AAC** 25	+	**APC** 25	-	**AAD** 25	+	**APD** 25	-	**AAG** 25	-	**APG** 25	+	**AAT** 25	-	**APT** 25	-
AAB 31	-	**APB** 31	-	**AAC** 31	-	**APC** 31	-	**AAD** 31	-	**APD** 31	-	**AAG** 31	-	**APG** 31	-	**AAT** 31	-	**APT** 31	-
AAB 32	-	**APB** 32	-	**AAC** 32	+	**APC** 32	-	**AAD** 32	+	**APD** 32	-	**AAG** 32	-	**APG** 32	+	**AAT** 32	-	**APT** 32	-
AAB 33	-	**APB** 33	-	**AAC** 33	-	**APC** 33	-	**AAD** 33	+	**APD** 33	-	**AAG** 33	-	**APG** 33	-	**AAT** 33	-	**APT** 33	-
AAB 34	-	**APB** 34	-	**AAC** 34	+	**APC** 34	-	**AAD** 34	+	**APD** 34	-	**AAG** 34	-	**APG** 34	-	**AAT** 34	+	**APT** 34	-
AAB 35	-	**APB** 35	-	**AAC** 35	+	**APC** 35	-	**AAD** 35	+	**APD** 35	-	**AAG** 35	-	**APG** 35	+	**AAT** 35	-	**APT** 35	-
AAB 41	-	**APB** 41	-	**AAC** 41	-	**APC** 41	-	**AAD** 41	-	**APD** 41	-	**AAG** 41	-	**APG** 41	-	**AAT** 41	+	**APT** 41	-
AAB 42	-	**APB** 42	-	**AAC** 42	-	**APC** 42	-	**AAD** 42	+	**APD** 42	+	**AAG** 42	-	**APG** 42	-	**AAT** 42	-	**APT** 42	-
AAB 43	-	**APB** 43	-	**AAC** 43	+	**APC** 43	-	**AAD** 43	-	**APD** 43	-	**AAG** 43	-	**APG** 43	+	**AAT** 43	-	**APT** 43	-
AAB 44	-	**APB** 44	-	**AAC** 44	-	**APC** 44	-	**AAD** 44	-	**APD** 44	-	**AAG** 44	-	**APG** 44	+	**AAT** 44	-	**APT** 44	-
AAB 45	-	**APB** 45	-	**AAC** 45	+	**APC** 45	-	**AAD** 45	-	**APD** 45	-	**AAG** 45	-	**APG** 45	-	**AAT** 45	-	**APT** 45	-
AAB 51	-	**APB** 51	-	**AAC** 51	+	**APC** 51	-	**AAD** 51	-	**APD** 51	-	**AAG** 51	-	**APG** 51	+	**AAT** 51	-	**APT** 51	-
AAB 52	-	**APB** 52	-	**AAC** 52	+	**APC** 52	-	**AAD** 52	+	**APD** 52	-	**AAG** 52	-	**APG** 52	+	**AAT** 52	-	**APT** 52	-
AAB 53	-	**APB** 53	-	**AAC** 53	-	**APC** 53	-	**AAD** 53	-	**APD** 53	-	**AAG** 53	-	**APG** 53	+	**AAT** 53	-	**APT** 53	-
AAB 54	-	**APB** 54	-	**AAC** 54	+	**APC** 54	-	**AAD** 54	+	**APD** 54	-	**AAG** 54	-	**APG** 54	+	**AAT** 54	-	**APT** 54	-
AAB 55	-	**APB** 55	-	**AAC** 55	-	**APC** 55	-	**AAD** 55	+	**APD** 55	-	**AAG** 55	-	**APG** 55	+	**AAT** 55	-	**APT** 55	-

Table 47 Screening of *Colletotrichum gloeosporioides* isolates from **mango** fruit for the production of *Glomerella cingulata*

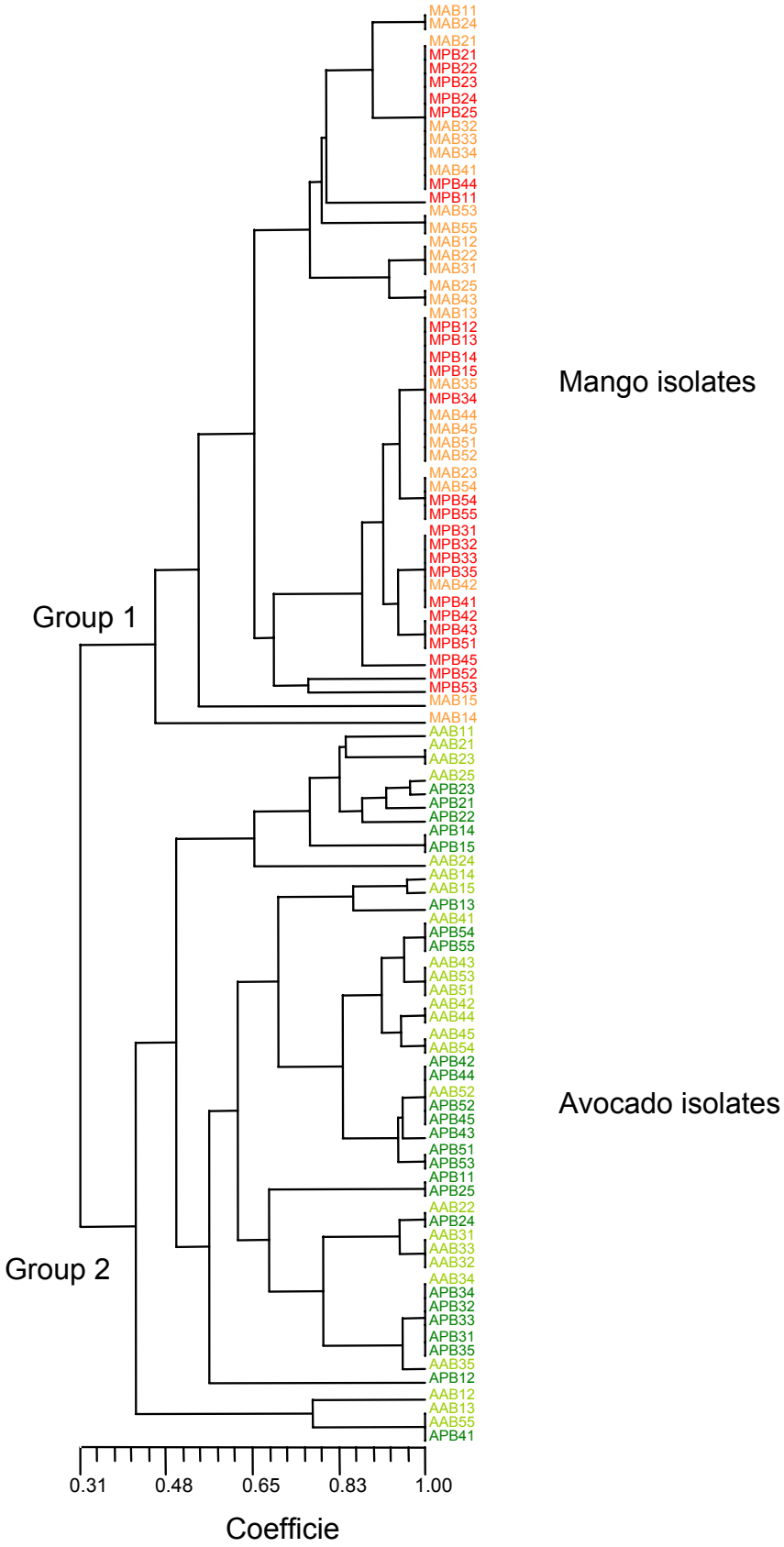
Mango Anthracnose Ayr		**Mango Pepper spot** *Ayr*		**Mango Anthracnose** Bangalow		**Mango Pepper spot** *Bangalow*		**Mango Anthracnose** Green Pigeon		**Mango Pepper spot** Green Pigeon	
MAA 11	-	**MPA** 11	-	**MAB** 11	-	**MPB** 11	-	**MAG** 11	-	**MPG** 11	-
MAA 12	-	**MPA** 12	-	**MAB** 12	-	**MPB** 12	-	**MAG** 12	-	**MPG** 12	-
MAA 13	-	**MPA** 13	-	**MAB** 13	-	**MPB** 13	-	**MAG** 13	-	**MPG** 13	-
MAA 14	-	**MPA** 14	-	**MAB** 14	-	**MPB** 14	-	**MAG** 14	-	**MPG** 14	-
MAA 15	-	**MPA** 15	-	**MAB** 15	-	**MPB** 15	-	**MAG** 15	-	**MPG** 15	-
MAA 21	-	**MPA** 21	-	**MAB** 21	-	**MPB** 21	-	**MAG** 21	-	**MPG** 21	-
MAA 22	-	**MPA** 22	-	**MAB** 22	-	**MPB** 22	-	**MAG** 22	-	**MPG** 22	-
MAA 23	-	**MPA** 23	-	**MAB** 23	-	**MPB** 23	-	**MAG** 23	-	**MPG** 23	-
MAA 24	-	**MPA** 24	-	**MAB** 24	-	**MPB** 24	-	**MAG** 24	-	**MPG** 24	-
MAA 25	-	**MPA** 25	-	**MAB** 25	-	**MPB** 25	-	**MAG** 25	-	**MPG** 25	-
MAA 31	-	**MPA** 31	-	**MAB** 31	-	**MPB** 31	-	**MAG** 31	-	**MPG** 31	-
MAA 32	-	**MPA** 32	-	**MAB** 32	-	**MPB** 32	-	**MAG** 32	-	**MPG** 32	-
MAA 33	-	**MPA** 33	-	**MAB** 33	-	**MPB** 33	-	**MAG** 33	-	**MPG** 33	-
MAA 34	-	**MPA** 34	-	**MAB** 34	-	**MPB** 34	-	**MAG** 34	-	**MPG** 34	-
MAA 35	-	**MPA** 35	-	**MAB** 35	-	**MPB** 35	-	**MAG** 35	-	**MPG** 35	-
MAA 41	-	**MPA** 41	-	**MAB** 41	-	**MPB** 41	-	**MAG** 41	-	**MPG** 41	-
MAA 42	-	**MPA** 42	-	**MAB** 42	-	**MPB** 42	-	**MAG** 42	-	**MPG** 42	-
MAA 43	-	**MPA** 43	-	**MAB** 43	-	**MPB** 43	-	**MAG** 43	-	**MPG** 43	-
MAA 44	-	**MPA** 44	-	**MAB** 44	-	**MPB** 44	-	**MAG** 44	-	**MPG** 44	-
MAA 45	-	**MPA** 45	-	**MAB** 45	-	**MPB** 45	-	**MAG** 45	-	**MPG** 45	-
MAA 51	-	**MPA** 51	-	**MAB** 51	-	**MPB** 51	-	**MAG** 51	-	**MPG** 51	-
MAA 52	-	**MPA** 52	-	**MAB** 52	-	**MPB** 52	-	**MAG** 52	-	**MPG** 52	-
MAA 53	-	**MPA** 53	-	**MAB** 53	-	**MPB** 53	-	**MAG** 53	-	**MPG** 53	-
MAA 54	-	**MPA** 54	-	**MAB** 54	-	**MPB** 54	-	**MAG** 54	-	**MPG** 54	-
MAA 55	-	**MPA** 55	-	**MAB** 55	-	**MPB** 55	-	**MAG** 55	-	**MPG** 55	-

B. Genetic diversity among Colletotrichum gloeosporioides *isolates*

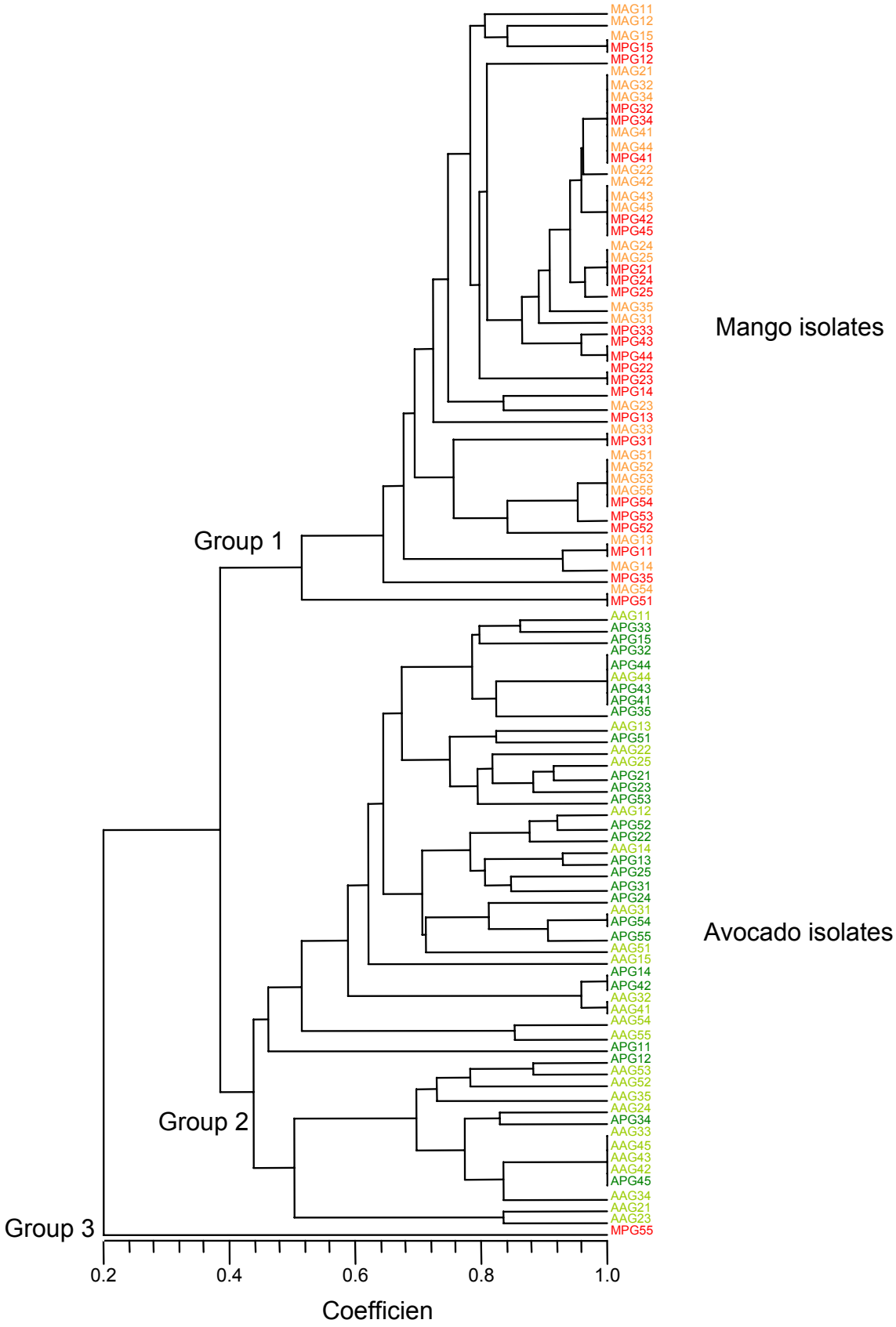
1. Genetic diversity among *Colletotrichum gloeosporioides* isolates

Each of the primers grouped the isolates similarly based on the respective DNA fingerprint patterns they generated. Comparison of the DNA fingerprints, both visually and by phenetic analysis, subdivided isolates into clonal lineages. These fingerprint patterns were extremely detailed and often had very little distance between bands. Without magnification, some of the bands were difficult to visualise, hence the gels were photocopied to almost double their size in order to score the bands more easily. These scores were then related back visually to the original fingerprint gels on a light-box for enhancement. The gels were scored 3 times for each of the 2 primers in order to provide consistent assessments across all of the isolates. The data presented is based on primer HIRH and the results were confirmed using data from primer RKMI. Ideally, gel fingerprints obtained from the other primers could have been used, however, comparing such a large number of isolates made this prohibitive. The main objective was to generate population data.

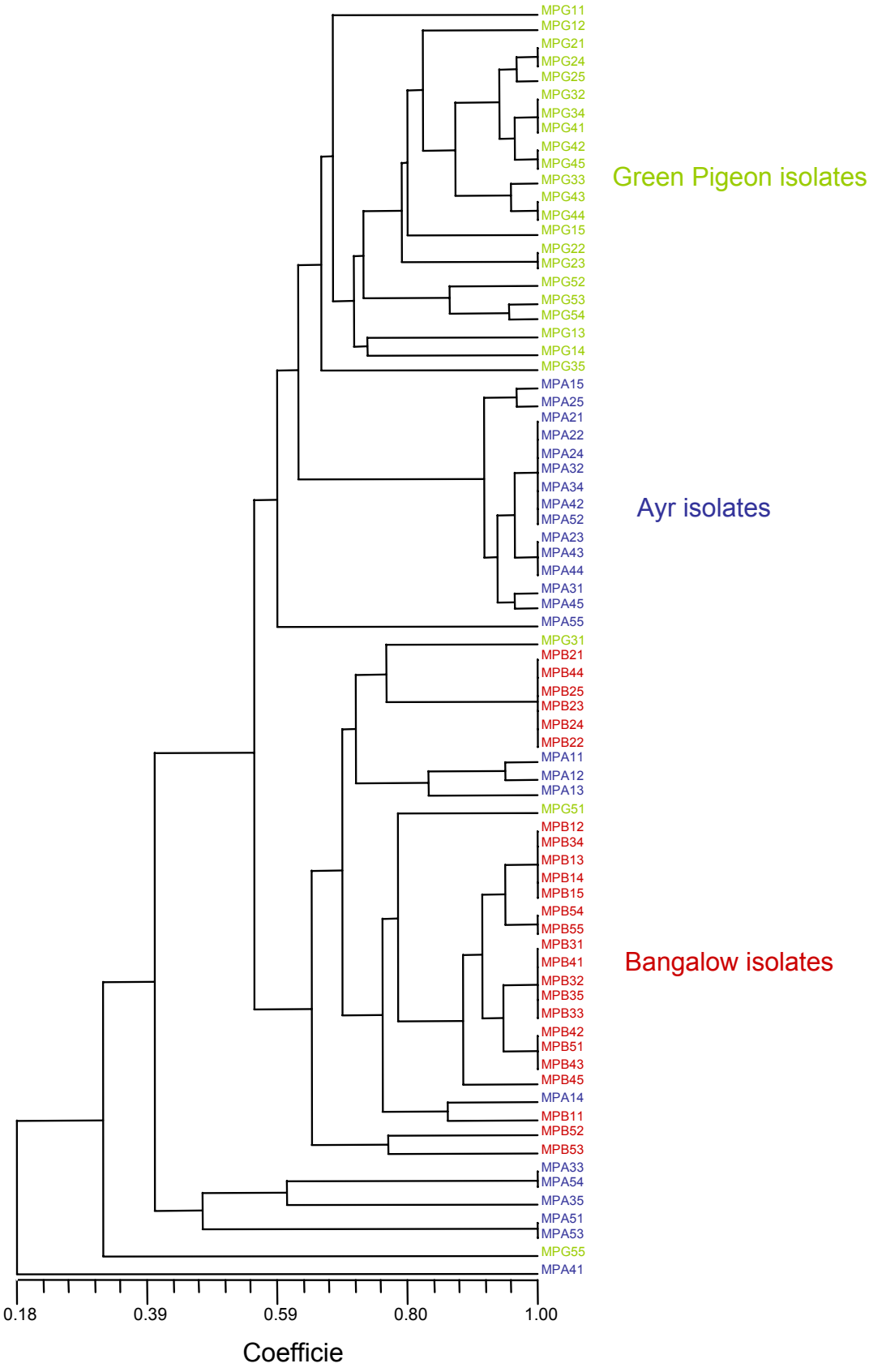
Comparing 350 isolates revealed the high level of variation among *Colletotrichum gloeosporioides* from mango and avocado within Australia. Among the mango isolates, many of the bands generated were monomorphic. The avocado isolates, however, had a large percentage of polymorphic bands. Cluster analysis clearly separated the mango isolates from the avocado isolates. It was possible to collect isolates of *C. gloeosporioides* from fruit from avocado orchards situated side by side with mango orchards on the same property. A property at Bangalow, NSW and also Green Pigeon, NSW were growing both avocados and mangoes. Dendograms 1 and 2 represent cluster analysis for isolates from Bangalow and Green Pigeon, respectively. Cluster analysis distinguishes isolates from mango into Group 1 and isolates from avocado into Group 2 and this is consistent for both isolates from both farms. A single isolate from a mango fruit from Green Pigeon formed a single outlying Group 3. These major groups had only a low level of genetic similarity between each other (approximately 0.3 for Bangalow isolates and 0.4 for Green Pigeon isolates). Within the main groups, isolates formed further clusters, with many isolates, especially mango isolates, being identical. Many isolates within clusters showed a high level of genotypic similarity (>0.7). Dendogram 3 represents cluster analysis of mango isolates from pepper spot from 3 geographic regions. Although not absolutely distinct, there are definite patterns in the dendogram. Most of the Green Pigeon isolates form a single group. There are also groups representing isolates from Ayr and Bangalow. Similarity for groups is moderate at 0.6, while similarities within clusters can be as high as 0.95. Although not all dendograms are presented, similar patterns were found throughout the *Colletotrichum gloeosporioides* populations.



Dendogram 1: *C. gloeosporioides* isolates from avocado pepper spot and anthracnose and mango pepper spot and anthracnose from **Bangalow**



Dendogram 2: *C. gloeosporioides* isolates from avocado pepper spot and anthracnose and mango pepper spot and anthracnose from **Green Pigeon**



Dendogram 3: *C. gloeosporioides* isolates from mango pepper spot from Bangalow, Green Pigeon and Ayr

B. Pathogenicity of pepper spot versus anthracnose isolates

A. Inoculation tests on detached avocado fruit in the laboratory

1. Preliminary inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado on detached mature 'Hass' avocado fruit

This experiment was conducted to test the inoculation methodology and to determine if the chosen inoculum concentration would be appropriate. The data were analysed as a randomised complete block (using the crates as replicates) and lesion incidence was scored as presence or absence of visible lesions at 3 inoculation sites per fruit.

The data (Figure 2) shows that all isolates were capable of producing lesions on the detached fruit under test conditions. Lesions first appeared as a blackening of the area where the spore suspension droplet was inoculated onto the fruit. Over time, lesions spread outwards becoming darker and more sunken and sometimes producing conidial masses in the centre, particularly in the humid test conditions (Plate 7).

Plate 7: Detached mature 'Hass' avocado fruit inoculated at 3 sites with *Colletotrichum gloeosporioides* (left) and water only (right). The 3 lesions shown on the fruit and the peel are typical anthracnose lesions

As the histogram (Figure 2) demonstrates, pepper spot isolate APC11 produced visible lesions at all inoculation sites while pepper spot isolates 24605 and APT21 produced lesions at less than and just over half the sites, respectively. Anthracnose isolate 23691 produced lesions on only half the inoculated sites and anthracnose isolates AAD11, AAT11 and AAT21 produced lesions on just over half the sites. However, these differences were not statistically significant.

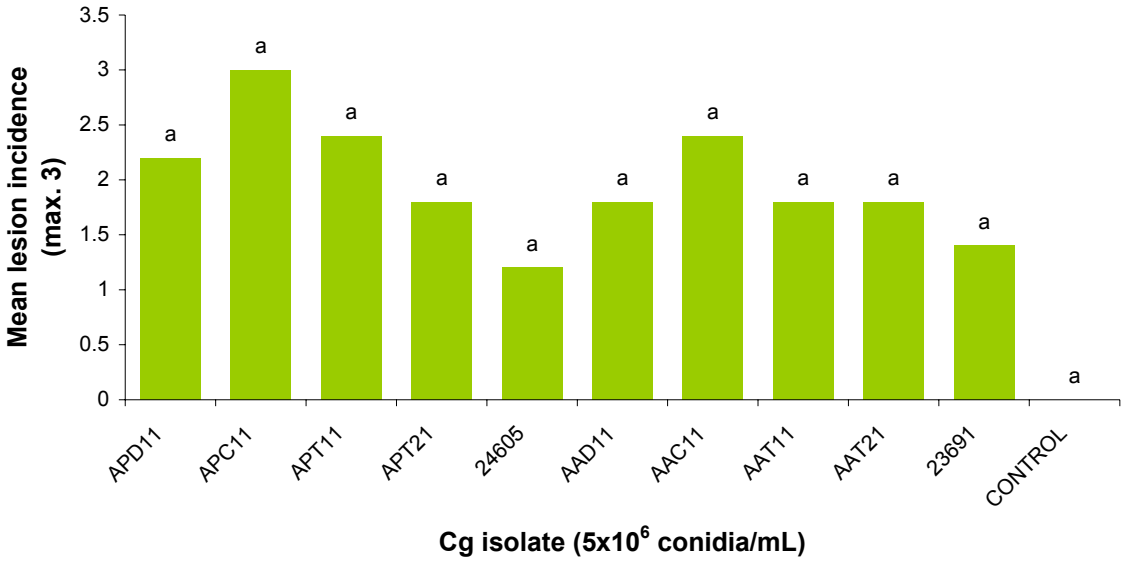


Figure 2: Anthracnose lesion incidence on detached avocado fruit inoculated with avocado pepper spot isolates (first 5 columns) and avocado anthracnose isolates (columns 6 to 10) plus water-inoculated control

There were some significant differences in lesion diameter (aggressiveness) between isolates (Figure 3). The avocado pepper spot isolates were generally more aggressive (i.e. formed larger lesions) than the avocado anthracnose isolates except for isolate 24605 (Figure 3). Isolate APC11 was significantly different from all but one anthracnose isolates (Figure 3). Overall, trends in lesion diameter corresponded with those of lesion incidence; isolates which produced more lesions tended to produce larger lesions.

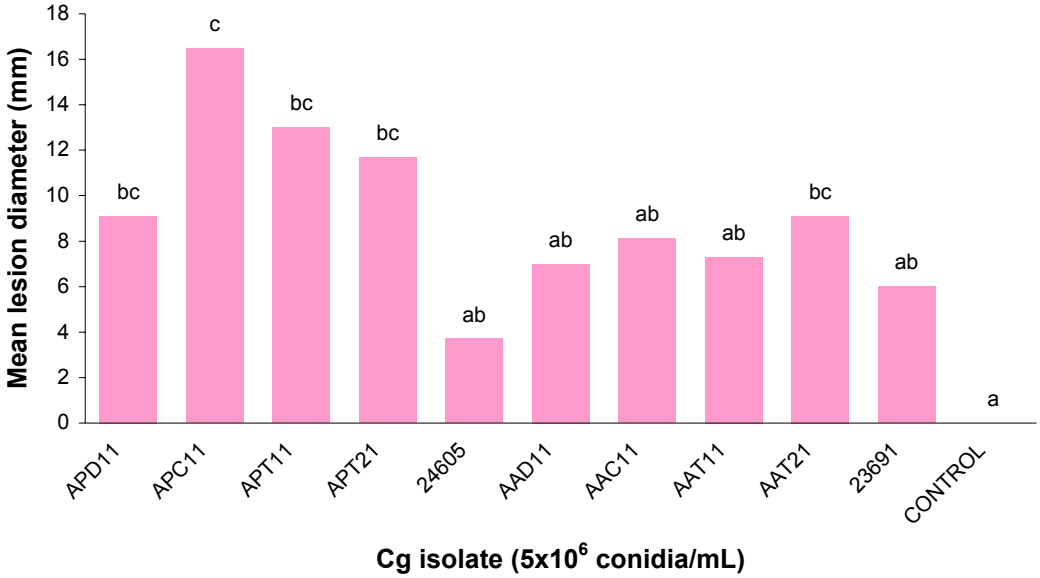


Figure 3: Mean anthracnose lesion diameter on detached mature 'Hass' avocado fruit inoculated with avocado pepper spot isolates (first 5 columns) and avocado anthracnose isolates (columns 6 to 10) plus water-inoculated control

2. Inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado and mango on detached seedless "cocktail" 'Fuerte' avocado fruit

The presence or absence of lesions (i.e. lesion incidence) was recorded daily for 9 days and a repeated measures analysis of variance was done to analyse the data over time. After 9 days there was a lot of natural infection and fruit rot and it was difficult in many cases to discern which lesions were the result of inoculation with *C. gloeosporioides* in the laboratory. Each fruit received a randomly allocated treatment (isolate) at the beginning of the experiment and the fruit were observed at successive occasions to determine how the treatment effects developed. In the analysis, there was a significant isolate*time interaction ($P<0.001$, LSD = 0.9684) indicating that the pattern of response of the isolates to time is different i.e. the significant differences between isolates will vary depending on the time under consideration. Figure 3 presents the lesion incidence data collected at 4 and 5 days after inoculation. At this time, fruit had ripened but were not over-ripe or rotting, and all potential lesions had developed at inoculated sites (Plate 8). Isolates were grouped according to symptom of origin (mango pepper spot, mango anthracnose, avocado pepper spot and avocado anthracnose), as indicated on the right side of the graph. Isolates were further grouped according to geographic origin (Green Pigeon, Bangalow, Ayr and others).

Plate 8: Avocado pepper spot isolate APB51 inoculated at 3 sites (indicated by black marked circles) on detached seedless "cocktail" 'Fuerte' avocado fruit. Blackening within the circles shows the initial inoculation zone and then the typical anthracnose symptom spreading outwards and into the flesh

More lesions developed on detached avocado fruit inoculated with avocado isolates than mango isolates (Figure 4). After 5 days, all avocado isolate groups had produced lesions at 50% of their inoculation sites. Half of the avocado isolate groups had produced lesions at 2 of the maximum 3 inoculation sites. After 5 days, most of the mango isolate groups had not even produced lesions at a third of inoculation sites. Mango pepper spot isolates from Ayr and mango anthracnose isolates from Bangalow were the exceptions, producing lesions at almost half the inoculation sites. All of the avocado isolates produced typical anthracnose lesions which ultimately became sunken over time. Approximately half of the mango isolates, however, produced a blackening of the skin surface of 1-2 mm in diameter and, while they discontinued spread and did not become sunken, they were counted as lesion incidence in this study (Plate 9).

Plate 9: Mango anthracnose isolate MAA11 inoculated at 3 sites (indicated by black marked circles) on detached seedless "cocktail" 'Fuerte' avocado fruit. Blackening within the circles shows the initial inoculation zone but lesions often progressed no further

Generally, most sites inoculated with mango isolates remained symptomless until 3 days after inoculation. Isolates MAA51 and MPB21 produced no symptoms until day 6 and isolates MPB41 and MPG11 did not cause any symptoms until 7 days after inoculation. It is possible that symptoms appearing at this stage might not be due to inoculation by *C. gloeosporioides* isolates as water-inoculated fruit was showing natural disease incidence by day 8. By day 4, mango pepper spot isolates from Ayr had the highest and most consistent incidence of lesions of all mango isolates tested, especially MPA11 with a mean incidence of 2. Of the avocado isolates, 9 pepper spot and 5 anthracnose isolates had an incidence mean of 2 and above at day 4, with the highest being a mean of 3 for APD21. After 4 days, there were many significant differences as indicated by the subscripts on the columns. Similarly, significant differences can be seen between many groups 5 days after inoculation.

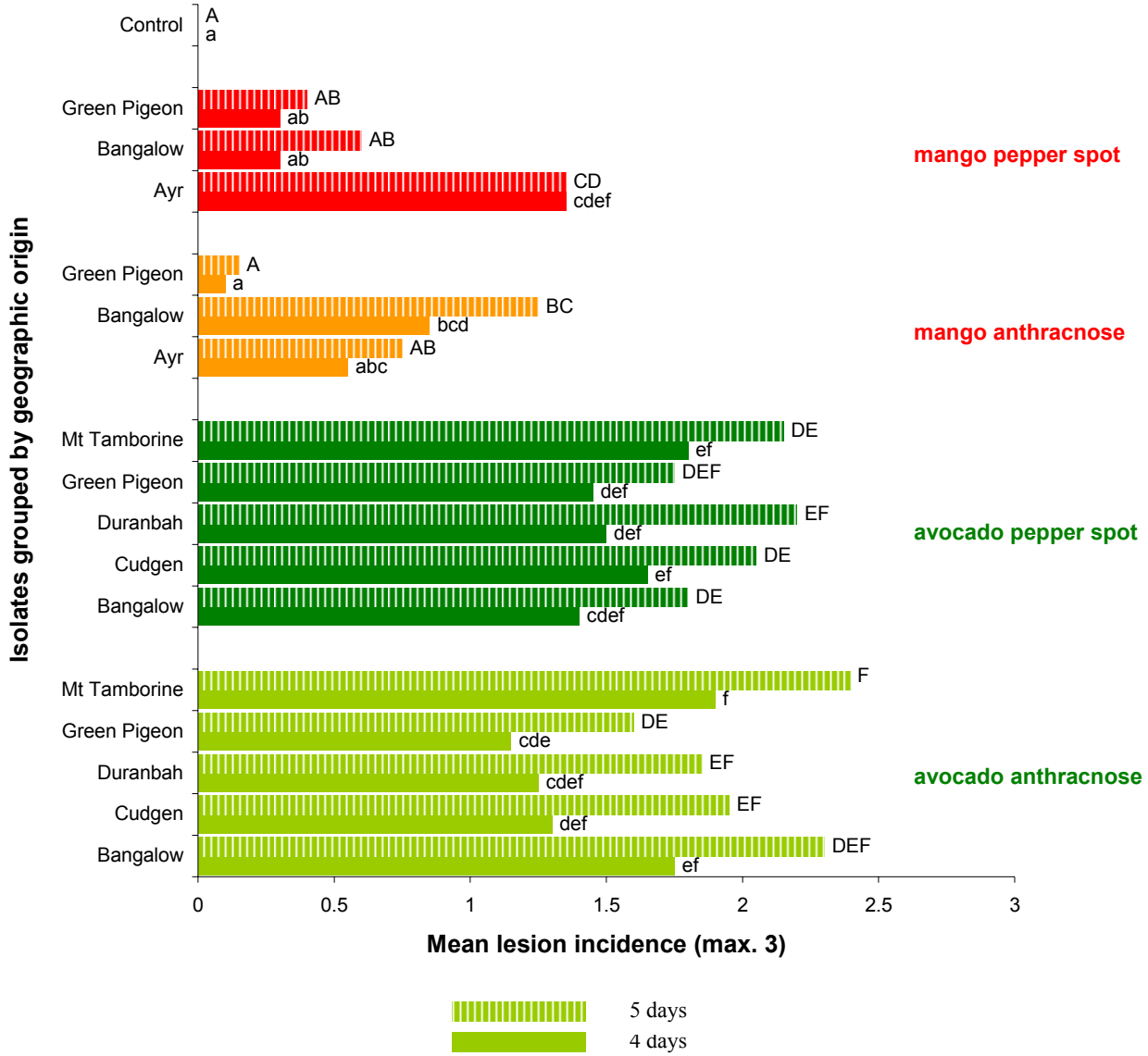


Figure 4: Mean incidence of anthracnose lesions (n=60) 4 and 5 days after inoculation with avocado isolates from pepper spot and anthracnose and mango isolates from pepper spot and anthracnose on detached "cocktail" 'Fuerte' avocado fruit. Isolates are grouped according to their fruit, symptom and place of origin. Columns with the same case letters are not significantly different at $P < 0.05$.

Table 48: Total mean anthracnose lesion incidence on detached "cocktail" 'Fuerte' avocado fruit according to fruit and symptom origins of *C. gloeosporioides* isolates (P<0.001) 4 and 5 days after inoculation

Origin of isolate	Day 4 mean* incidence	Day 5 mean* incidence
avocado anthracnose	1.47 a	1.84 a
avocado pepper spot	1.50 a	1.70 a
mango anthracnose	0.50 b	0.50 b
mango pepper spot	0.60 b	0.63 b

*mean has been adjusted to account for missing fruit

When data from Figure 4 is further grouped into four categories for isolates based on 'fruit type' origin and 'symptom type' origin (i.e. disregarding geographic origin), Table 48 is produced. Comparisons of lesion incidences due to inoculation with *C. gloeosporioides* isolates from avocado pepper spot and anthracnose and mango pepper spot and anthracnose (Table 48), 4 days and 5 days after inoculation, show that there were significant differences between isolates from avocado and isolates from mango but there were no significant differences between isolates from pepper spot and isolates from anthracnose from either fruit.

Lesion size is a further indicator of isolate aggressiveness. Again, there was a significant isolate*time interaction ($P<0.001$) indicating that the pattern of response of the isolates to time is different i.e. the significant differences between isolates will vary depending on the time under consideration. As Figure 5 shows, there was some variability between growth rates 4 days after inoculation and 5 days after inoculation. Some groups of isolates remained unchanged in growth rate over the 24 h period (e.g. AAB and MAG) while others showed remarkable increase in growth from day 4 to day 5 (e.g. APG).

Lesions arising from inoculations with avocado isolates were larger than inoculations with mango isolates, although after 9 days mango pepper spot isolates from Ayr produced large lesions (27mm) on avocado fruit. Figure 5 shows the trend for avocado isolates to produce larger lesions 4 and 5 days after inoculation. Although not measured, it was observed that most of the lesions less than 3mm were extremely slow to expand and generally did not penetrate deeply into the tissue. This scenario was particularly prevalent with mango isolates.

Four days after inoculation, the largest mean diameter was produced by isolate AAB11 at 11.3mm. This was followed by isolate MPA41 at 6.1mm, AAB31 (5.3mm), APB51 (5.1mm), AAB21 (4.4mm) and AAC21, APC11, APT21 (4mm). Of the 30 mango isolates, only 4 had mean diameters of 2.0mm and above after 4 days. Two of these were pepper spot isolates from Ayr and two were anthracnose isolates from Ayr. Of the 50 avocado isolates, 26 had mean diameters of 2mm and above after 4 days. Within the avocado isolate group, all 5 pepper spot isolates from Mt Tamborine measured over 2.0mm, followed by 4 pepper spot isolates from Cudgen, 3 pepper spot isolates from Green Pigeon, 3 anthracnose isolates from Bangalow and 3 anthracnose isolates from Mt Tamborine.

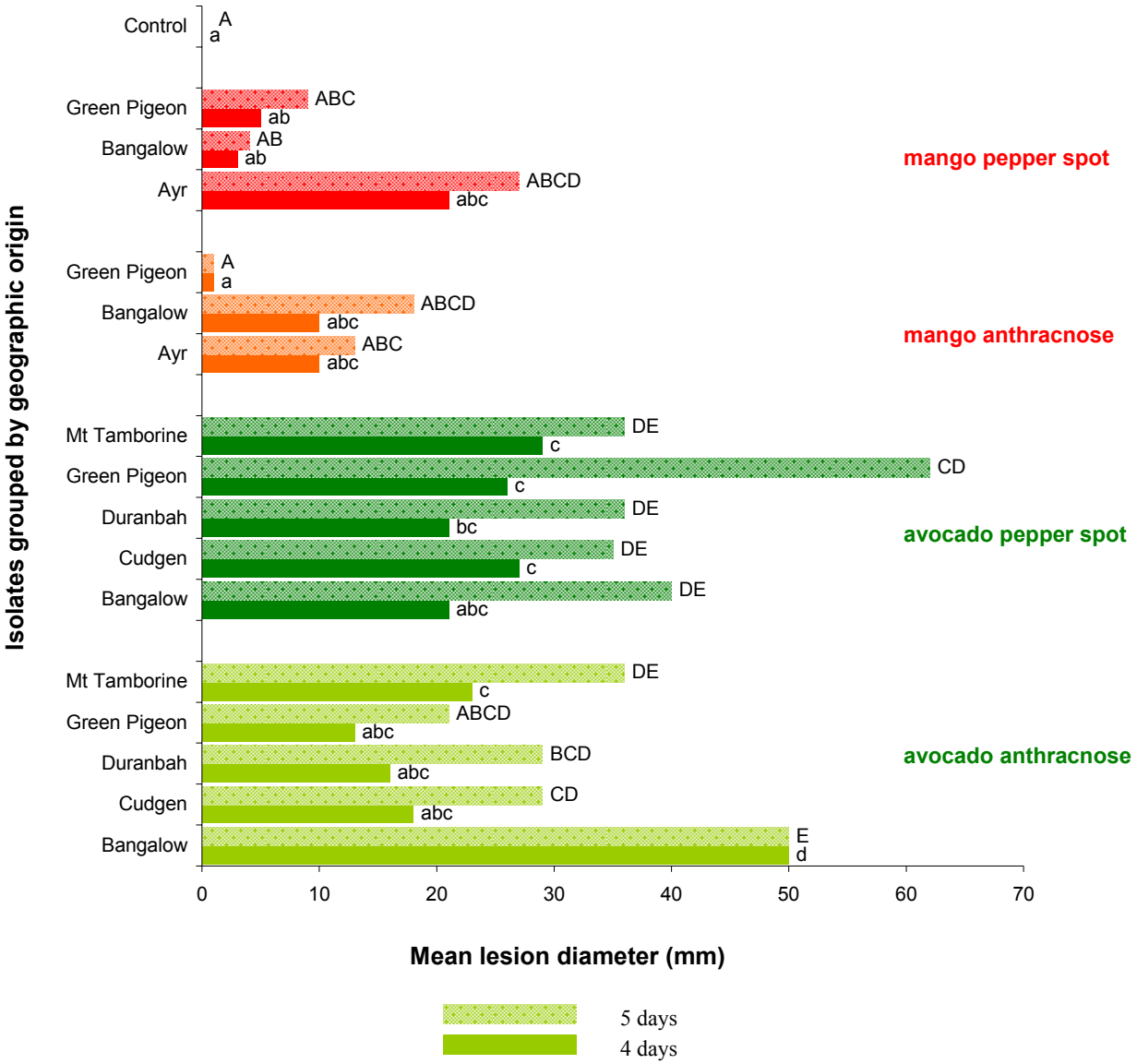


Figure 5: Mean diameter of anthracnose lesions (n=60) 4 and 5 days after inoculation with avocado isolates from pepper spot and anthracnose and mango isolates from pepper spot and anthracnose on detached "cocktail" 'Fuerte' avocado fruit. Isolates are grouped according to their fruit, symptom and place of origin. Columns with the same case letters are not significantly different at $P < 0.05$.

Table 49: Total mean anthracnose lesion diameter on detached "cocktail" 'Fuerte' avocado fruit according to fruit and symptom origins of *C. gloeosporioides* isolates (P<0.001) 4 and 5 days after inoculation

Origin of isolate	Day 4 mean* diameter	Day 5 mean* diameter
avocado anthracnose	2.33 a	3.51 a
avocado pepper spot	2.32 a	3.70 a
mango anthracnose	0.66 b	1.02 b
mango pepper spot	0.79 b	1.31 b

*mean has been adjusted to account for missing fruit

When data from Figure 5 is further grouped into four categories for isolates based on 'fruit type' origin and 'symptom type' origin (i.e. disregarding geographic origin), Table 49 is produced. Comparisons of lesion diameters due to inoculation with *C. gloeosporioides* isolates from avocado pepper spot and anthracnose and mango pepper spot and anthracnose (Table 49), 4 days and 5 days after inoculation, show that there were significant differences between isolates from avocado and isolates from mango but there were no significant differences between isolates from pepper spot and isolates from anthracnose from either fruit. In general, trends in lesion incidence data follow a similar pattern to trends in lesion diameter data; isolates which produced the most lesions also tended to produce the largest lesions.

3. Inoculation tests on avocado nursery plants in the glasshouse

Pepper spots were not observed on leaves but they were present on the petioles. Pepper spot symptoms appeared as small, shiny, raised, black lesions measuring less than 0.5mm in diameter when they became visible to the naked eye. Symptoms are identical on fruit, pedicels and petioles (Plates 10 and 11). Symptoms were expected to appear after 4-6 weeks, however, this was not the case. After leaving the plants for 6 months until October, by which time the roots were potbound and the plants very stressed, symptoms finally appeared and the petioles were rated for severity of pepper spots, according to the rating scale described in Methods and Materials. When pepper spot lesions were picked from the petioles and plated onto SPDA, *Colletotrichum gloeosporioides* was isolated from most culture sites.

Plate 10: Pepper spot lesions after inoculation with *Colletotrichum gloeosporioides* on 'Hass' avocado branch (diameter ca. 7mm) in the glasshouse (left)

Plate 11: Pepper spot lesions in closer detail (right)

Avocado pepper spot isolates generally produced the most pepper spot disease on petioles, with mean ratings ranging from 3 for Bangalow and Cudgen isolates to 3.3 for Duranbah isolates to just over 4 for Mt Tamborine isolates compared with the other three isolate groups (Figure 6). Avocado pepper spot isolates from Green Pigeon, however, were significantly

less aggressive than the avocado pepper spot isolates from the other regions with a mean rating of 1.04. This contrasts with data from the 'Fuerte' "cocktail" experiment where these same isolates, although originally isolated from pepper spots, were not significantly more or less aggressive than other avocado isolates when inoculated onto detached fruit and assessed for anthracnose development. Avocado anthracnose isolates produced the next highest ratings, ranging from 1.5 to 2.5, with mango isolates the lowest, with individual petioles often rating 0 (Figure 6). There were no significant differences between mango isolates from anthracnose or pepper spot from any geographic grouping (Figure 6).

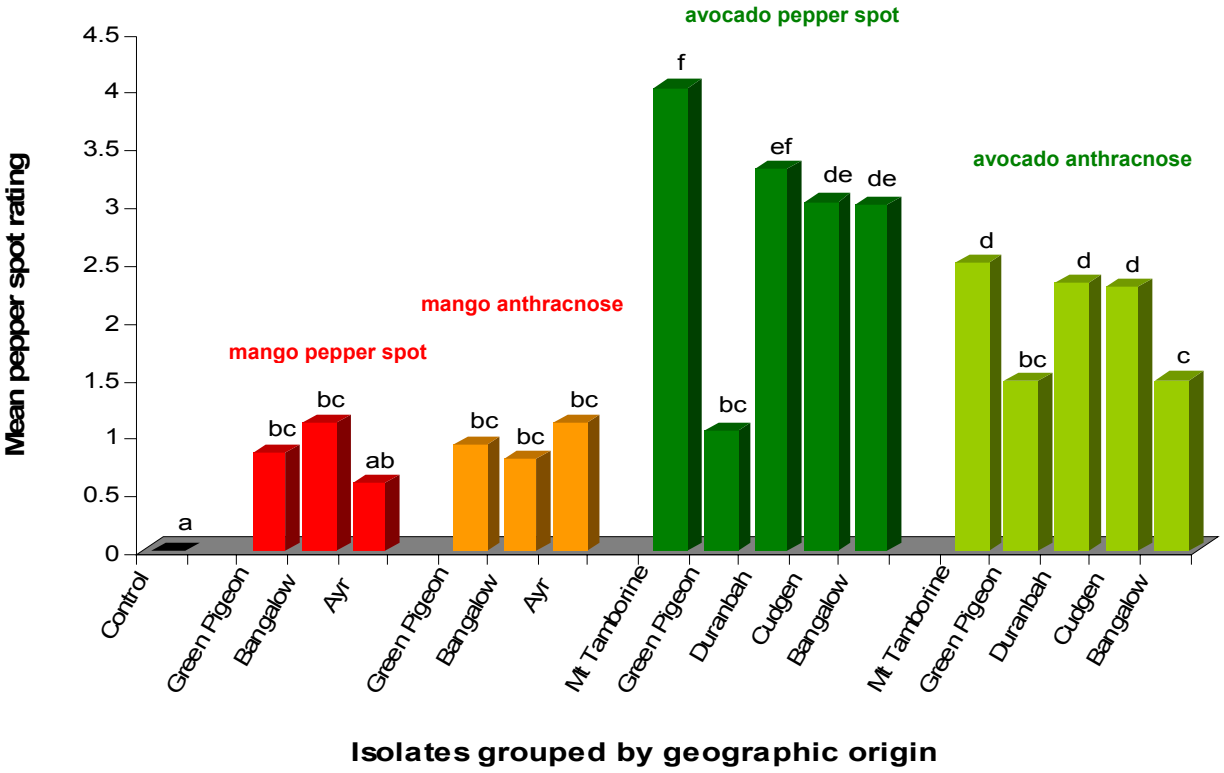


Figure 6: Mean pepper spot lesion rating (n=25) on petioles of 'Hass' avocado plants in the glasshouse 6 months after inoculation with avocado and mango isolates of *C. gloeosporioides*

Table 50 summarises total mean data for isolates from avocado and mango. The severity of pepper spot on avocado petioles was significantly more severe when inoculated with isolates originating from avocado pepper spot lesions than from avocado anthracnose lesions and mango (pepper spot and anthracnose) lesions (Table 50). Avocado anthracnose isolates produced pepper spot symptoms more severely than mango (pepper spot and anthracnose) isolates whilst there were no significant differences in pepper spot severity between mango pepper spot and mango anthracnose isolates.

Table 50: Total mean pepper spot ratings on petioles of 'Hass' avocado plants in the glasshouse 6 months after inoculation with avocado and mango isolates of *C. gloeosporioides*. Isolates are grouped according to fruit and symptom origin.

Origin of isolate	mean* pepper spot rating
avocado anthracnose	2.00 b
avocado pepper spot	2.88 c
mango anthracnose	0.94 a
mango pepper spot	0.84 a

*mean has been adjusted to account for missing fruit

B. Inoculation tests on avocado fruit in the field

1. Preliminary inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado on 'Hass' avocado fruit in the field at Mt Tamborine

This trial was initiated early in the project with some of the avocado isolates available at that time. Fruit and pedicels were assessed for the presence or absence of pepper spot lesions from 2 weeks after inoculation. Symptoms appeared as small, shiny, raised, black lesions measuring less than 0.5mm in diameter (Plate 12).

Plate 12: Relatively severe pepper spot on the upper portion of avocado fruit

Over time and particularly in conjunction with exposure to the sun, many of these lesions tended to coalesce to form larger lesions. Generally, however, especially in the first few months when fruit were growing rapidly, lesions numbers often dropped as fruit skin expansion lead to pepper spot reduction. Due to the incidence of natural disease on the fruit, there were low ratings of pepper spot on the water-inoculated fruit.

Figures 7 and 8 show pepper spot rating differences on fruit and pedicels, respectively, over a 4 week period, 2 weeks after inoculation. For both fruit and pedicel data, the interaction between isolate and time was significant, indicating that the isolates did not respond in the same way over time. The severity rating for many isolates remained more or less constant while for others it increased or decreased with the passage of time. Therefore, there may have been no significant difference initially but by the end of the experiment there were significant differences. Pepper spot severity ratings on the pedicel from inoculation with APT11 was

significantly higher than for other isolates and was higher than its rating on fruit. APT11 had one of the highest ratings at the final assessment on fruit, along with AAT11 and AAT21. Interestingly, all these 3 isolates were originally isolated from fruit from this same orchard at Mt Tamborine the previous year. On the other hand, isolate AAC11, originally isolated from an avocado anthracnose lesion, caused less pepper spot compared with all other isolates except isolate 23691 on both fruit and pedicels (Figures 7 and 8). Isolates inoculated onto pedicels generally caused higher ratings than on fruit. The pedicels seemed to be more susceptible to pepper spot development. Overall, isolates originating from pepper spot lesions did not appear to be any more aggressive on fruit than isolated derived from anthracnose lesions, or vice-versa (Table 51). Pedicels, however, did tend to show significant differences after 4 and 6 weeks between isolates originating from anthracnose and isolates originating from pepper spot (Table 52).

Table 51: Total mean **pepper spot** ratings on 'Hass' avocado **fruit** in the field 2, 4 and 6 weeks after inoculation with selected avocado isolates of *C. gloeosporioides* (isolates are grouped according to symptom origin)

Origin of isolate	Mean* pepper spot rating		
	2wks	4wks	6wks
avocado anthracnose	0.67 a	0.77 a	1.00 a
avocado pepper spot	0.95 a	0.96 a	1.17 a

*mean has been adjusted to account for missing fruit

Table 52: Total mean **pepper spot** ratings on 'Hass' avocado **pedicels** in the field 2, 4 and 6 weeks after inoculation with selected avocado isolates of *C. gloeosporioides* (isolates are grouped according to symptom origin)

Origin of isolate	Mean* pepper spot rating		
	2wks	4wks	6wks
avocado anthracnose	0.67 a	1.08 a	0.96 a
avocado pepper spot	1.05 a	1.67 b	1.86 b

*mean has been adjusted to account for missing fruit

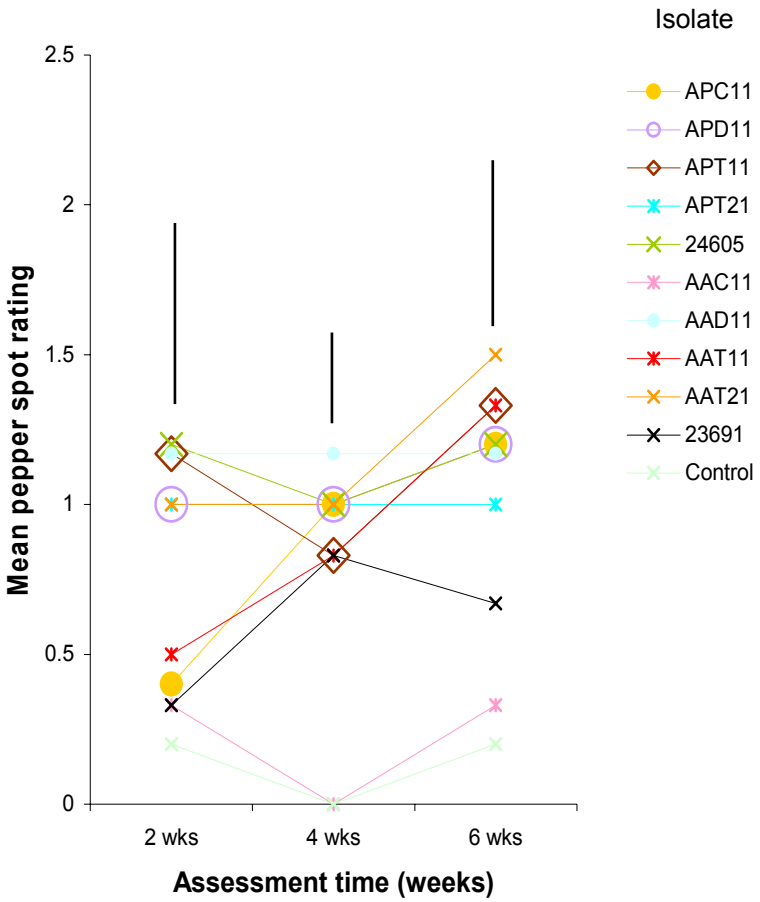


Figure 7: Development of pepper spot on small 'Hass' avocado fruit in the field after inoculation with *C. gloeosporioides* isolates from avocado anthracnose and avocado pepper spot (bar = LSD between isolates at that point in time)

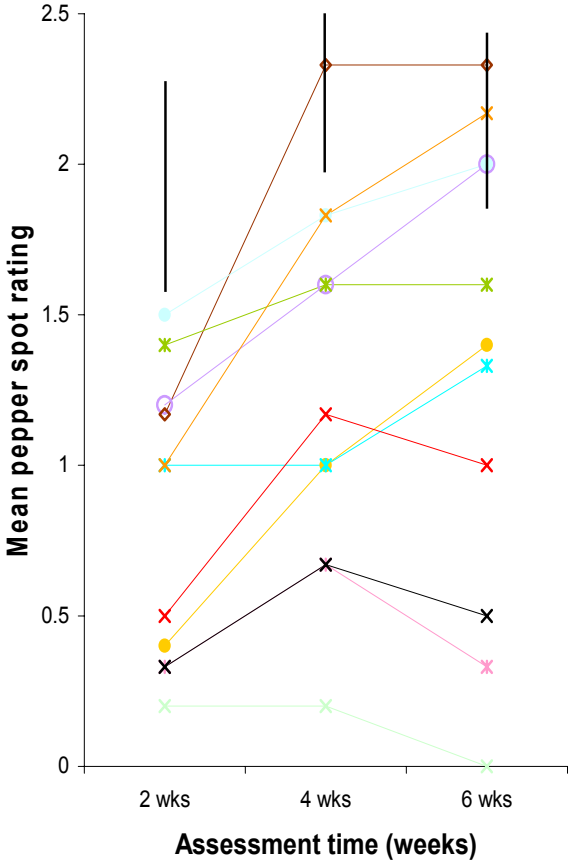


Figure 8: Development of pepper spot on pedicels of small 'Hass' avocado fruit in the field after inoculation with *C. gloeosporioides* isolates from avocado anthracnose and avocado pepper spot (bar = LSD between isolates at that point in time)

2. Further inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado on 'Hass' avocado fruit in the field (Mt Tamborine)

This trial commenced in April 2001 (autumn) and assessments were made 4 weeks later (May), then again in June and July. Fruit, although not fully mature, were approximately 80-100mm in length at the final assessment. Pedicels were not assessed in this or further experiments as the purpose of this work was to ultimately compare symptoms of pepper spot with symptoms of anthracnose caused by the *C. gloeosporioides* isolates on avocado fruit. In order to present data more clearly, anthracnose and pepper spot isolates were separated on two axes in the figure. There was evidence of natural infection as the water-inoculated fruit showed a gradual increase in disease severity over the 12 weeks; this only reaches a rating of 0.5, however.

As the data show (Figure 9), isolate AAG11 consistently produced a higher rating of pepper spot on fruit than other anthracnose isolates, although statistically there were no significant differences between any of the isolates. Infection rates increased in most isolates after inoculation, except for AAD11 which still had a rating of 0 after 4 weeks along with the control. By the 8 week assessment, all inoculated fruit had a pepper spot rating from 0.6 for 23691 up to almost 1.2 for AAG11. Between 8 weeks and 12 weeks, from June to July, severity tended to plateau or even decrease. This could be due to the fact that the fruit are growing and symptoms are no longer visible, and no further infections are appearing. Pepper spots may be being assimilated into the fruit skin as the fruit grow at this time through cell division and cell enlargement. This would account for the fluctuations in ratings every 2 weeks as fruit grow at variable rates. The time effect was significant.

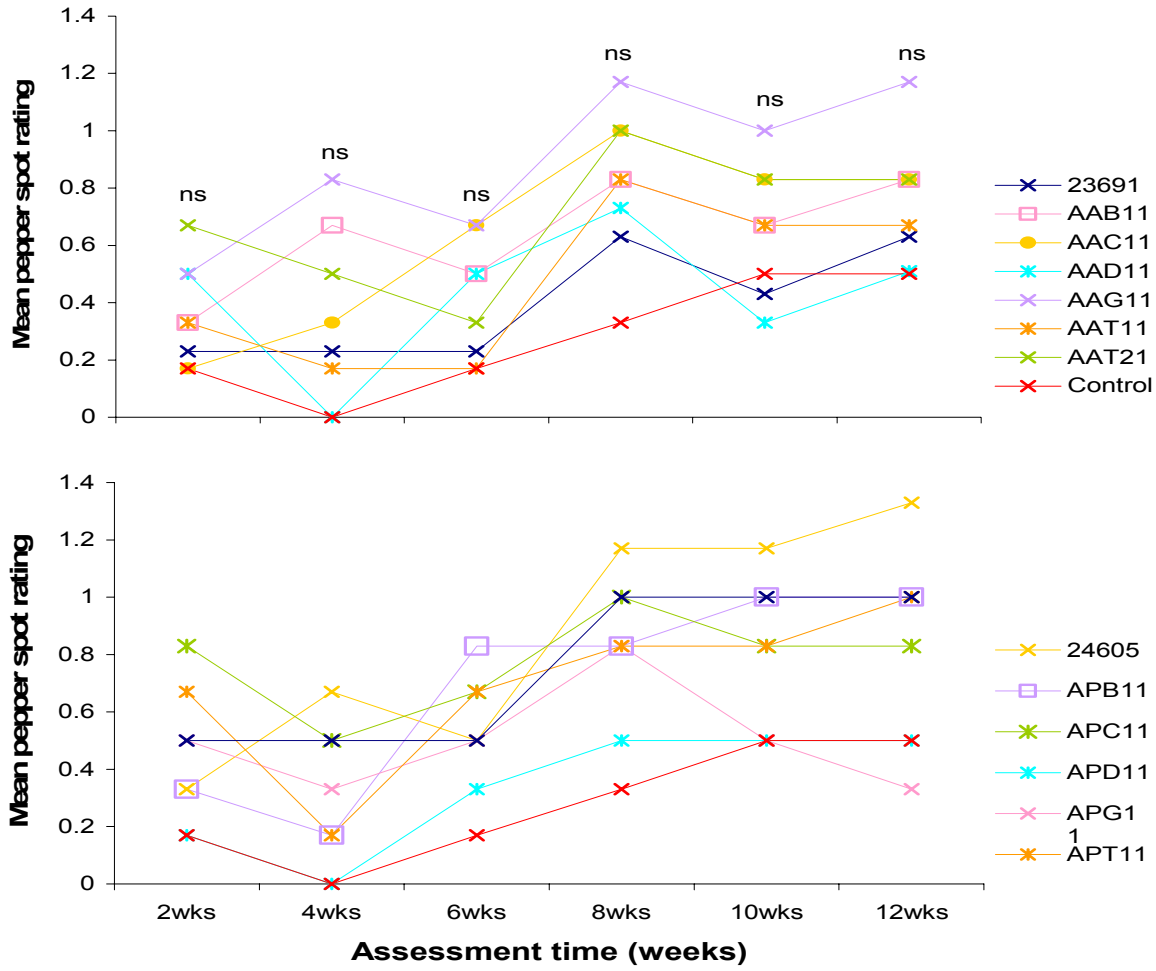


Figure 9: Mean **pepper spot** symptoms on 'Hass' avocado **fruit** in the field after inoculation with anthracnose isolates (above) and pepper spot isolates (below) of *C. gloeosporioides* in **autumn** (ns = no significant difference at P=0.05 between all isolates)

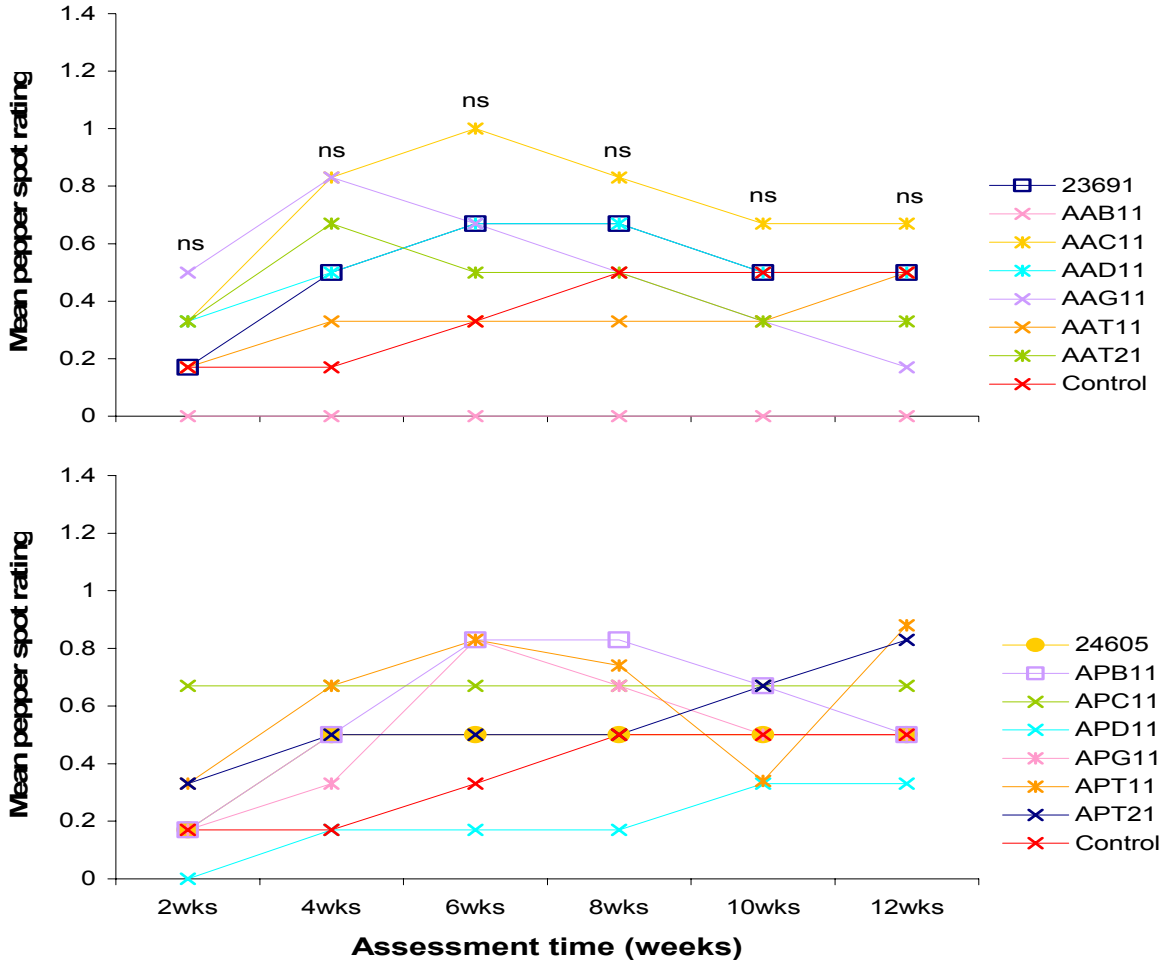


Figure 10: Mean **pepper spot** symptoms on 'Hass' avocado **fruit** in the field after inoculation with anthracnose isolates (above) and pepper spot isolates (below) of *C. gloeosporioides* in **winter** (ns = no significant difference at P=0.05 between all isolates)

The pepper spot isolates showed similar trends to the anthracnose isolates, with the similar plateau or decline in severity after 8 weeks (Figure 10). The data analysis for all isolates revealed that there was not a significant interaction between time and isolate, nor were there any significant isolate differences. This means there were no significant differences in aggressiveness between anthracnose and pepper spot isolates causing pepper spot symptoms at any of the 6 assessment times (Table 53). Again, there was a significant difference in development of pepper spot over time after inoculation.

Table 53: Total mean **pepper spot** ratings on 'Hass' avocado **fruit** in the field after inoculation with pepper spot isolates of *C. gloeosporioides* in **autumn** (isolates are grouped according to symptom origin)

Origin of isolate	Mean* pepper spot rating					
	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks
avocado anthracnose	0.39 a	0.39 a	0.44 a	0.90 a	0.70 a	0.79 a
avocado pepper spot	0.48 a	0.33 a	0.57 a	0.88 a	0.83 a	0.86 a

*mean has been adjusted to account for missing fruit

3. Further inoculation of selected *Colletotrichum gloeosporioides* isolates from avocado on 'Hass' avocado fruit in the field (Mt Tamborine)

This was a repeat of experiment 2. While the previous experiment commenced in April (autumn), fruit for this trial were inoculated in July (winter) with assessments continuing into October. Fruit were fully mature and ready for harvest by the final assessment. As Figure 11 shows, overall pepper spot ratings due to anthracnose and pepper spot isolates, respectively, at this stage of fruit maturity did not reach a mean rating of 1, which was less than the previous trial.

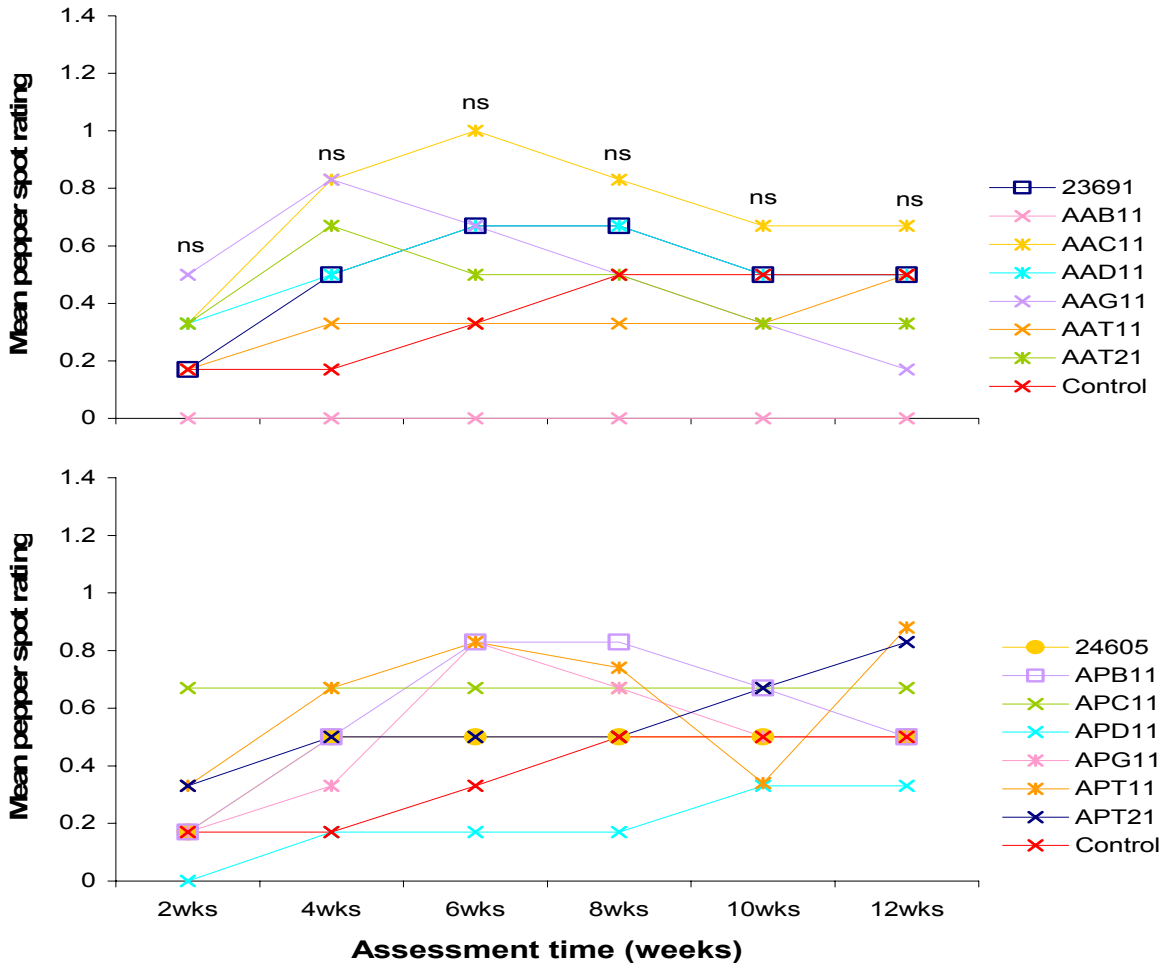


Figure 11: Mean **pepper spot** symptoms on 'Hass' avocado **fruit** in the field after inoculation with anthracnose isolates (above) and pepper spot isolates (below) of *C. gloeosporioides* in **winter** (ns = no significant difference at P=0.05 between all isolates)

Ratings for many isolates, especially anthracnose isolates, actually declined after the 4 week assessment and it is assumed that fruit were still able to continue cell growth under the site of the pepper spot lesion, which probably eventually detaches from the fruit. It has been commented that avocado fruit are unusual in that cell division continues slowly for as long as the fruit is on the tree (Schroeder 1953). Ratings for some isolates were actually less than for the water-inoculated fruit, which rate due to some natural infection on the trees. There were no significant isolate differences nor was there a significant interaction between time and isolate. This means again, there were no significant differences in aggressiveness between anthracnose and pepper spot isolates causing pepper spot symptoms (Table 54). The effect of time on the level of pepper spot ratings was, however, significant.

Table 54: Total mean **pepper spot** ratings on 'Hass' avocado **fruit** in the field after inoculation with pepper spot isolates of *C. gloeosporioides* in **winter** (isolates are grouped according to symptom origin)

Origin of isolate	Mean* pepper spot rating					
	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks
avocado anthracnose	0.26 a	0.52 a	0.55 a	0.50 a	0.38 a	0.38 a
avocado pepper spot	0.26 a	0.48 a	0.62 a	0.59 a	0.54 a	0.58 a

*mean has been adjusted to account for missing fruit

4. Inoculation of *Colletotrichum gloeosporioides* isolates from avocado and mango on 'Hass' avocado fruit in the field (Duranbah)

This trial was carried out in November 2003 (late spring) and fruit were on average 2cm in length at this stage (4-8 weeks after fruit set). In fruit this young, pepper spot symptoms tended to appear within 2 weeks. Figure 12 represents data taken 6 weeks after inoculation and shows that there was a small amount of natural pepper spot infection on water-inoculated fruit on the trees at the assessment time (January). Pepper spot was significantly more severe on fruit inoculated with avocado isolates than with mango isolates (Figure 12). Water-inoculated fruit had a mean rating of 0.63, where approximately half the fruit had ratings of 0 and half had ratings of 1. Pepper spot tends to occur only on some branches on a tree and not necessarily on all trees, so it was difficult to predict which fruit would remain free of naturally occurring disease when selecting fruit for inoculation, especially that early after fruit-set.

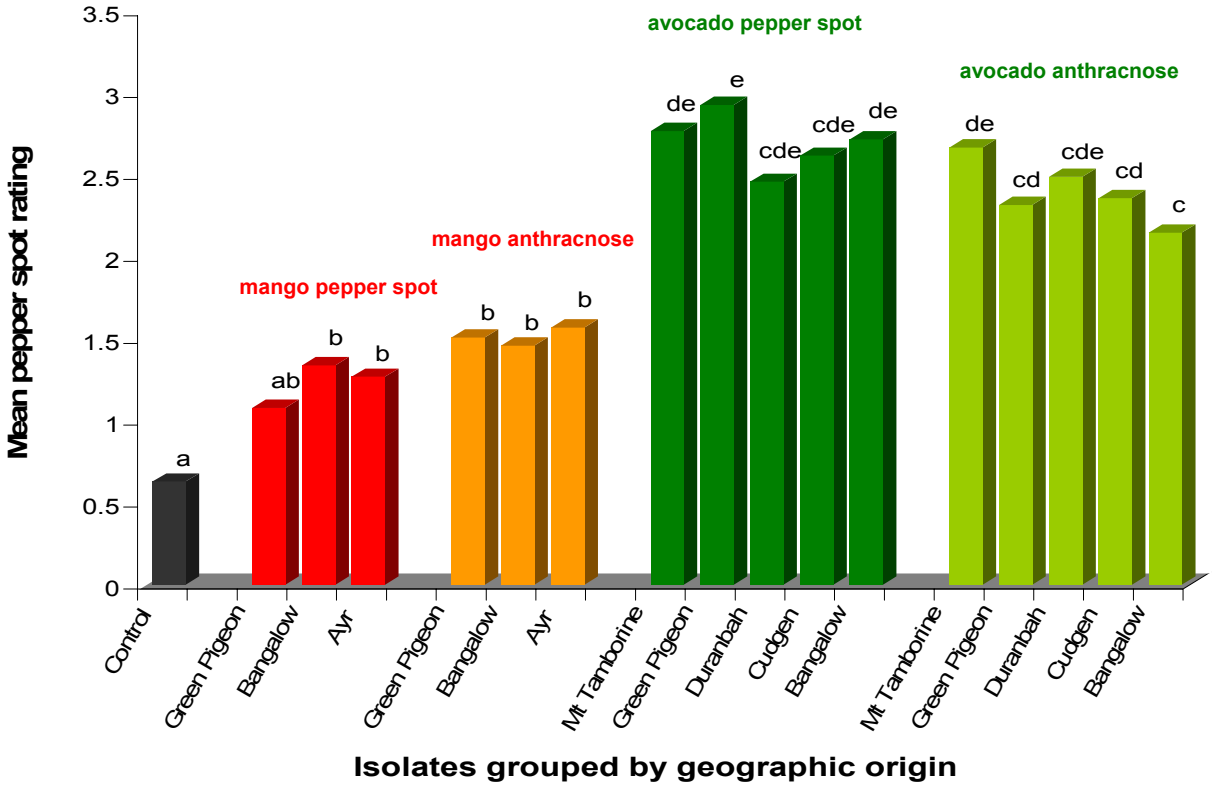


Figure 12: Mean pepper spot severity (n=25) 6 weeks after inoculation with avocado and mango *C. gloeosporioides* isolates on 'Hass' avocado fruit in the field at Duranbah

Table 55: Total mean pepper spot ratings on immature 'Hass' avocado fruit in the field 6 weeks after inoculation with avocado and mango isolates of *C. gloeosporioides*. Isolates are grouped according to fruit and symptom origin.

Origin of isolate	Mean* pepper spot rating
avocado anthracnose	2.40 b
avocado pepper spot	2.70 c
mango anthracnose	1.51 a
mango pepper spot	1.23 a

*mean has been adjusted to account for missing fruit

The mean pepper spot ratings for avocado isolates were significantly higher than for mango isolates (Table 55). The severity of pepper spot on fruit inoculated with avocado pepper spot isolates was significantly more severe than fruit inoculated with avocado anthracnose isolates (Table 55). However, there were no significant differences in pepper spot severity between mango pepper spot and mango anthracnose isolates. These trends were also observed on petioles of nursery trees in the glasshouse (Table 50).

C. Factors affecting pepper spot development

A. Effect of Colletotrichum gloeosporioides *inoculum concentration and fruit maturity on the development of pepper spot symptoms on avocado fruit and pedicels*

From October 2001, two pepper spot isolates (APT11 and APD11) were inoculated onto avocado fruit and pedicels in the field at 3 inoculum concentrations at 11 inoculation times (17.10.01, 30.10.01, 13.11.01, 27.11.01, 17.12.01, 15.01.02, 12.02.02, 12.03.02, 16.04.02, 14.05.02, 18.06.02). Fruit were inoculated at each of the 11 inoculation times with the selected isolate and spore concentration. At each inoculation time, 5 trees were selected and each treatment and control were inoculated onto 5 fruit each. Fruit were assessed for the presence or absence of pepper spot lesions from 2 weeks after inoculation, while pedicels were only assessed at the final 12 week assessment. Symptoms appeared as small, shiny, raised, black lesions measuring less than 0.5mm in diameter.

Fruit Assessments
The key findings from this experiment were as follows (Table 56):

- There were very low levels of pepper spot on a few fruit inoculated with water only.
- The higher concentrations of spore suspension resulted in more severe pepper spot.
- Regardless of fruit maturity when inoculated, pepper spot severity tended to increase slowly until 8 weeks after inoculation, then maintain that level of severity for the rest of the assessment period.
- Fruit inoculated in mid January and mid February consistently had more severe pepper spot than fruit inoculated at other times, regardless of spore concentration used. Conversely, fruit inoculated in June and December consistently had low pepper spot incidences.
- Comparison of the water-inoculated control with the isolates was always significant.
- Pepper spot ratings between isolates APD11 and APT11 was not significant for early inoculations, but became significant from December inoculation to March inoculation and then became non-significant again for late inoculations.
- There were always significant differences when comparing the 3 spore concentrations of isolates APD11 and APT11.
- The interaction between the 2 different isolates and spore concentration was not significant i.e. the 2 isolates behaved in the same way for each concentration.

The effect of the treatments and concentrations over time were as follows:

- The results of the water-inoculated control across time was significant compared to the mean of the isolates combined as would be expected. The control ratings remained low and fairly constant while the pepper spot symptoms increased over time.
- A comparison of the pepper spot ratings for isolates APD11 and APT11 across time was not significant for early inoculations but was significant for inoculation times from January to April.
- The resulting pepper spot ratings due to the three different spore concentrations across time was significant at every inoculation time (the 2 lower concentrations seemed to respond differently to the highest concentration).
- Looking at the interaction between the 2 isolates and 3 different concentrations across time, there were no significant results in any of the analyses.

Table 56: Differences of mean pepper spot data within and between variables of avocado **fruit** inoculated with various concentrations of suspensions of *Colletotrichum gloeosporioides* at 11 inoculation dates with assessment of fruit over 12 weeks

Inoculation date											
	Oct 17	**Oct 30**	**Nov 13**	**Nov 27**	**Dec 17**	**Jan 15**	**Feb 12**	**Mar 12**	**Apr 16**	**May 14**	**Jun 18**
Isolates x Control	<0.001 s	<0.001 s	0.014 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s
Isolate APD11 x Isolate APT11	0.191 ns	0.664 ns	0.788 ns	0.867 ns	0.045 s	<0.001 s	<0.001 s	0.011 s	0.183 ns	0.598 ns	0.425 ns
Concentration of inoculum	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s
Isolate x Time	0.096 ns	0.927 ns	0.541 ns	0.446 ns	0.577 ns	<0.001 s	0.01 s	0.046 s	0.017 s	0.188 ns	0.002 s
Concentration x Time	0.022 s	<0.001 s	0.004 s	<0.001 s	0.2 ns	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s
LSD across Concs*	0.3179	0.3989	0.6147	0.2968	0.2915	0.2552	0.2556	0.2402	0.2215	0.1577	0.134
LSD: Conc x Time**	0.2543	0.2771	0.6138	0.2372	0.2539	0.2442	0.2292	0.2101	0.1838	0.1064	0.0959
Isolate x Conc	0.325 ns	0.501 ns	0.556 ns	0.950 ns	0.962 ns	0.015 ns	0.101 ns	0.490 ns	0.534 ns	0.678 ns	0.048 s
Isolate x Conc x Time	0.105 Ns	0.725 ns	0.660 ns	0.712 ns	0.549 ns	0.615 ns	0.836 ns	0.572 ns	0.415 ns	0.378 ns	0.766 ns

's' is significant and 'ns' is not significant.
* LSD comparing means across all 3 concentrations of isolates across time. Therefore, compares lines on each graph (Figures 5.2 to 5.7) with each other .
** LSD comparing means within a concentration across time

Figure 13 reveals that there was some initial pepper spot naturally occurring on the fruit at very low levels of 0 to 0.5 but this remained fairly constant over the 12 week rating periods (staying below 1) with no exceptional changes for immature or mature fruit.

The series of graphs (Figures 14 to 19) show the mean pepper spot ratings of avocado fruit inoculated with the 3 concentrations (5 x 10^4, 5 x 10^6, 5 x 10^8 conidia/mL) of *C. gloeosporioides* spore suspensions of for the 2 isolates, APD11 and APT11 at the 11 inoculation dates with assessment of fruit over 12 weeks and pedicels at the final assessment time only.

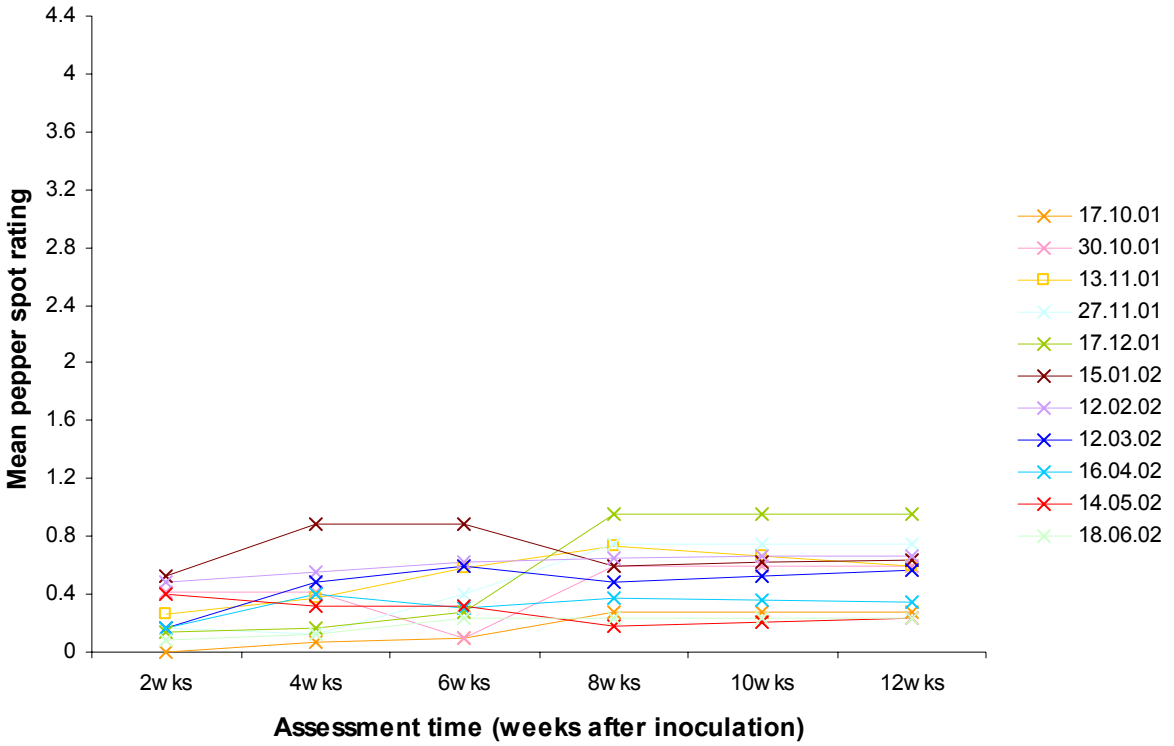


Figure 13: Mean pepper spot ratings of water-inoculated (**control**) avocado **fruit** at 11 inoculation dates (as shown in the legend) with assessment of fruit over 12 weeks

Two weeks after inoculation with *Colletotrichum gloeosporioides* at 5 x 10^4 conidia/mL (Figures 14 and 15), disease ratings were already slightly higher than the control fruit, but only achieved a maximum rating of about 1 by APD11. In most cases, ratings tended to increase or level out slightly over 12 weeks, regardless of when fruit were inoculated. Only fruit inoculated with isolate APD11 at 3 inoculation times (February, March and April) showed a negligible decline in pepper spot over the 12 weeks while fruit inoculated with isolate APT11 in January, April and May showed negligible declines in pepper spot over 12 weeks. Overall ratings were relatively low over the 12 week period, remaining below 1.3 for isolate APD11 and just on 1.6 for isolate APT11. Pepper spot ratings tended to reach a maximum by 8 weeks after inoculation after which time, ratings tended to remain constant.

Figures 16 and 17 show that the initial pepper spot ratings of fruit inoculated at 5 x 10^6 conidia/mL were only slightly higher than for the lower spore concentration, reaching only around 1.1 for both isolates at some inoculation times but only reaching a rating of 0.4 for both isolates APD11 and APT11 inoculated in December. Ratings for isolate APD11 only increased to a maximum rating of about 1.7 after 8 weeks, similar to the low spore

concentration ratings. Fruit inoculated with APT11, on the other hand, showed pepper spot symptom ratings up to almost 2.8 after 8 weeks. The notable inoculation time was January (mid summer) which increased rapidly from 6 to 8 weeks from about 1.4 to almost 2.8 for isolate APT11. Fruit inoculated in February showed the next highest pepper spot rating with a severity rating of around 2. Fruit inoculated in late October, February and April showed symptom declines from 8 to 12 weeks after inoculation with isolate APD11. For isolate APT11, pepper spot ratings for early October inoculations showed the most dramatic decline from just under 2 after 6 weeks to around 1.6 after 8 weeks. February, April and May inoculations for isolate APT11 showed negligible rating declines from 8 to 12 weeks.

Inoculation with spore suspensions of 5 x 10^8 conidia/mL (Figures 18 and 19) resulted in considerably higher initial pepper spot and most ratings tended to increase over time. After 2 weeks, pepper spot ratings ranged from about 1 to 1.7 for isolate APD11 and 0.9 to about 1.7 for isolate APT11. Inoculation of fruit in January (hottest and driest month of the year) followed by February resulted in outstanding increases in pepper spot over the 12 weeks for isolate APD11 with ratings of just over 3.2 and around 3 respectively. Both actually reached their rating peaks at 8 weeks and then plateaued for the rest of the assessment period. Similar results were recorded for isolate APT11 with fruit inoculated in January and February having the highest pepper spot severity. Fruit inoculated with APT11 in January showed an increase in pepper spot from a mean of about 1.7 after to 2 weeks to about 2.9 after 6 weeks followed by a rapid increase to about 4.1 at the 8 week assessment time. This rating then remained unchanged until the final assessment at 12 weeks. Fruit inoculated in February saw a fairly steady pepper spot increase from about 1 at the 2 week stage to about 3.1 after 6 weeks, 3.3 after 8 weeks and a negligible increase to the final 12 week assessment time.

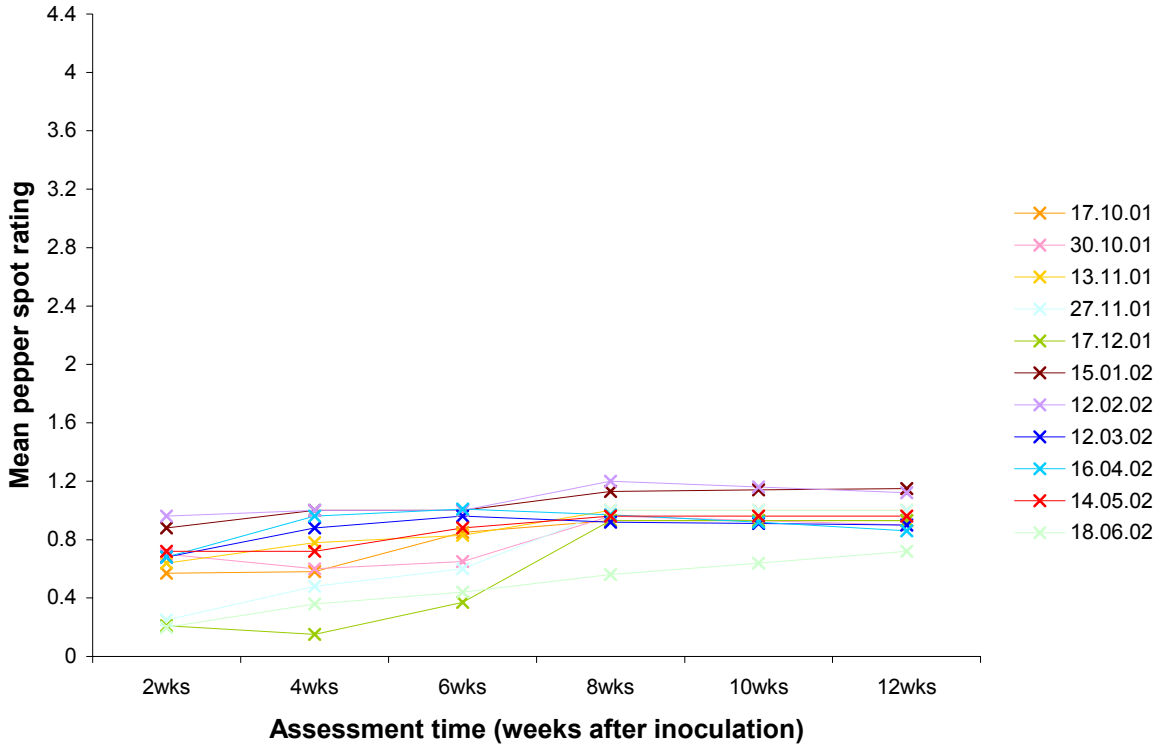


Figure 14: Mean pepper spot ratings of avocado **fruit** inoculated with 5 x **10^4** conidia/mL suspension of *Colletotrichum gloeosporioides* isolate **APD11** at 11 inoculation dates (as shown in the legend) with assessment of fruit over 12 weeks

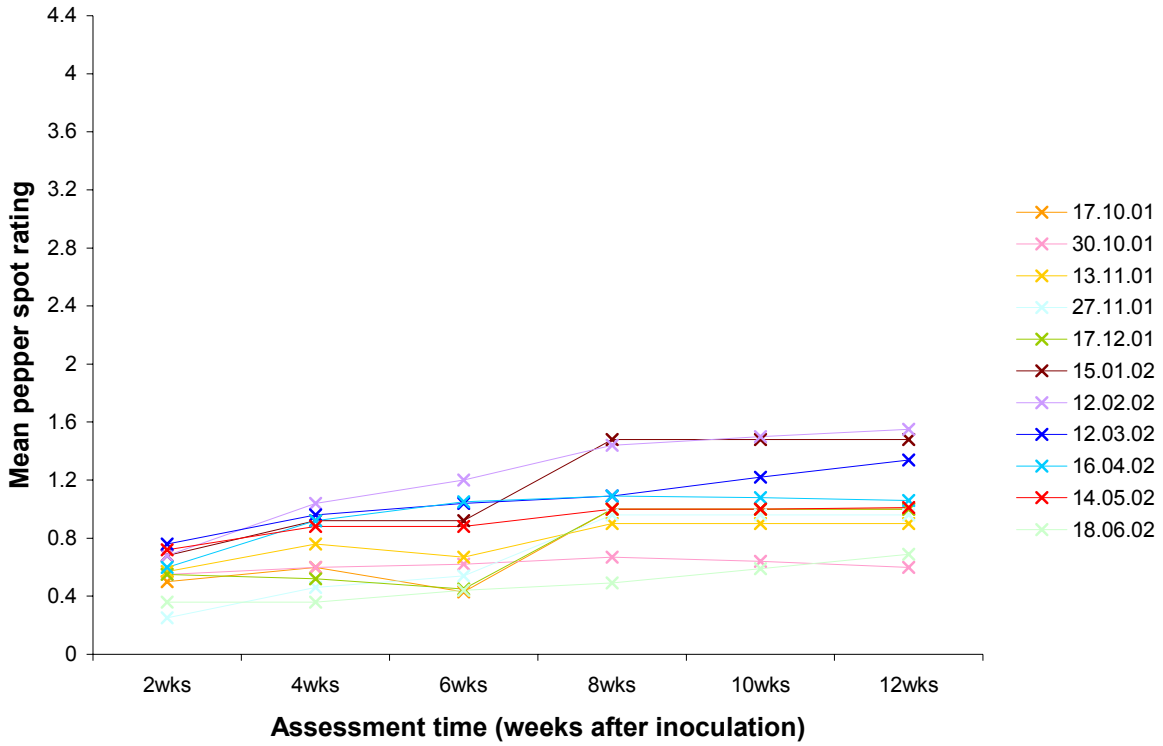


Figure 15: Mean pepper spot ratings of avocado **fruit** inoculated with 5 x $\mathbf{10^4}$ conidia/mL suspension of *Colletotrichum gloeosporioides* isolate **APT11** at 11 inoculation dates (as shown in the legend) with assessment of fruit over 12 weeks

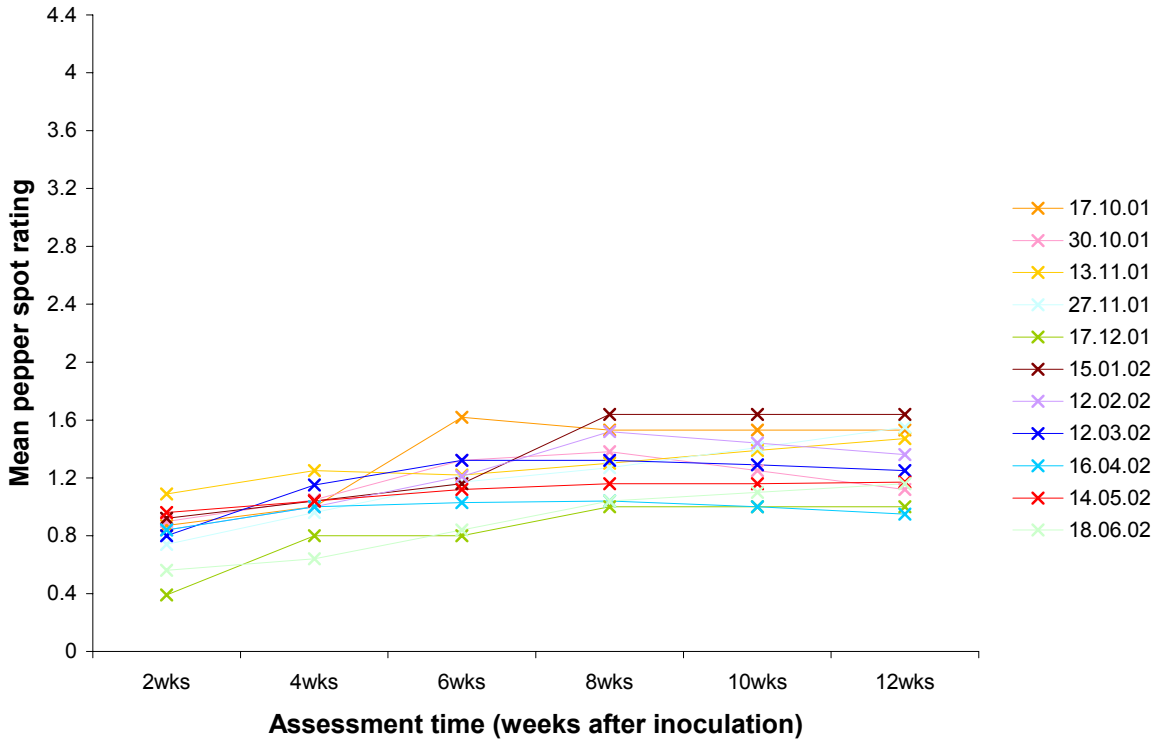


Figure 16: Mean pepper spot ratings of avocado **fruit** inoculated with 5 x $\mathbf{10^6}$ conidia/mL suspension of *Colletotrichum gloeosporioides* isolate **APD11** at 11 inoculation dates (as shown in the legend) with assessment of fruit over 12 weeks

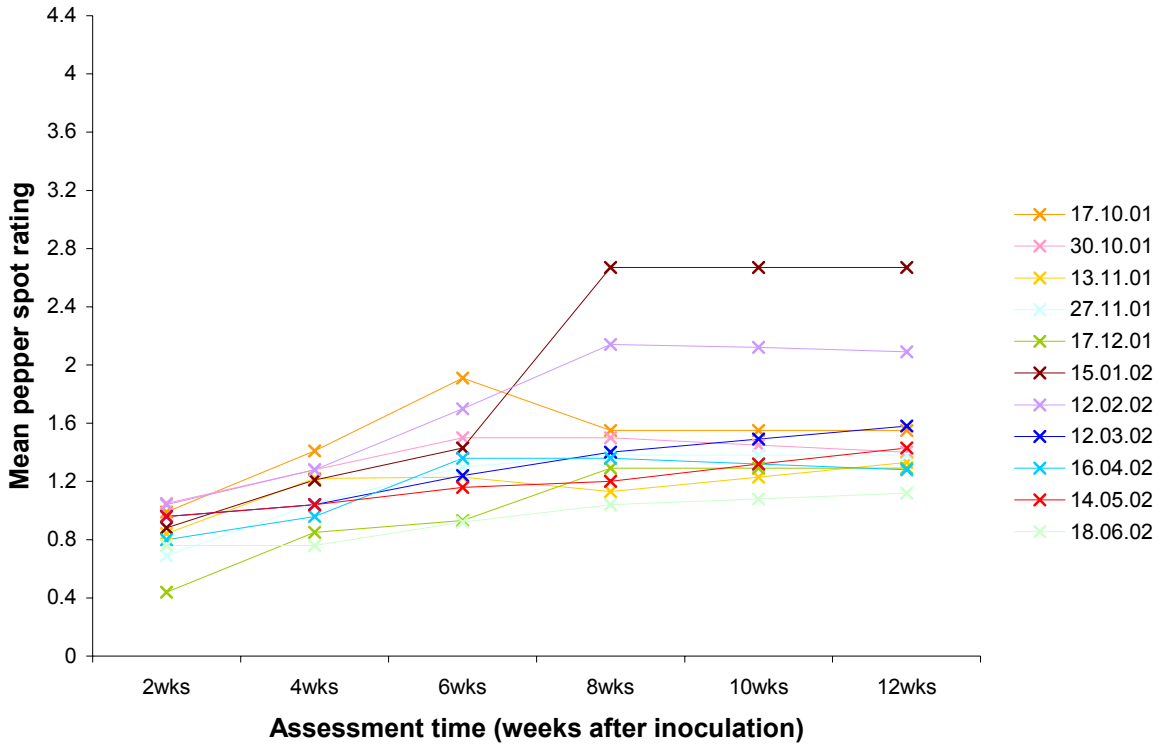


Figure 17: Mean pepper spot ratings of avocado **fruit** inoculated with 5 x $\mathbf{10^6}$ conidia/mL suspension of *Colletotrichum gloeosporioides* isolate **APT11** at 11 inoculation dates (as shown in the legend) with assessment of fruit over 12 weeks

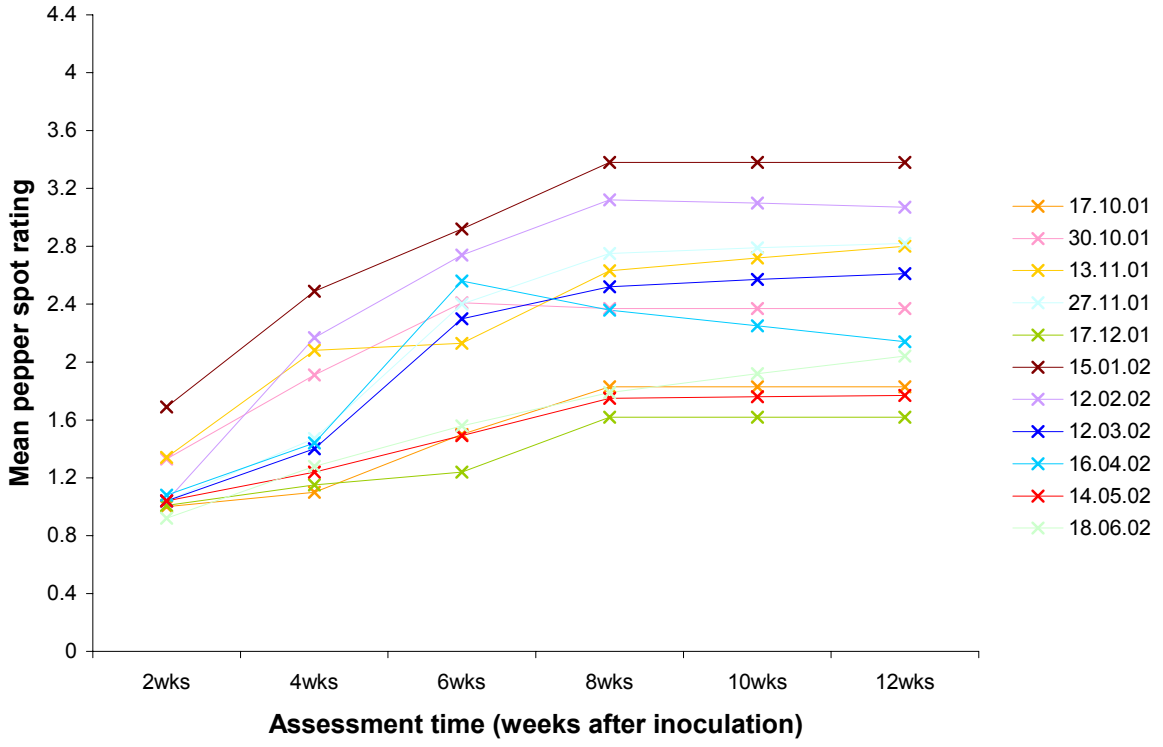


Figure 18: Mean pepper spot ratings of avocado **fruit** inoculated with 5 x $\mathbf{10^8}$ conidia/mL suspension of *Colletotrichum gloeosporioides* isolate **APD11** at 11 inoculation dates (as shown in the legend) with assessment of fruit over 12 weeks

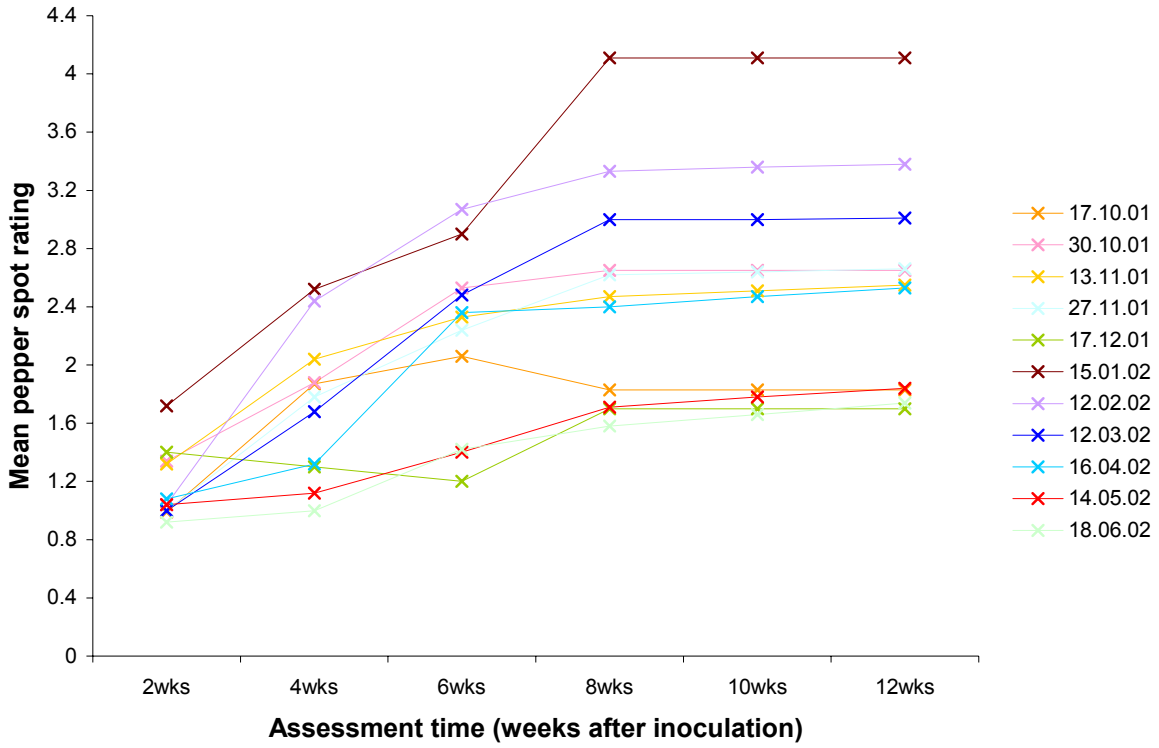


Figure 19: Mean pepper spot ratings of avocado **fruit** inoculated with 5 x **10^8** conidia/mL suspension of *Colletotrichum gloeosporioides* isolate **APT11** at 11 inoculation dates (as shown in the legend) with assessment of fruit over 12 weeks

Pedicels assessments

Fruit and their pedicels were inoculated as described and, while pepper spot ratings were recorded over 12 weeks for the fruit, disease ratings were only made for pedicels at the final 12 week assessment time (Plate 13). There were significant differences (Table 57) ($P<0.05$) between most of the 3 spore suspension concentrations at each of the inoculation times. Only on 3 occasions were there no significant differences, occurring between inoculum concentrations of 5 x 10^4 and 5 x 10^6 in December, March and April pedicel inoculations. The interaction between isolates and concentration was not significant not were there any significant differences between the 2 different isolates, APD11 and APT11.

Plate 13: Field inoculation of small fruit with pepper spot at a spore suspension concentration of 5 x 10^6 conidia/mL resulting in a rating of 5 on the pedicel.

Table 57: Differences of mean pepper spot data within and between variables of avocado **pedicels** inoculated with various concentrations of suspensions of *Colletotrichum gloeosporioides* at 11 inoculation dates with assessment of pedicels over 12 weeks

Inoculation date											
	Oct 17	**Oct 30**	**Nov 13**	**Nov 27**	**Dec 17**	**Jan 15**	**Feb 12**	**Mar 12**	**Apr 16**	**May 14**	**Jun 18**
Isolate APD11 x Isolate APT11	0.338 ns	0.652 ns	0.948 ns	0.391 ns	0.908 ns	0.024 s	0.359 ns	0.367 ns	0.948 ns	0.284 ns	0.045 s
Concentration	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	0.001 s	<0.001 s	<0.001 s	<0.001 s
Isolates x Conc	0.437 ns	0.106 ns	0.533 ns	0.909 ns	0.597 ns	0.493 ns	0.098 ns	0.695 ns	0.710 ns	0.127 ns	0.998 ns
Concentration 5 x 10^4 x Concentration 5 x 10^6	0.005 s	0.004 s	<0.001 s	<0.001 s	0.068 ns	<0.001 s	0.003 s	0.267 ns	0.068 ns	0.018 s	0.031 s
Concentration 5 x 10^4 x Concentration 5 x 10^8	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	0.002 s	<0.001 s	<0.001 s	<0.001 s
Concentration 5 x 10^6 x Concentration 5 x 10^8	0.002 s	<0.001 s	<0.001 s	<0.001 s	<0.001 s	0.011 s	<0.001 s	0.017 s	0.001 s	<0.001 s	0.004 s

's' is significant and 'ns' is not significant.

Graph patterns are similar for both isolates, APD11 and APT11 (Figures 20 and 21). Pedicel pepper spot ratings should correlate closely with fruit pepper spot ratings taken after 12 weeks. However, an important difference between fruit and pedicel data was that pedicels were subject to a condition known as 'ring neck'. This is a physiological disorder characterised by premature death of pedicel tissue and is partly attributed to water deficit in the tree during fruit development (Whiley *et al*. 1986). Therefore, data replicates for mid summer was reduced due to an inability to rate many of the pedicels due to their corky appearance (Plates 14 and 15).

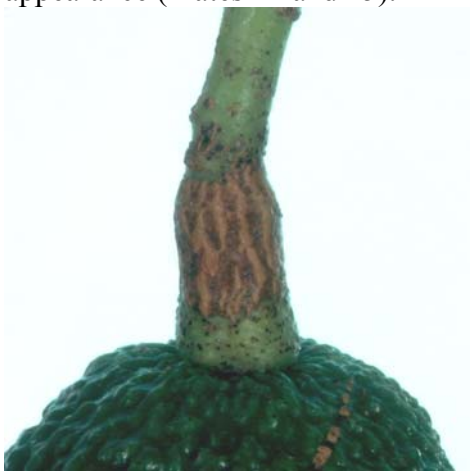


Plate 14: Early stages of 'ring neck' on a 'Hass' avocado pedicel inoculated with a pepper spot isolate. Note the formation of pepper spot symptoms below the corky area.

Plate 15: More severe 'ring neck' on a 'Hass' avocado pedicel inoculated with a pepper spot isolate. The pedicel is beginning to crack and pepper spot symptoms are no longer apparent

There were only 3 pedicels affected by 'ring neck' from the time of inoculation to the 12 week assessment time for pedicels inoculated from October through to December. Twelve weeks after pedicels were inoculated in mid January, there were 50 pedicels affected by 'ring neck' and, therefore, impossible to assess. Forty-eight pedicels were affected from the February inoculation, 31 for March, 21 for April, 19 for May and 23 for June. It might be speculated that fruit with pedicels most affected by 'ring neck' due to water stress might also be fruit (and pedicels) with the highest pepper spot ratings, but since these pedicels cannot be assessed, the comparison of fruit data with pedicel data does not necessarily correlate. Inoculation with isolate APD11 saw a dramatic peak in pepper spot severity for its February inoculation at 5 x 10^8 conidia/mL. Both isolates had suffered equal fruit losses due to ring neck and typical fruit fall, however, this loss of data could account for the irregularity.

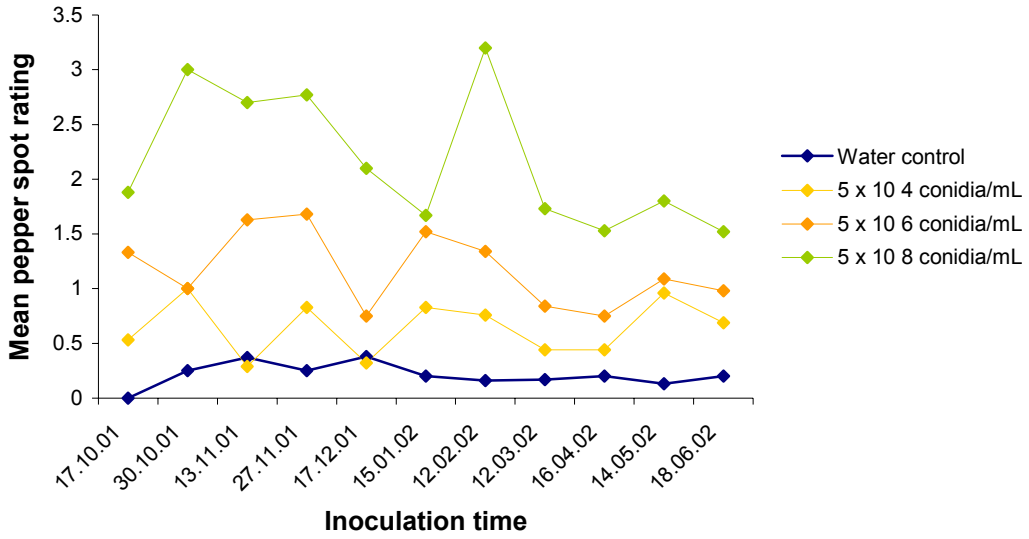


Figure 20: Mean pepper spot ratings of avocado **pedicels** inoculated at 11 separate times with 3 spore concentrations of *C. gloeosporioides* isolate **APD11** and a water control (as shown in the legend) with a single assessment after 12 weeks

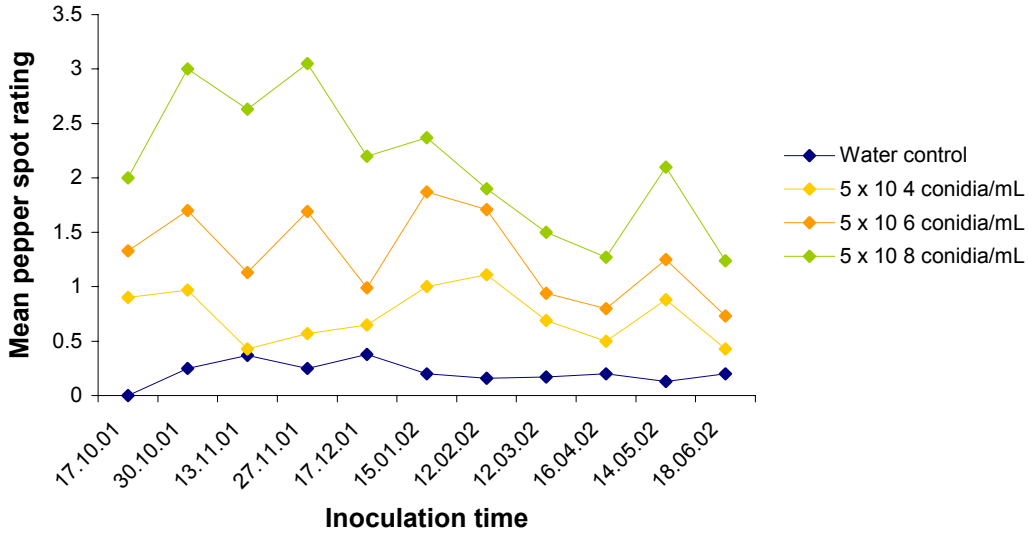


Figure 21: Mean pepper spot ratings of avocado **pedicels** inoculated at 11 separate times with 3 spore concentrations of *C. gloeosporioides* isolate **APT11** and a water control (as shown in the legend) with a single assessment after 12 weeks

Fruit and pedicels isolations

Inoculated fruit and their pedicels were harvested at the final assessment time and taken to the laboratory to re-isolate inoculated fungi and to account for any naturally occurring *C. gloeosporioides* isolates (there were no isolations from fruit from the final June inoculation). Four pepper spot lesions were taken from skin and pedicel of each fruit along with green flesh samples and grown on SPDA. For water-inoculated control fruit, any spots or green tissue was excised from the fruit and also grown on SPDA.

Overall, there were significant differences ($P<0.001$) between the water-inoculated control fruit isolations and isolations from pepper spots of the fruit and pedicels inoculated with fungal spore suspensions (as well as samples taken from green tissue of fruit). Natural

disease levels appeared to fluctuate as shown (Figures 22 and 23) by the water-inoculated control data. Data for the fruit inoculated at 5 x 10^4 conidia/mL also tended to fluctuate while medium and high inoculum concentration data (5 x 10^6 and 5 x 10^8 conidia/mL) remained fairly consistently high with isolation of *C. gloeosporioides* from lesions reaching almost to the maximum of 4 on some occasions.

There were no significant differences between the 2 isolates, APD11 and APT11, for isolations from fruit (P=0.306) (Figures 22 and 23), pedicels (P=0.767) (data not shown) or green tissue (P=0.283) (data not shown) nor were the interactions between isolate and inoculum concentration significant for isolations from fruit (P=0.824), pedicels (P=0.097) or green tissue (P=0.679). For fruit isolations, there was a significant difference between inoculum concentrations of 5 x 10^4 and 5 x 10^6 conidia/mL (P<0.001) as well as between concentrations of 5 x 10^4 and 5 x 10^8 conidia/mL (P<0.001). There were no significant differences between inoculum concentrations of 5 x 10^6 and 5 x 10^8 conidia/mL (P=0.253). For pedicel isolations, there were significant differences between all concentrations (P<0.001). For green tissue isolations, there were no significant differences between inoculum concentrations of 5 x 10^4 and 5 x 10^6 conidia/mL (P=0.132). There was, however, a significant difference between inoculum concentrations of 5 x 10^4 and 5 x 10^8 conidia/mL (P<0.001) as well as between concentrations of 5 x 10^6 and 5 x 10^8 conidia/mL (P=0.002).

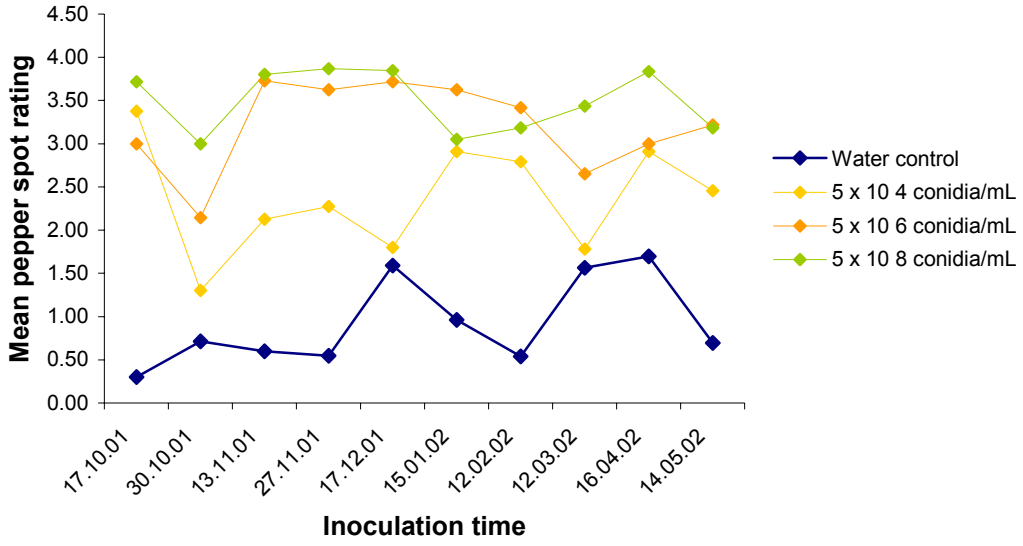


Figure 22: Isolation of *C. gloeosporioides* from pepper spot lesions of avocado **fruit** produced 12 weeks after inoculation with 3 concentrations of **APD11** and a water control (as shown in the legend) at 10 separate times of the year

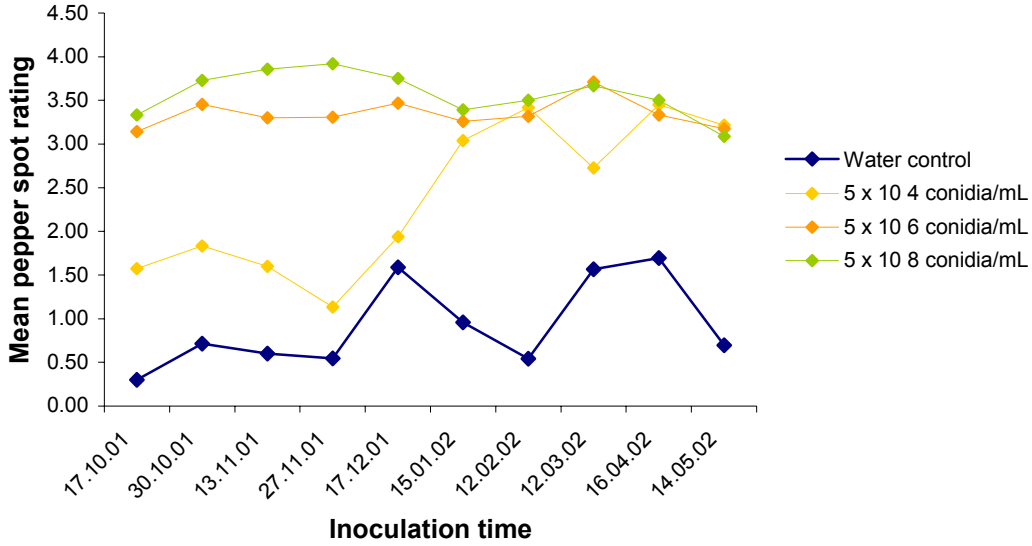


Figure 23: Isolation of *C. gloeosporioides* from pepper spot lesions of avocado **fruit** produced 12 weeks after inoculation with 3 concentrations of **APT11** and a water control (as shown in the legend) at 10 separate times of the year

Re-isolation of the fungus from fruit tended to stay relatively constant and this was especially evident in the results gained from isolate APT11 (Figure 23) at the medium and high inoculum concentrations. The recovery of the fungus from pepper spots inoculated early in the season with the lowest inoculum concentration (5 x 10^4 conidia/mL) tended to be the lowest for both isolates.

1. Inoculation of petioles of 'Hass' on several nursery avocado rootstocks in the glasshouse with lower concentrations of *Colletotrichum gloeosporioides* spore suspensions, and treatment of petioles with spore germinating fluid (SGF)

Petioles were inoculated on March 17. However, after 3 months (May 18th) no symptoms have been observed. It is possible this is because the trees are not stressed as problems visualising symptoms on healthy trees has been encountered before.

2. Measurement of pH of spore suspensions of *C. gloeosporioides*

As Table 58 shows, pH of spore suspensions tends to decrease as spore concentration increases, although there is only a consistent decline in pH for isolate APT11. pH for both isolates APC41 and APD11 tends to increase at a spore concentration of 5 x 10^4 conidia/mL. The pH of the sterile distilled water used in these experiments was 7.11.

B. Effect of nitrogen fertilisation and rootstock on avocado pepper spot disease susceptibility

1. Preliminary trial (2001-2002) to evaluate the effect of nitrogen fertiliser level on pepper spot development after *Colletotrichum gloeosporioides* inoculation on 'Hass' avocado fruit on two different rootstocks

This trial was carried out in early summer when fruit were 4-5cm in length. The presence or absence of pepper spot symptoms was assessed from 2 weeks after *C. gloeosporioides* inoculation and continued for 20 weeks (Figures 24 and 25). The 'Duke 6' rootstock trees

showed no significant interaction between nitrogen level and time (P=0.167) and no significant nitrogen level effect (P=0.172). There was, however, a significant time effect on the incidence of pepper spot (P<0.001) i.e. pepper spot became more severe with time after inoculation. The 'Velvick' rootstock trees showed no significant interaction between nitrogen level and time (P=0.147) and no significant nitrogen level effect (P=0.146). As for 'Duke 6' rootstock trees, there was a significant time effect. Some water-inoculated control fruit showed a pepper spot rating of 1 but the means only reached a maximum of 0.6 for nil nitrogen trees at the last 2 assessment times (data not shown). At the first assessment, all water-inoculated fruit were pepper spot free and by the second assessment, control fruit mean pepper spot rating was only 0.2.

In summary, although pepper spot ratings increased significantly over time, nitrogen fertilisation had no significant effect. Generally, however, for the higher nitrogen fertiliser application, the incidence of pepper spot tended to be lower for both rootstocks. In addition, there were no significant differences (P<0.05) between the 2 different rooststocks used in this experiment.

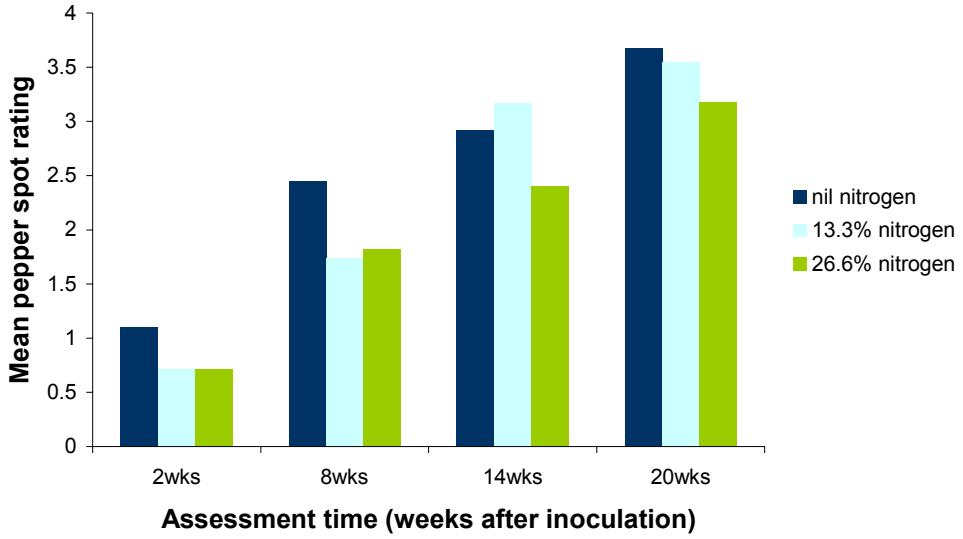


Figure 24: The effect of 3 nitrogen fertiliser levels and **'Duke 6'** rootstock on pepper spot severity due to *C. gloeosporioides* on 'Hass' avocado fruit (LSD shown in Table 14)

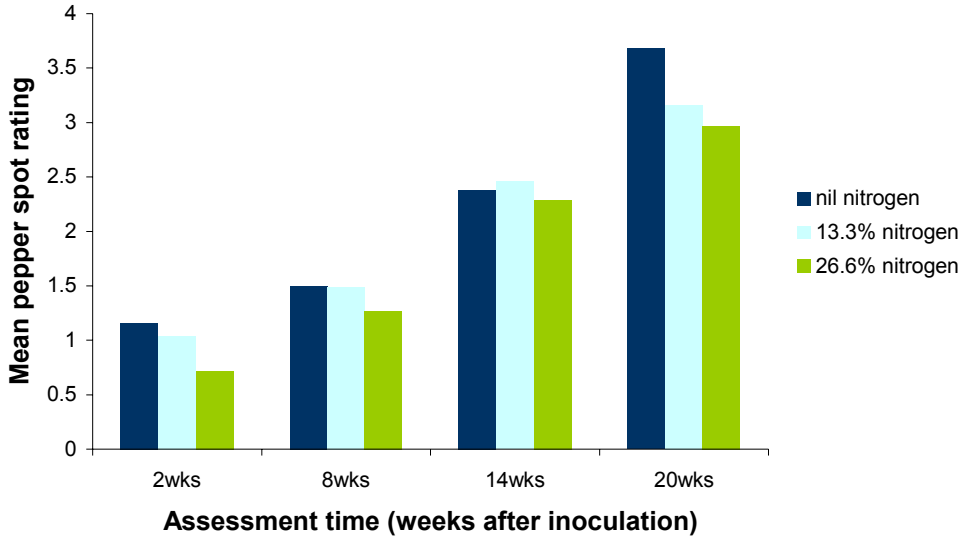


Figure 25: The effect of 3 nitrogen fertiliser levels and **'Velvick'** rootstock on pepper spot severity due to *C. gloeosporioides* on 'Hass' avocado fruit (LSD shown in Table 14)

When data was analysed for pepper spot severity for the 2 different rootstocks and the 3 different nitrogen levels at each different assessment time (Table 58), there were some significant differences at $P<0.05$ as indicated by the subscript. Fruit assessment after 2 weeks and 20 weeks, resulted in no significant differences between rootstocks 'Duke 6' and 'Velvick' and fertiliser levels had no effect on pepper spot rating either. After 8 weeks, pepper spot ratings for fruit from rootstock 'Velvick' at 26.6% are significantly lower than all 'Duke 6' fruit regardless of nitrogen level. Likewise, fruit from 'Duke 6' trees which had nil nitrogen fertiliser, showed significantly higher pepper spot severity than all other fruit. After 14 weeks, pepper spot ratings for fruit from rootstock 'Duke 6' at nil and 13.3% nitrogen were significantly different from 26.6% 'Duke 6' fruit all 'Velvick' fruit.

Table 58: The effect of rootstock and nitrogen level on mean pepper spot severity on 'Hass' avocado fruit at each time of assessment

		Assessment time			
Rootstock	**Fertiliser**	**2wks**	**8wks**	**14wks**	**20wks**
Duke 6	26.6% nitrogen	0.72	1.82 b*	2.40 a	3.58
Duke 6	13.3% nitrogen	0.72	1.74 b	3.17 b	3.52
Duke 6	nil nitrogen	1.10	2.45 c	2.92 b	3.75
Velvick	26.6% nitrogen	0.72	1.27 a	2.29 a	2.96
Velvick	13.3% nitrogen	1.04	1.49 ab	2.46 a	3.18
Velvick	nil nitrogen	1.16	1.50 ab	2.38 a	3.67
	LSD	0.190	0.343	0.300	0.347

*Means with the same subscript at each assessment time were not significantly different at a 5% level ($P<0.05$).

2. Trial to evaluate the effect of nitrogen fertiliser level on pepper spot on 'Hass' avocado fruit on two different race rootstocks (2002-2003)

Inoculations were carried out for this trial in January 2003 (mid summer) and March 2003 (early autumn). This trial was a split plot design with 2 treatment factors: nitrogen level (nil nitrogen and 26.6% nitrogen) and treatment (isolate inoculated and control) with each nitrogen plot (tree) split into two (one each for the inoculated and control treatments).

According to the statistical analysis of data from the January inoculations for the 'Duke 6' rootstock fruit (Figure 26), there were no significant differences due to nitrogen levels ($F_{1,8}$=0.09, P=0.777) and no significant differences in the interaction between nitrogen level and APD11 inoculation treatment (P=0.817). The pepper spot incidence due to APD11 treatment was significantly different to the water-inoculated control fruit (P<0.001). For the 'Velvick' rootstock fruit (Figure 27), there were also no significant differences due to nitrogen levels ($F_{1,8}$=1.76, P=0.221) and no significant differences in the interaction between nitrogen level and APD11 inoculation treatment (P=0.071). The pepper spot incidence on inoculated fruit was significantly different ($F_{1,8}$=44.56, P<0.001) to the water-inoculated control fruit.

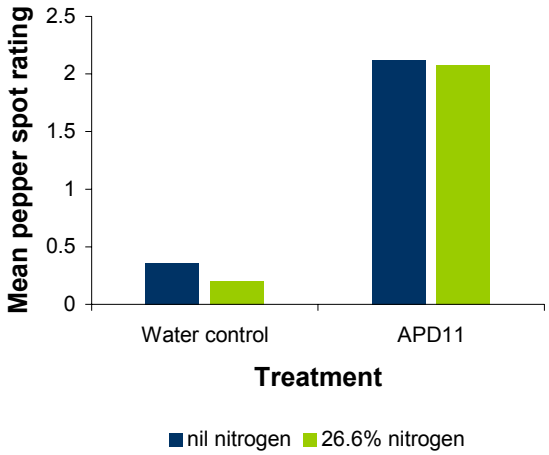


Figure 26: Effect of rootstock **'Duke 6'** and nitrogen level (nil or 26.6%) on development of pepper spot due to *C. gloeosporioides* in mid summer (**January** 2003)

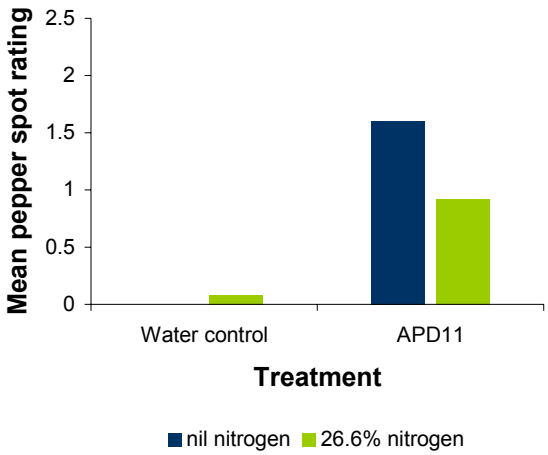


Figure 27: Effect of rootstock **'Velvick'** and nitrogen level (nil or 26.6%) on development of pepper spot due to *C. gloeosporioides* in mid summer (**January** 2003)

Figures 26 and 27 show that rootstock 'Duke 6' fruit tended to have more pepper spot than rootstock 'Velvick' fruit and the difference was significant (P<0.001). 'Duke 6' fruit had more pepper spot on the water-inoculated control fruit as well as on fruit inoculated with fungal conidia, regardless of nitrogen level than on 'Velvick' rootstock fruit. Overall, pepper spot levels tended to be lower with higher nitrogen level especially on 'Velvick' rootstock fruit.

As shown in Figures 28 and 29, fruit inoculated in autumn (March 2003) had a greater incidence of naturally occurring pepper spot than when fruit were inoculated in January. Most of the natural disease was on defined whole branches which increased the overall mean data. The data collected pepper spot severity for 'Duke 6' fruit inoculated with isolate APD11 was similar in both the January trial and the March trial for nil nitrogen trees. The same trend occurred for 26.6% nitrogen trees. For 'Velvick' fruit, there was little change from around 1.6 in pepper spot severity of fruit on nil nitrogen trees inoculated with isolate

APD11. There was, however, an increase in pepper spot severity of APD11-inoculated fruit from about 1 in January to almost 2 in March for 26.6% nitrogen trees.

Data analysis for autumn inoculations of the 'Duke 6' rootstock fruit (Figure 28) showed no significant differences due to nitrogen levels ($F_{1,8}$=0.23, P=0.644) and no significant differences in the interaction between nitrogen level and APD11 inoculation treatment (P=0.658). The pepper spot severity due to APD11 inoculation treatment was significantly different to the water-inoculated control fruit ($F_{1,8}$=14.66, P=0.005). For the 'Velvick' rootstock fruit (Figure 29), there were also no significant differences due to nitrogen levels ($F_{1,8}$=1.04, P=0.339) and no significant differences in the interaction between nitrogen level and APD11 inoculation treatment (P=0.572). Pepper spot ratings for APD11-inoculated fruit were significantly higher than for water-inoculated fruit ($F_{1,8}$=7.85, P<0.023). Differences recorded for pepper spot severity occurring on the 2 different rootstocks were not significant (P=0.572).

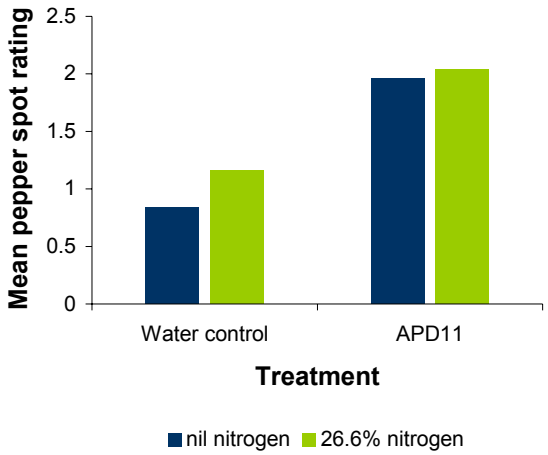


Figure 28: Effect of rootstock **'Duke 6'** and nitrogen level (nil or 26.6%) on development of pepper spot due to *C. gloeosporioides* in mid summer (**March** 2003)

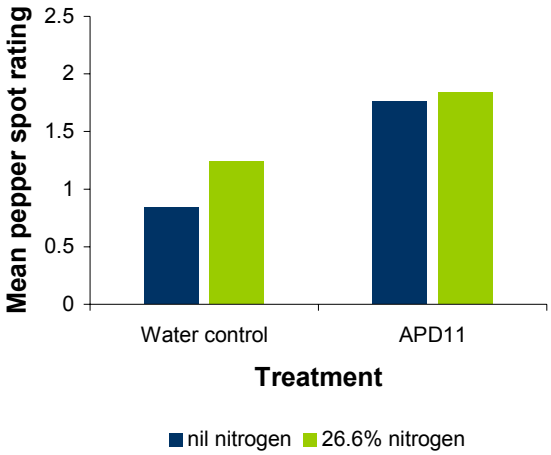


Figure 29: Effect of rootstock **'Velvick'** and nitrogen level (nil or 26.6%) on development of pepper spot due to *C. gloeosporioides* in mid summer (**March** 2003)

3. Effect of pepper spot infection following *C. gloeosporioides* inoculation on the subsequent development of postharvest anthracnose during fruit ripening

Fruit from inoculation trials described above were left on the trees until harvest in August 2003, at which time they were re-assessed for pepper spot severity. Fruit were ripened at 22^0C (65% RH), peeled at eating ripe stage, and assessed for anthracnose incidence. Anthracnose lesion severity was estimated as a percentage of fruit surface area affected by disease. Anthracnose symptoms appear as small, circular, brown lesions that enlarge and become sunken and black. The purpose of assessing for anthracnose disease was to gauge any possible effects of pepper spot inoculation on the potential for promoting resistance in the fruit against anthracnose. At the same time, it was necessary to determine if anthracnose could develop from pepper spot lesions after harvest.

Statistically there were no correlations between pepper spot incidence and anthracnose development and from the data (Table 59) it can be noted that 'Duke 6' rootstock control fruit had higher levels of anthracnose than 'Velvick' at both high nitrogen and nil nitrogen treatments. APD11-inoculated fruit generally had higher levels of anthracnose in addition to higher pepper spot severity.

Table 59: Anthracnose severity in avocado fruit (%) at eating ripe stage.

		Water-inoculated control fruit		**APD11-inoculated fruit**	
		Pepper spot rating	Anthracnose severity	Pepper spot rating	Anthracnose severity
Velvick	***Hi N***	***1.5***	13.3	2.17	21.3
Velvick	***Nil N***	***1.3***	6.47	1.92	9.6
Duke 6	***Hi N***	***1.52***	16.29	2.2	33.1
Duke 6	***Nil N***	***0.96***	15.25	2.48	14.8

pepper spot rating out of possible maximum 5, anthracnose severity as %
NB. Fruit were re-assessed for pepper spot at time of harvest and, therefore, differ slightly from March ratings.

C. Effect of avocado rootstock cultivars Anderson 8 (Guatemalan), Anderson 10 (Guatemalan), Nabal (Guatemalan) and Parida 1 (Mexican) rootstocks on pepper spot disease susceptibility

1. Field inoculation

Fruit inoculations for this trial were carried out in January and then again in March 2003 on 4 different rootstocks. The analysis was done as a completely randomised design. Although the data for water-inoculated control fruit is not included in the graph (Figure 30), there was virtually no natural pepper spot on any fruit in January. In March, the presence of natural pepper spot was negligible; 'Parida 1' had a water-inoculated control fruit rating of 0.2, 'Anderson 8' ('A8') and 'Anderson 10' ('A10') rated 0.3 and 'Nabal' rated 0.8.

For the mid summer inoculation data, there was a significant rootstock by APD11 inoculation treatment interaction ($F_{3,32}$=4.09, P=0.014). There was a significant difference between rootstocks ($F_{3,32}$=4.70, P=0.008) and there were significant differences in pepper spot severity between the water-inoculated control fruit and APD11-inoculated fruit for each of the rootstocks ($F_{1,32}$=212.73, P<0.001). As expected, there were no significant differences

between the water-inoculated control fruit for the different rootstocks. Rootstock 'Parida 1' had a significantly higher average rating than the other 3 rootstocks which did not differ from each other. For the autumn inoculation data, the interaction between rootstock and APD11 inoculation treatment was non-significant ($F_{3,32}$=0.34, P=0.798). There was also no significant difference between rootstocks ($F_{3,32}$=2.58, P=0.071). However, the pepper spot rating difference between the water-inoculated control fruit and APD11 inoculation treatment fruit was significant ($F_{1,32}$=87.52, P<0.001).

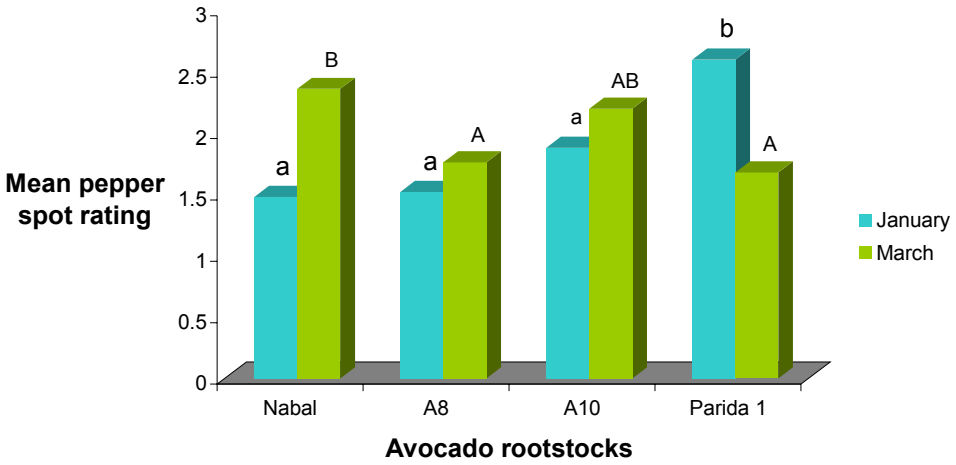


Figure 30: Effect of 'Hass' grafted onto different avocado rootstocks on the severity of pepper spot due to *C. gloeosporioides* inoculations in mid summer (January: LSD=0.500) and early autumn (March: LSD=0.529) [(means with the same subscript were not significantly different (excluding control data) at a 5% level (P<0.05)]

2. Effect of avocado rootstock on mineral concentrations of leaves and the relationship with pepper spot disease incidence and severity after inoculation with *C. gloeosporioides* on 'Hass' avocado fruit

Analysing the data as a completely randomised design, significant differences amongst rootstocks were found for B (P=0.042), Cu (P=0.015), Fe (P=0.030) and P (P=0.009) levels in leaves (Table 60). Rootstock 'A8' had a very high level of copper in the leaves compared with other rootstock leaves and rootstock 'Nabal' had a very high iron level in its leaves. Mineral content data was also obtained for fruit skin taken from all the trees at the site which included 10 trees of each rootstock. There were no significant differences of minerals among any of the rootstocks except for phosphorous, which had a lower level in rootstock 'A8' fruit. Additional data also revealed that, for the same year, there were no significant differences among rootstocks for anthracnose incidence or severity nor were there significant differences on crop load and fruit size. On comparing the fruit skin mineral data and anthracnose disease data with results from pepper spot rating data (not statistically), on a percentage of the same trees, there were no correlations.

Table 60: Mineral analysis of leaves of 'A8', 'A10', 'Nabal' and 'Parida 1' rootstocks in May 2003

Mineral	units	P	LSD	Rootstock			
				'A8'	'A10'	'Parida 1'	'Nabal'
B	mg/g	0.042	6.633	28.8 bc*	21.8 a	22.2 ab	29.4 c
Cu	mg/kg	0.015	25.99	50.34 b	13.66 a	8.22 a	19.10 a
Fe	mg/kg	0.030	28.67	98.2 a	97.6 a	98.2 a	135.6 b
P	%	0.009	0.0159	0.19 bc	0.2 c	0.18 ab	0.17 a
Mg	%	0.311	0.0797	0.43	0.41	0.36	0.42
Ca	%	0.176	0.3998	1.50	1.75	1.33	1.62
K	%	0.972	0.2638	0.91	0.96	0.94	0.91
Mn	mg/kg	0.181	230.8	536	326	456	556
N	%	0.175	0.325	2.76	2.98	2.96	2.68
S	%	0.320	0.0591	0.26	0.27	0.26	0.31
Zn	mg/kg	0.406	7.62	35.4	33.0	36.6	39.2
Mg+Ca/K	ratio	0.873	0.938	2.18	2.35	2.01	2.29
N/Ca	ratio	0.117	0.5017	1.85	1.78	2.27	1.72

*Means with the same subscript (in green) within each rootstock were not significantly different at a 5% level ($P<0.05$).

3. Effect of pepper spot infection following *C. gloeosporioides* inoculation on the subsequent development of postharvest anthracnose during fruit ripening

Fruit from inoculation trials described above were left on the trees until harvest in August 2003. Fruit were harvested, assessed for pepper spot severity, ripened at 22^0C (65% RH) and peeled at eating ripe stage. Ripe fruit were assessed then for anthracnose incidence. Anthracnose lesion severity was estimated as a percentage of fruit surface area affected by disease. Anthracnose symptoms appear as small, circular, brown lesions that enlarge and become sunken and black.

Statistically there were no correlations between pepper spot and anthracnose and from the data (Table 61) it can be noted that both 'A10' and 'Parida 1' rootstocks have more severe anthracnose and pepper spot than 'Nabal' and 'A8' rootstocks. According to mineral analyses, both rootstocks had lower levels of boron and copper. Anthracnose was more severe in APD11-inoculated fruit than water-inoculated control fruit except for rootstock 'Nabal'. This may suggest that some of the inoculations may have formed quiescent infections that resumed development after harvest.

Table 61: Anthracnose severity on fruit infected with pepper spot harvested from various rootstocks

	Water-inoculated control fruit		APD11-inoculated fruit	
	Pepper spot rating	*Anthracnose severity*	Pepper spot rating	Anthracnose severity
Nabal	0.66	31.39	2.6	22.7
A8	0.32	12.5	2.6	33.5
A10	0.3	60.8	3.66	72.2
Parida 1	0.81	69.8	3.56	83.5

pepper spot rating out of maximum possible 5, anthracnose severity as %

D. Effect of skin pH on the susceptibility of 'Hass' avocado to pepper spot disease after inoculation with Colletotrichum gloeosporioides

This trial commenced in November 2002 on a block of 4 year old 'Hass' avocado fruit on cv. Edranol trees, using 2 pepper spot isolates (APT11 and APD11) at an inoculum concentration of 5 x 10^6 conidia/mL at 7 monthly inoculation times from November through to May 2003. Inoculated fruit were harvested 2 weeks after inoculation and pH measurements of fruit mesocarp tissue were recorded.

In this analysis over time, there was a significant effect due to inoculation time (P<0.001) (Figure 31). When analysing the data at each inoculation time the trial design was set up as a randomised block design with three treatments (isolates + water-inoculated control) and five replicates (trees). The mean pH of the three readings for each fruit was used. There were significant differences between the treatments at only one inoculation time (30 Jan 2003; P=0.018) with the water-inoculated control fruit having a significantly higher pH than either APD11 or AAD11 inoculated fruit. Means with the same letter (Figure 31) were not significantly different at the 5% level (LSD=0.150) which indicates that there were significant differences in the changes in pH across inoculation dates. This is true for both isolate inoculation treatments and the water-inoculation control. There was no significant interaction between isolate and inoculation time (P=0.872) nor was there a significant isolate effect (P=0.786).

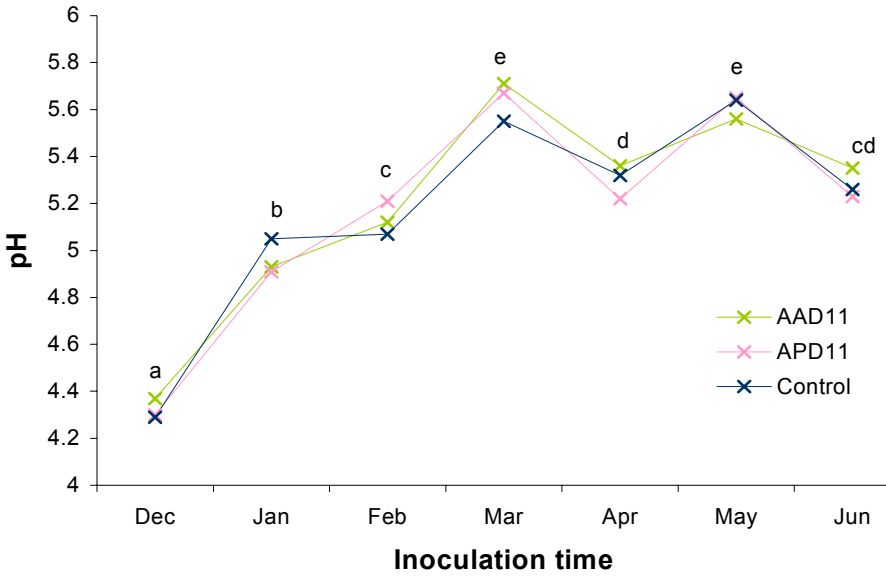


Figure 31: Mean pH of avocado mesocarp tissue of 'Hass' fruit from December to June [means with the same subscript across time (with both isolate and water-inoculated control means combined) are not significantly different at the 5% level (LSD=0.150)]

Because there was a distinct (rather than random) pattern in the selection of it was of concern to the biometrician that changes in pH observed over time might actually be a function of the position of the inoculated trees in the field rather than a time effect. A uniformity trial was done in March 2004 whereby fruit from all of the trees used previously was harvested at one time and their pH measured. Figure 32 represents the results and indicates that on a single day in December, all fruit tended to have a fairly constant pH of approximately 6, which confirms that all trees used in the previous pH experiment were uniform. Interestingly, in the previous year, at this time the pH was approximately 4.3. Avocado trees tend to crop every 2

years and there was very little fruit available for the second experiment. Hence, the metabolism of the tree, and consequently the fruit pH, may be quite different in this year.

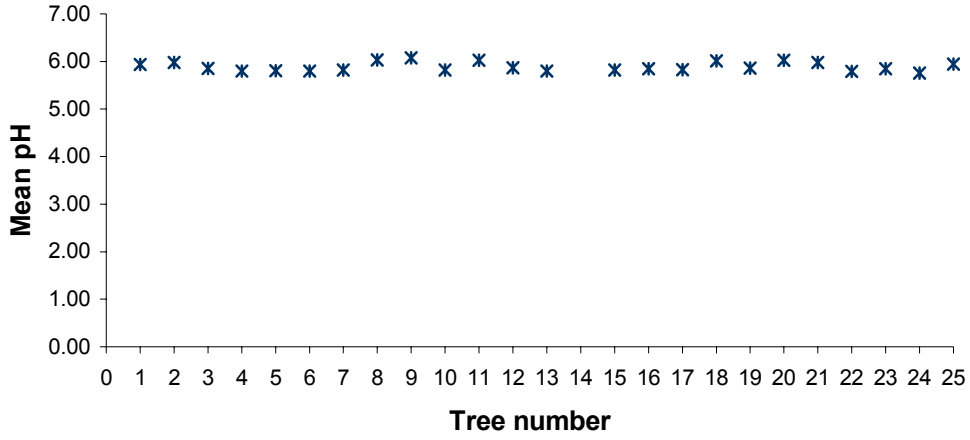


Figure 32: Mean pH of fruit from trees in December 2003 (no fruit on Tree 14)

As Table 62 shows, pH of spore suspensions tends to decrease as spore concentration increases, although there is only a consistent decline in pH for isolate APT11. pH for both isolates APC41 and APD11 tends to increase at a spore concentration of 5 x 10^4. The pH of the sterile distilled water used in these experiments was 7.11.

Table 62: pH measurements of spore suspensions of *C. gloeosporioides* prepared at various concentrations

Spore concentration/mL	APC41	APD11	APT11
5 x 10^2	6.24	6.71	7.65
5 x 10^3	6.37	6.84	7.03
5 x 10^4	6.63	6.98	6.56
5 x 10^5	6.17	6.74	6.35
7 x 10^5	-	-	6.33
9 x 10^5	6.25	-	-
2.5 x 10^6	-	6.79	-
5 x 10^6	6.16	6.27	6.23
5 x 10^8	5.76	6.40	6.20

F. Histopathology of Colletotrichum gloeosporioides *causing pepper spot infection on avocado fruit*

1. Field inoculation

For this trial, 4 month old fruit were inoculated with spore suspensions of pepper spot isolates of *C. gloeosporioides* at 1 x 10^8 conidia/mL and were harvested after 24 h, 48 h, 72 h and 7 days and transported to the laboratory.

2. Light Microscopy (LM)

A section through the avocado peel at 4 months after fruit set (fruit are ca. 40mm in length) shows the structure of the outer layers and the cells beneath. Fruit grow rapidly at this stage so some variability in anatomy is to be expected. The outer layer of the exocarp (peel) is a wax layer which can be difficult to detect after the sample fixation process. The wax layer protects the cuticle (3-6µm thick). Below the cuticle is a layer of epidermal cells. Immature fruit have a single layer of elongated epidermal cells, but as fruit matures and cell division occurs, often at uneven rates, these cells become irregular in appearance. Epidermal cells contain cytoplasm, vacuoles, phenolic compounds. There are 1-3 layers of hypodermal cells below the epidermis and beneath these are several layers of parenchyma cells and a layer of sclerenchyma or stone cells limiting the inner surface of the peel. The parenchyma cells contain chloroplasts, tannin and some oil. Throughout the mesocarp tissue there are specialised oil cells. The oil cells, or idioblasts, are distinguished by their large size and lignified walls. The mesocarp is completely permeated with conductive tissue, the vascular system. A thin layer next to the seed coat is the endocarp (Biale and Young 1971).

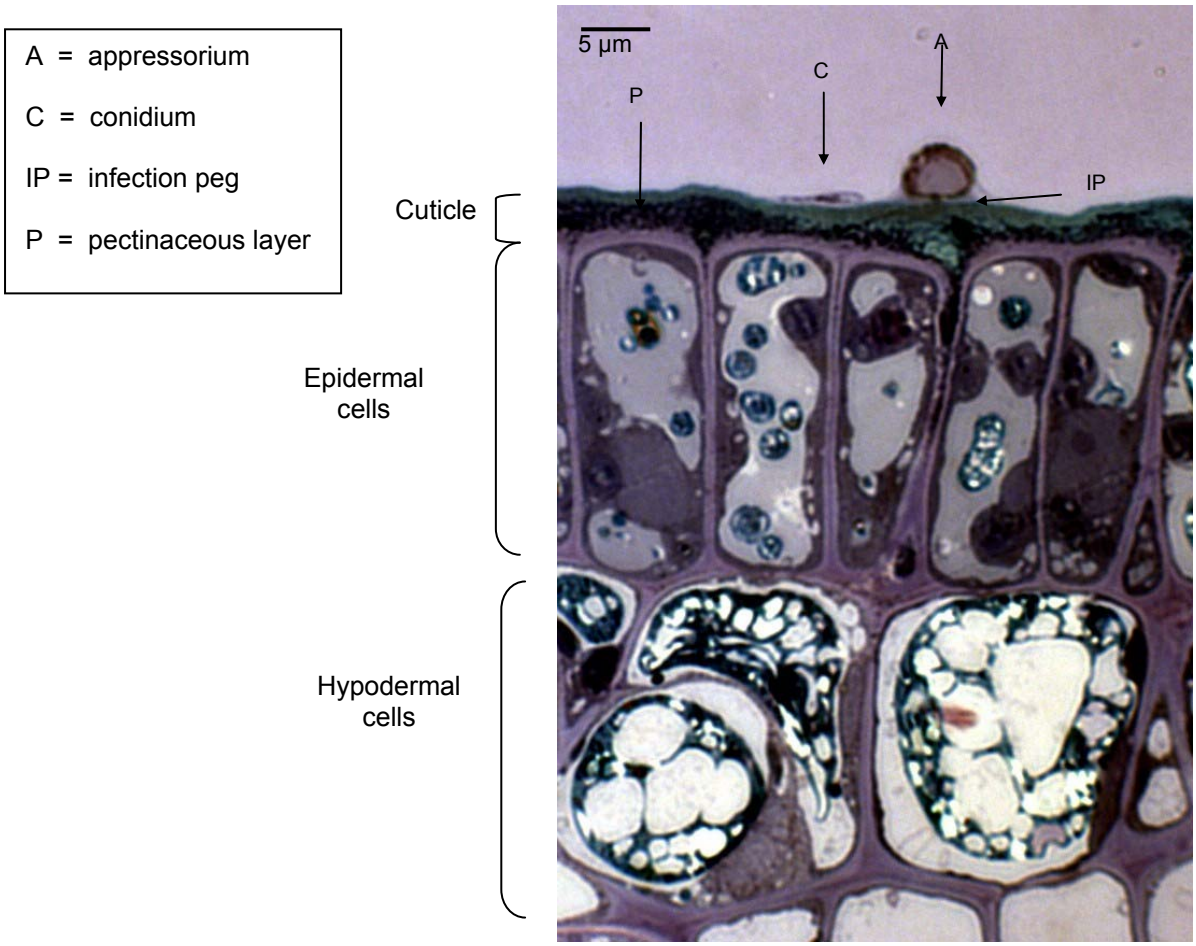


Plate 16: Conidium and appressorium on the surface of avocado fruit 7 days after inoculation with a pepper spot isolate of *Colletotrichum gloeosporioides* (wax layer not present on this sample)

Both light microscopy and transmission electron microscopy of samples taken from 24 hrs up to 7 days after fungal inoculation, determined that the fungus penetrated no further than the cuticle. This was concluded after examining approximately 500 sites of penetration.

Plate 16 shows the conidium on the surface of the avocado peel 7 days after inoculation onto the fruit surface. From the conidium via a germ tube, an appressorium has developed and an infection peg is penetrating into the cuticle of the fruit. Samples examined both earlier and later than 7 days (from preliminary work) also concluded that no further penetration was achieved by the infection peg.

3. Transmission Electron Microscopy (TEM)

More detail is shown using TEM (Plate 17) to observe a section through an appressorium. The embedding process causes distortion of the appressorium but the infection peg can be clearly seen penetrating into the cuticle and no further 7 days after inoculation with *Colletotrichum gloeosporioides*.

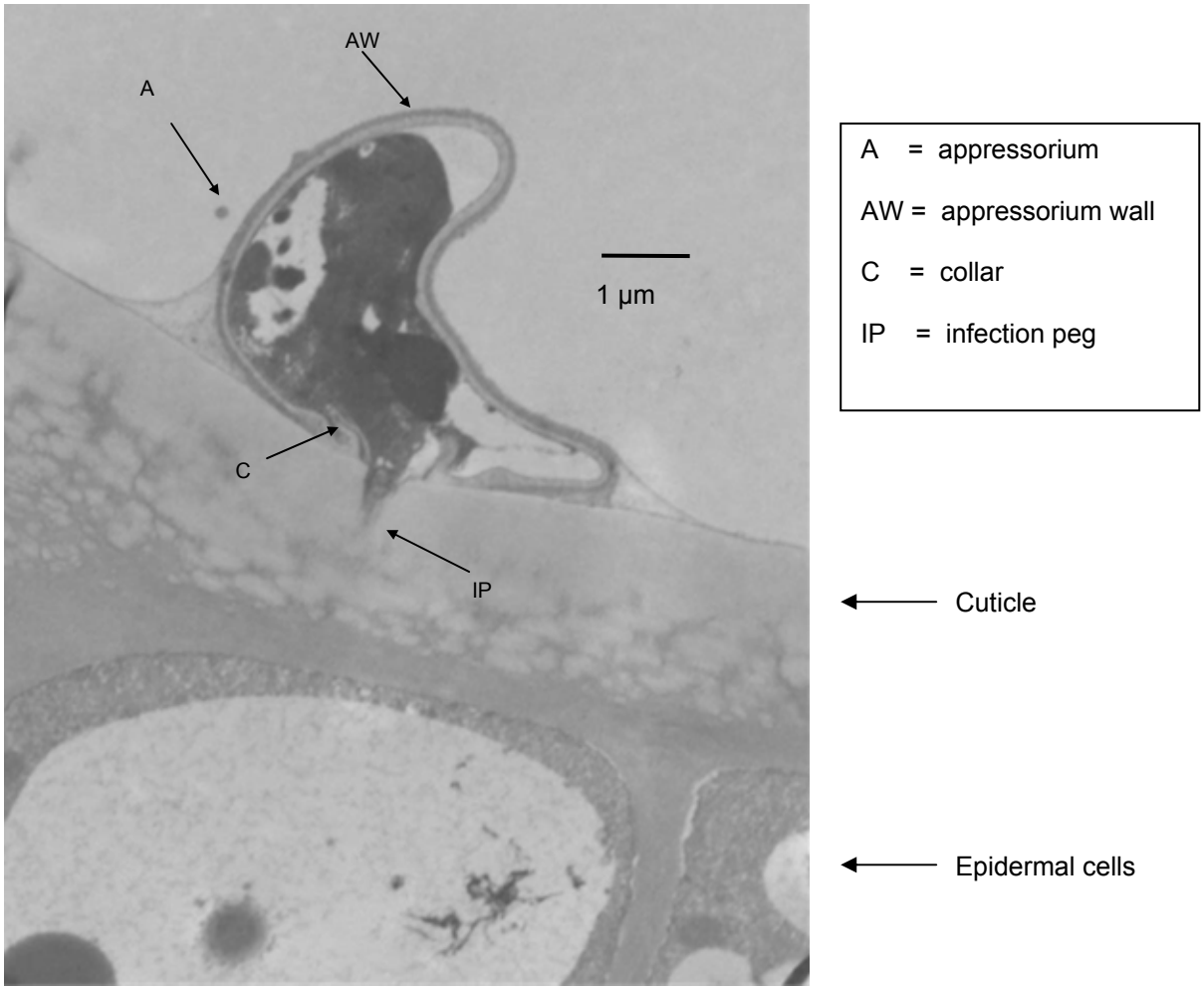


Plate 17: TEM shows penetration of the infection peg less than 1µm into the cuticle 7 days after inoculation of fruit with *Colletotrichum gloeosporioides*

3.5 Cross-Protection Studies

A. Root dip experiment

C. gloeosporioides was capable of systemically colonising root and stem tissue of avocado seedlings as the fungus was able to be isolated from stem pieces at 1 cm from the hypocotyl.

B. Seedling transmission experiment

The fungus was only occasionally detected at the hypocotyl, indicating poor transmission of the fungus endophytically from the fruit to the seed to the seedling in this experiment. Problems were encountered with seedling growth and it would be worthwhile to repeat this experiment in the future.

4. DISCUSSION

Rootstock and Nutrition Studies

Tree phenology, as expected, was influenced by nitrogen fertilisation. Withholding nitrogen fertiliser resulted in tree canopies that were paler green in colour and with fewer vegetative flushing terminals.

Withholding nitrogen fertiliser also resulted in small but significant decreases in leaf N and Ca concentrations. The difference in leaf Ca concentration was most likely due to fewer vegetative flushing terminals occurring on these trees. The reduced number of flushing terminals would reduce the sink strength of the terminals and thus draw less Ca. Nitrogen fertiliser form (ammonium vs nitrate) however, did not significantly affect tree phenology, crop load, mineral concentration or disease levels.

While there were no significant fertiliser effects on fruit shelf life or disease there was trend evident for the nil nitrogen treatment to have lower anthracnose levels. This corresponds with a reduced N concentration in the skin tissue and more favourable balances of N/Ca (lower) and Ca+Mg/K (higher).

Rootstock had a significant effect on vegetative flushing, canopy colour and crop load. This effect was most likely due to inherent differences in assimilate partitioning between the rootstocks. 'Velvick' maintained a larger crop load than 'Duke 6' throughout the growing season and as a consequence appeared to also have fewer vegetative terminals flushing. This more favourable leaf to fruit ratio may also explain why 'Velvick' trees had higher concentrations of Ca and a lower concentrations of N in the leaves and therefore more favourable balances of N/Ca (lower) and Ca+Mg/K (higher). Fruit skin tissue also had more favourable balances of Ca+Mg/K. In turn, the better balance of minerals in the West Indian 'Velvick' rootstock trees may explain why there was less postharvest anthracnose compared with fruit from the Mexican 'Duke 6' rootstock trees. This rootstock effect, however, was lost in the second season, possibly due to the lack of significant differences in fruit skin mineral concentrations and the very high disease incidences (>75%). Another possible explanation is that, as the trees age, their natural ability to defend themselves may decline and thus we would start to see smaller differences between rootstocks in disease resistance. Our earlier study (Willingham et al. 2001) supports this theory as we found different aged rootstock material to have different concentrations of antifungal dienes. Ungrafted 'Velvick' trees were found to have diene concentrations 10 times higher than ungrafted 'Duke 6' in the nursery while 3½ year old 'Hass' trees on 'Velvick' were found to have diene concentrations 3 times higher than 'Duke 6' but 8 year old 'Hass' trees on 'Velvick' were found to have diene concentrations only 1.5 times higher than 'Duke 6'.

Fruit skin pH levels did not reveal significant rootstock differences. However, withholding nitrogen fertiliser did result in a significantly lower fruit skin pH level in the second season experiment which correlates with these fruit tending to have less postharvest anthracnose. Fruit skin pH was also shown to significantly increase during ripening in 'Hass' which confirms the Israeli research conducted on other varieties (Yakoby et al. 2000).

In the other rootstock study, the Guatemalan ('A8', 'A10', 'Nabal') rootstocks were also found to be superior for disease control compared with the Mexican ('Parida 1') rootstock. However, rootstock effects were not apparent in the last two seasons, possibly due to heavier

crop loads on 'Parida 1'. The increased crop load on 'Parida 1' would theoretically improve the leaf to fruit ratio and thus the nutrient partitioning between the leaves and fruit. This means the fruit would have a better balance of N and Ca which we have shown to be important for anthracnose susceptibility.

In both rootstock studies, strong correlations between disease levels and mineral nutrient concentrations were evident. This indicates that while treatment differences between the different nitrogen fertiliser regimes or rootstocks may not always be significant, the balance of nutrients in the tree still has an important predictable impact on postharvest disease levels.

New Product Studies

The currently most commonly used copper formulations by the avocado industry, namely copper oxychloride and copper hydroxide (Kocide®), were found to provide the same level of disease control as any of the new formulations tested, with the exception of Kocide® Liquid Blue which was inferior for anthracnose disease control. The phytotoxicity experiments demonstrated no phytotoxicity problems when using the new copper formulations in conjunction with phosphonate sprays. However, it is important that any new copper products available in the future are tested for their compatibility with phosphonate use as phytotoxicity (eg., canopy loss) can occur.

Field and postharvest applications of host defence promoter compounds such as Bion® and soluble silicon (PhotoFinish®) were disappointing as they failed to show significant disease reductions. A different soluble silicon product called Kasil® (potassium silicate), with a higher concentration of soluble silicon has been sourced and is currently being evaluated in the new avocado project AV04001. Instead of dipping fruit, trees have been injected and preliminary results so far have shown a significant reduction in postharvest disease.

The South African developed product, Biocoat® was not found to be effective in reducing disease despite different application methods.

Harvesting Method Studies

To determine if harvesting methods had an effect on the development of stem-end rot, trials were conducted at three locations and two time periods. When fruit were mature and from healthy, non-stressed trees harvesting method had no effect on the development of postharvest diseases. Fruit which were just mature and snap harvested from drought stressed trees had significantly higher levels of stem-end rot than fruit which were clip harvested. Where the fruit from the drought stressed trees were more mature, harvesting method did not have any effect on the development of stem-end rot. In cases where trees are stressed, such as drought or badly affected by Phytophthora root rot, and fruit are only just mature it is best to clip harvest the fruit rather than snap harvest the fruit.

Snap harvesting fruit in wet weather significantly increased the severity and incidence of anthracnose at the stem-end of the fruit. Fruit should not be harvested in wet conditions as they are more susceptible to skin damage (Hofman *et al.*, 2002) however, if fruit must be harvested in wet weather, fruit should be clip harvested. Snap harvesting exposes the stem-end flesh and may provide an entry point for infection by *Cg*.

Treating trees with copper 5 days prior to harvesting did not decrease the development of stem-end rot. In fact, it significantly increased the incidence of side anthracnose in fruit which were harvested in wet conditions in the morning. It also increased the incidence of anthracnose in the fruit which were harvested in the afternoon although not significantly. The trees were very thoroughly sprayed with copper, to an extent which may have damaged the surface of the fruit and hence caused more postharvest anthracnose. The increase in the levels of anthracnose in the morning could be because the fruit were harvested wet which may have caused more skin damage and hence a greater incidence anthracnose.

A second possibility for the increase in the levels of anthracnose is that the copper spray killed any beneficial microorganisms living on the surface of the fruit. Stirling *et al.* (1999) found that a single application of copper fungicide caused a least a 10-fold decrease in the population of fungi, yeasts and bacteria on the surface of avocado leaves. Beneficial fungi, yeasts or bacteria may have been providing a level of biological control of anthracnose.

Pepper Spot Studies

A. DNA fingerprinting

Molecular methods have been utilised for differentiating between species and genotypes of *Colletotrichum* from many temperate and tropical fruit hosts (Liyanage *et al.* 1992, Mills *et al.* 1992b, Freeman *et al.* 1996, Freeman and Shabi 1996, Ureña-Padilla *et al.* 2002) including avocado (Hodson *et al.* 1993, Hayden *et al.* 1994, Freeman *et al.* 1996, Freeman and Shabi 1996). DNA analysis has shown that genetically distinct populations occur within *C. gloeosporioides* but it has also highlighted the discrepancies within population studies (Hodson *et al.* 1993).

From the cluster analyses of isolates of *Colletotrichum gloeosporioides* , it is apparent that geographic origin of isolates has an effect on clustering as does fruit of origin. It is likely that there are small micro-populations of isolates within trees and orchards. The genetic similarity of the genotypes within each lineage ranged from 50 to 100%. Within each lineage of *Colletotrichum gloeosporioides*, the DNA fingerprint pattern of each genotype differed by only one or a few fragments. The similarity of the DNA fingerprint patterns suggests that the genotypes of *Colletotrichum gloeosporioides* have evolved by mutation within each clonal lineage.

It has been shown that isolates of *C. gloeosporioides* from mango did not produce the sexual stage of the fungus, *Glomerella cingulata*, in culture. Isolates from avocado were more variable with the sexual stage produced from some anthracnose isolates from Duranbah and from pepper spot isolates from Green Pigeon. From these teleomorph studies as well as the molecular analysis it is concluded that mango isolates probably comprise a clonal population which was probably spread throughout the world from a single source, most likely India, the site of origin of mango.

Research indicates that isolates from mango might comprise a pathogenically and genetically distinct population of *C. gloeosporioides* (Alahakoon *et al.* 1994a, Hayden *et al.* 1994, Hodson *et al.* 1993). Analyses of RFLPs and RAPDs show that mango strains tend to be more host specific and genetically uniform than avocado strains (Waller and Bridge 2000, Mills *et al*. 1992). Isolates that were genetically similar to those from avocado were found

infrequently on mango (Alahakoon *et al.* 1994b). The mango isolates were not found on the other crops and usually were virulent only on mango. Alahakoon *et al.* (1994b) suggests that the mango isolates comprise a population of *C. gloeosporioides* which was disseminated throughout the world from a single source, perhaps as an endophyte.

Mills *et al*. (1992) reported that isolates of *C. gloeosporioides* from avocado produced a number of restriction fragment length polymorphisms (RFLPs) when probed with an rDNA clone, while no variation was apparent amongst mango isolates. Their results indicate that isolates infecting mango show less diversity than those infecting other hosts suggesting common ancestry and relatively recent distribution (Mills *et al.* 1992a). This is compared with avocado and papaya isolates which show extensive variation.

Hodson *et al.* (1993) demonstrated considerable variation in rDNA and mtDNA polymorphism within sets of *C. gloeosporioides* isolated from different tropical fruits. The rDNA and mtDNA RFLPs of geographically widely separated strains from mango were found to be almost identical, while those from avocado, banana, and papaya varied considerably.

There are two possibilities for the origin of *Colletotrichum gloeosporioides*. The first suggests that the pathogen coevolved with avocado (Mexican, West Indian, Guatemalan origins) and mango (NE Indian and SE Asian origins) in its country of origin and has been introduced to Australia with planting material. The second idea is that the pathogen evolved independently from local populations of *Colletotrichum gloeosporioides* and attacked the host plant as it was introduced into Australia. Results indicate that most lineages of *Colletotrichum gloeosporioides* have coevolved with their respective fruit. Although the mango isolates from Ayr are relatively isolated (~1200km), they don't appear to have an obviously distinct clade from mango isolates from northern NSW.

B. Pathogenicity of pepper spot versus anthracnose isolates

A series of field, glasshouse and laboratory experiments were used to characterise *Colletotrichum gloeosporioides* isolates based on host specificity, pathogenicity and aggressiveness when inoculated onto avocado fruit, pedicels and petioles under specific conditions. All isolates, to varying degrees, had some effect on unripe avocado fruit and pedicels on the tree at all stages of maturity as well as on detached ripening fruit. Likewise, all isolates had some effect on petioles of nursery avocado trees, but not their leaves. This research has demonstrated differences in disease outcome when avocado is inoculated with isolates of *C. gloeosporioides* from avocado and mango. All isolates were pathogenic at the high inoculum levels ($5x10^6$ conidia/mL) used in the experiments but they varied in their relative aggressiveness. The mango isolates were significantly and repeatedly less aggressive than the avocado isolates, and significant differences were demonstrated between avocado anthracnose and avocado pepper spot isolates, especially for certain isolates.

Isolates of *C. gloeosporioides* inoculated on detached avocado fruit in the laboratory were more aggressive, i.e. caused more severe disease, than on unripe fruit in nature. Once an avocado fruit is harvested, the ripening processes begin. In the *Colletotrichum gloeosporioides*/avocado relationship, this means that the quiescent fungus normally attached to the fruit surface can recommence growth as the ripening ethylene levels increase and antifungal compounds and possibly other defences diminish. Therefore, in these experiments

the fungus was being introduced onto a fruit which was already ripening and subsequently more vulnerable to direct invasion. It is important to note that these lesions were produced without wounding of the fruit surface, the preferred method of many researchers; they were, however, incubated at constant ambient temperature and high humidity. They were also inoculated with an unnaturally high spore concentration and were also inoculated after harvest when the fruits' defence mechanisms were already on the decline as ripening begins. Although some individual fruit may have been more susceptible to ripening fruit rot, each isolate tested included 4 fruit replicates per isolate with 3 lesion sites per fruit, thus each category [e.g. avocado pepper spot isolates from Green Pigeon (APG)] contained a total of 20 fruit and 60 inoculation sites. This level of replication should negate any individual fruit effects. There was a clear distinction between mango isolates and avocado isolates with disease incidence being lower after inoculation with mango isolates even under these artificial conditions. There were no considerable differences observed between pepper spot and anthracnose isolates in all incidences and severity of the symptoms they produced on "cocktail" 'Fuerte' fruit. Basically, the data concluded that pepper spot isolates were as capable as anthracnose isolates of causing anthracnose in ripening fruit. Also, anthracnose isolates were capable of causing pepper spot to similar levels as pepper spot isolates in this detached fruit study.

The production of pepper spot lesions (although less severe) by mango isolates on the leaf petioles of 'Hass' avocado nursery trees and 'Hass' fruit in the field may again be due to the high inoculum density used in the inoculation experiments. It can be speculated that this is an incompatible host response where isolate growth was inhibited and hypersensitive cell death triggered. This implies that genetic recognition is occurring at some stage of the interaction but this cannot be confirmed. The same host response resulting in the production of pepper spot lesions occurred following inoculation with the avocado isolates (ie. anthracnose and pepper spot) but pepper spot production was significantly more severe. The pepper spot isolates were significantly more aggressive than the anthracnose isolates in symptom development. However, it is possible that there is no distinction between the pathogenicity of anthracnose versus pepper spot isolates in the induction of pepper spot symptoms. Just as quiescent *Colletotrichum gloeosporioides* has likely co-evolved to develop a mechanism to use the host's ripening ethylene as a signal to reactivate the infection process at a time when antifungal compound levels have become less effective (Ardi *et al.* 1998), so too it seems that avocado may have further evolved to recognise the invading pathogen via other defences not yet adequately examined.

In the glasshouse inoculation study, it was found that pepper spot symptoms did not appear until 6 months after inoculation of avocado nursery plants. By this stage, plants were potbound and stressed and symptoms were very prominent. In a previous study, Willingham *et al.* (2000) found that pepper spot symptoms developed in 4-6 weeks on twigs and petioles following inoculation with an avocado pepper spot isolate with an inoculum density of 3 x 10^6 conidia/mL. A possible explanation for the delay in the current study was that it took longer for diene levels to fall, due to reduced plant vigour due to the potbound conditions. Petioles do not have the ripening processes of fruit, and diene levels may only decline as leaves senesce. Levels of diene in petioles have not been studied specifically, but levels are generally higher in leaf tissue than in skin (Carman *et al*. 1999). It has also been considered that the plants used in the Willingham *et al*. (2000) study were under stress in the glasshouse prior to inoculation with *C. gloeosporioides* (Willingham 2005, pers. comm.). Willingham *et al.* (2000) did not indicate which rootstock of 'Hass' nursery trees was used for their study, although the leaves produced by the rootstock released anise aroma when crushed, thus

indicating Mexican race origin. Further research by Willingham *et al.* (2001) established that fruit from 'Hass' grafted to West Indian 'Velvick' rootstock are less susceptible to *C. gloeosporioides* infection probably due to a higher concentration of diene. In the current experiment the nursery trees were grafted to 'Velvick' which may have easily delayed further penetration by the fungus through passive antifungal defence. As the plants aged, deteriorated and became stressed, the symptomless quiescent structures may have resumed growth and a typical hypersensitive response could have been activated. Signalling systems and chemical processes occurring in leaf petioles could be different to those occurring in fruit and hence the atypical response.
Another possibility is that the delay in appearance of symptoms on petioles may be due to an inhibition of appressorium germination or inhibition of germinated appressorium penetration of the petiole. Appressoria have been reported as the quiescent structures for *C. musae* and banana (Muirhead and Deverall, 1981) and with *Colletotrichum gloeosporioides* in papaw (Persley and Ploetz, 2003) and passionfruit (Manicom *et al.* 2003). With this explanation, once penetration has occurred, a typical hypersensitive response (HR) is produced. This explanation also implies that the hypersensitive response occurs when the pathogen resumes growth after quiescence; however, the fungus would have to survive at an earlier stage of plant penetration.

In the field experiments, symptoms of pepper spot became visible after about 2 weeks. These results suggested that pepper spot may have resulted from the avocado host preventing the establishment of incompatible *C. gloeosporioides* isolates by hypersensitive cell death. The HR involves cell death around the infection site at the point of entry and results in a localised zone of dead cells. The pepper spot lesion appeared to contain further growth of the fungus but did not kill it (as the fungus was readily isolated from the lesions). The hypothesis was that the more aggressive isolates escape or suppress this hypersensitive response by penetrating further and quickly becoming quiescent. In fact, it is more reasonable to suggest that the isolates which become quiescent and ultimately produce anthracnose are probably less aggressive and do not trigger a HR. This is a compatible interaction between the host and the pathogen. One pathogenicity gene has been identified in *C. gloeosporioides*, and studies of mutants of this gene suggest that it has a role in either suppressing or avoiding a host hypersensitive response during the primary infection process (Manners *et al.* 2000). More research is required to establish if *R* and *Avr* genes are involved in the relationship between avocado and *C. gloeosporioides*.

There do not appear to be any unique characteristics of isolate groups from the various geographical locations, although avocado isolates from Green Pigeon did tend to display slight variations and cultures on SPDA were inclined to be darker and slightly slower growing.

All of these experiments demonstrate the heterogeneity of *C. gloeosporioides* populations. It was thought that patterns of aggressiveness amongst the isolate populations would be more apparent. However, it appears that the relationship between avocado and *Colletotrichum gloeosporioides* is complex and that, while *C. gloeosporioides* has evolved infection strategies to best infect avocado fruit, the host may also be optimising its defence mechanisms. Although part of the one heterogenous population, there were significant differences in aggressiveness between avocado isolate groups in certain experiments. This may have been due to differences in host part or age and hence defence capabilities. It may be hypothesised that young fruit (Duranbah field experiment) and petioles under stress (nursery experiment) which both showed differences in aggressiveness between isolates were

not able to resist infection as well as older more mature fruit (Mt. Tamborine field experiments) which failed to show the same differences. Thus, the differences between isolate groups were perhaps in part, a reflection of host capability to resist infection rather than an absolute difference in pathogen aggressiveness.

It is acknowledged that some experimental work inoculating mango fruit with the same isolates used in these experiments would have drawn some interesting conclusions. Alahakoon *et al.* (1994a, 1994b), Freeman and Shabi (1996), Freeman *et al.* (1996), and Adaskaveg and Hartin (1997) found that isolates of *C. gloeosporioides* obtained from a specific host were more pathogenic to that crop than others. It would be expected that avocado isolates could produce anthracnose symptoms on detached mango fruit but it is not so definite that they could produce pepper spot symptoms on mango fruit (known as 'tear stain') on the tree.

C. Factors affecting pepper spot development

Experiments revealed that the factors influencing disease on avocado trees tended to have the same impact for both pepper spot and anthracnose disease incidence. Any differences there might have been were subtle. The overall conclusion is that the pepper spot symptom is due to a complex system of events. These events are somehow different to the quiescent relationship between *Colletotrichum gloeosporioides* causing anthracnose on avocado fruit.

Inoculation of avocado trees in the field with *C. gloeosporioides* throughout the season of fruit development revealed variations depending on the time of year. Generally, fruit inoculated in mid summer were more susceptible to pepper spot infection. Fruit maturity may also play a role as growth rate tended to slow down at this stage as well. Young fruit (1-2cm long) tended to produce symptoms more rapidly but it was observed that over time the number of pepper spots actually decreased as the fruit outgrew them. However, in many cases, the number of spots could also increase as the fungus was not killed and could trigger later host responses. It is known that disease resistance can depend on the developmental stage at which the host is exposed to the pathogen, however, the bases of this developmental control are virtually unknown (Whalen 2005). Antifungal compounds known as dienes present in the tissue of avocado fruit are thought to contribute to resistance to pathogen invasion of unripe fruit (Prusky *et al*. 1991a). It is possible that the level of dienes is less in young fruit, therefore, resulting in increased susceptibility of fruit to pepper spot. As fruit mature, so too levels of dienes may increase sufficiently to arrest fungal growth leading to quiescence. The amount of diene available may also depend on the fruit cultivar, plant age, and environmental conditions (Prusky *et al*. 2000). It has been reported that pepper spot incidence in avocado orchards tends to coincide with an increase in the age of the trees regardless of tree health. Fruit from older trees tend to have higher levels of postharvest disease and it has been suggested that diene levels decrease with tree age (Willingham et al. 2001). Whether or not this compound is involved in the pepper spot scenario is not known.

Inoculum concentration also has an effect. Alahakoon *et al.* (1994) found that the extent of cross-infection appeared dependent on inoculum density and it is known that the germination of conidia of *C. gloeosporioides* tends to be low *in vitro* due to the crowding effect or inhibitors (Bailey *et al.* 1992) which inhibit germination and appressorium formation until conidia are well dispersed in a favourable environment (Liu and Kolattukudy 1999). Conidia of *C. gloeosporioides* release a thin film of glycoprotein ECM (extracellular matrix) at the spore-substratum interface, which may consolidate the initial attachment of conidia

(Hutchison *et al.* 2002). The conidia of *C. gloeosporioides* need a hard surface contact for 2 hr before they can differentiate into appressoria in response to host signals such as surface wax and the ripening hormone ethylene (Dean 1997, Kim *et al*. 2000a). The molecular signalling involved in the induction of this differentiation is poorly understood. At a low spore concentration, infection levels stayed relatively low over time. As the spore concentration is increased, disease severity over time appeared to increase. Fruit inoculated with spores at 5 x 10^8 conidia/mL had higher pepper spot ratings. Although spores at this concentration would not germinate in water due to inhibitors in the extracellular matrix, once in contact with the fruit surface, this higher concentration must increase the chances of a few spores surviving and germinating before the suspension dries out or runs off the fruit. It was noted in the previous section that isolates of *C. gloeosporioides* could adhere to the waxy fruit surface and their pedicels (and seemingly to petioles) but not to the leaves of avocado. Pedicels tended to consistently produce higher levels of pepper spot symptoms than fruit. The reason for this is not known, although it is probably a physiological characteristic. The data collected for the pedicel assessments over time was distorted by the prevalence of "ring neck" due to water stress.

Time of year for inoculation of fruit did not appear to have any effect on the survival of fungal spores and subsequent formation of pepper spots, at the medium and high inoculum concentrations. If there was to be any reduction in spore survival it would be expected at the hottest time of the year when desiccation of the fruit surface would be rapid and fruit surface temperatures high. On the contrary, there was an increase in pepper spot symptoms at this time, with only a slight dip in fungal recovery from fruit 12 weeks after inoculation. The recovery of the fungus from pepper spots inoculated early in the season with the low inoculum concentration (5 x 10^4 conidia/mL) of both isolates tended to be the lowest. Although these fruit were inoculated in late spring months, they were harvested in late summer by which time, perhaps, environmental conditions had reduced the viability of the fungus within the pepper spot.

Measurements of the pH of spore suspensions found that as spore concentration is increased, the pH of the suspension decreased becoming slightly more acidic. In studies of germination and appressorium formation in mango, Kuo (1999) found that if the spore concentration was above 10^6 spores/mL, the germination percentage was reduced to 0% over 18hrs, but could reach 100% when the spore concentration was adjusted to 10^5 spores/mL. The addition of nutrients could enhance the germination rate.

It was found that the pH of fruit skin increased over time, with fruit inoculated with *Colletotrichum gloeosporioides* having slightly higher skin pH than water-inoculated control fruit. In studies by Yakoby *et al.* (2000b), it was observed that two avocado cultivars relatively resistant to *C. gloeosporioides* attack had pericarp pHs of less than 5.5 which did not increase during ripening. They suggested that host pH regulates the secretion of pectate lyase which may affect *C. gloeosporioides* pathogenicity. Wang *et al.* (2004) recently observed that production of the reactive oxygen species (ROS), H_2O_2, by *C. gloeosporioides* is also dependent on pH in avocado/*C. gloeosporioides* interactions. They found that H_2O_2 was produced at pH 5 but not at pH 7. *C. gloeosporioides* can also secrete ammonia locally into the host tissue, resulting in a pH increase that enables enzymatic secretion and enhanced virulence (Prusky *et al.* 2001). In the current studies, 'Edranol' (Guatemalan) rootstock fruit had an early season mean pH as low as 4, which indicates that *C. gloeosporioides* would be capable of producing H_2O_2, which can be a precursor to the development of a hypersensitive response. In this project we also found that for fruit from 'Hass' grafted to 'Velvick' and

'Duke 6' rootstocks, the pH increased significantly during ripening although with no significant differences between the two rootstocks. However, the skin of fruit from 'Hass'/'Duke 6' treated with high ammonium had a significantly higher pH than fruit from the other fertiliser regimes for 'Hass'/'Duke 6'. Increasing anthracnose did correlate (not significantly) with increase in pH levels but it has been ascertained (Willingham 2003) that increased nitrogen fertilisation also results in increased anthracnose incidence. Increase in pepper spot did correlate with increasing pH, however, any relationship cannot be assumed without further investigation. It was intended to correlate pH recordings with diene measurements using HPLC from fruit peel, however, the methodology for this work is still in the development stage and it is anticipated that results can be obtained in the future.

As mentioned in the Introduction, 'Velvick' rootstock fruit tend to have less anthracnose than 'Duke 6' rootstock fruit (Willingham *et al*. 2001) and this trend seemed to follow for pepper spot incidence. Similarly, pepper spot disease was highest on 'Hass' fruit from 'Parida 1' Mexican rootstock fruit which is consistent with reports that fruit from 'Hass' develop less anthracnose when grafted to Guatemalan rootstocks compared to fruit from 'Hass' grafted to Mexican rootstocks (Willingham *et al*. 2001). Lower nitrogen levels seemed to correlate with increased pepper spot symptoms which is opposite to the situation in the development of anthracnose. When plants receive excess nitrogen fertilisation, production of secondary metabolites such as those involved in defence mechanisms is overshadowed by the production of primary metabolites involved in areas such as photosynthesis, thus leading ultimately to increased postharvest anthracnose incidence. This increase in nitrogen and subsequent vegetative growth of the trees results in fuller tree canopies and, therefore, less sun exposure of fruit. Therefore, while fruit are still on the tree, the hypersensitive response (HR) is reduced resulting in fewer pepper spot symptoms.

Another objective of this chapter was to determine if the presence of pepper spot offered cross protection against postharvest anthracnose. The results showed no evidence for induced resistance. In some cases, there was increased susceptibility. The hypersensitive response does not prevent quiescent infections from resuming growth. In fact, it is possible that viable *C. gloeosporioides* still contained within pepper spots at the time of harvest may then be able to recommence growth into the ripening fruit once it can overcome the pepper spot barrier. It would be ideal to repeat this experiment over more than one season and to correlate it with other aspects such as rootstock, mineral content, pH and so on. Although Willingham *et al*. (2004) reported a reduction in anthracnose in fruit from stressed trees, the fruit were very small and took a long time to ripen. The trees were rated for health on a visible scale with no consideration of physiological constraints. It has since been determined that the entire orchard was unhealthy and more comparative assessments on a range of orchards would be recommended. Because pepper spot appears to be an indicator of tree stress, it would be necessary to investigate further the effects of tree stress on the development of postharvest anthracnose along with any correlations with preharvest pepper spot incidence.

A general observation during the pepper spot studies, was that pepper spot symptoms appeared to be more prevalent on trees under stress. This was the case for the glasshouse inoculation experiment where plants became potbound and nutrient deficient over 6 months. Field observations also revealed a tendency for fruit on sunny exposed branches to be more susceptible to pepper spot infection. This was particularly obvious on trees stressed by *Phytophthora cinnamomi* root rot. These trees tend to have sparse canopies and sunburnt fruit and, therefore, greater levels of pepper spot disease on the branches, pedicels and fruit.

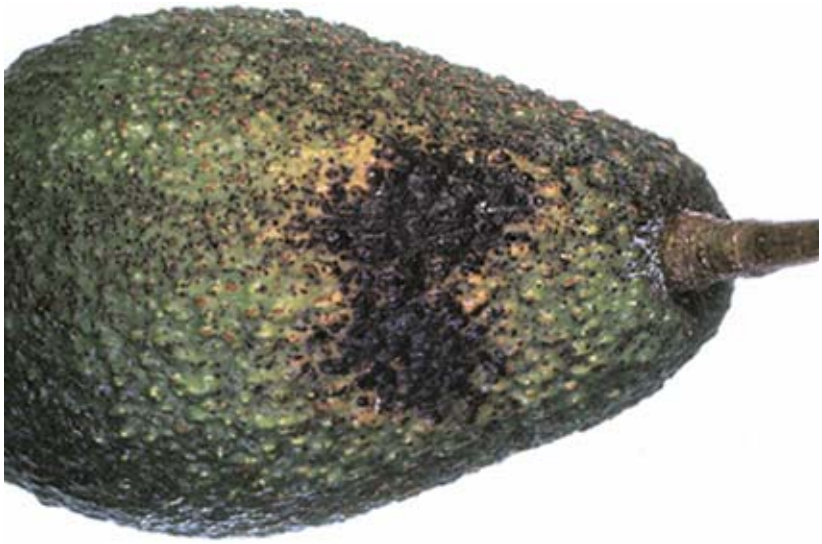


Plate 18: Section of 'Hass' avocado fruit affected by sunburn and associated pepper spot symptoms

Predisposition to disease is generally thought to be increased with exposure to stress (Joyce *et al*. 1998) due to a reduced ability of the host to mount defence reactions. Therefore, if the pepper spot symptom is a hypersensitive response (a host defence mechanism) then the implication is that an increase in tree stress is actually amplifying this host defence mechanism. This is a counter argument.

A possible theory is that all *C. gloeosporioides* isolates probably trigger HR but it is contained and invisible in healthy trees, involving only 1 or 2 cells, while surrounding cells remain viable and intact. Isolates are either prevented from growing or continue on to form quiescent infections. Elicitation of HR requires live pathogens and the timing and the magnitude of the visible HR in plants is often pathogen-dosage dependent (Devadas and Raina 2002). If the pathogen concentration falls below a certain threshold, then there is no visible HR (Turner and Novacky 1974). In stressed trees, fruit resilience is compromised (reduced chemical and physical barriers) and more host cells collapse. Fruit from stressed trees might have water deficit stress, nutrient deficiency and imbalance, and toxicities (e.g. chloride). At high temperatures, there could be increased cell desiccation, alterations in biochemical reactions and enzyme systems, and disrupted membranes. Therefore, fruit from stressed trees possibly have a reduced defence capacity via preformed antifungal dienes and mechanical barriers. Although stressed fruit are still able to elicit an HR, it conceivably involves more cells with the response being less contained. With the resilience of the fruit cells being compromised, the death of cells would be further reaching and the host response would then become clearly visible as a pepper spot lesion. Some quiescent infection will probably still occur in stressed fruit and then produce postharvest anthracnose.

Plate 19: Severe sunburn on an exposed side of a small 'Hass' avocado fruit with associated pepper spots coalescing on the fruit surface

It has been mentioned previously that reactive oxygen species (ROS) are involved in the HR typical of plant-pathogen incompatible interactions. ROS are also generated under adverse conditions (Dat *et al*. 2000). High and low temperature exposure cause accumulation of ROS and increased H_2O_2 as do exposure to high light intensities, drought and salt stress, copper toxicity and pathogen attack (Dat *et al*. 2000, Pontier *et al.* 1999). This oxidative burst is triggered within minutes of fungal infection (Van Camp *et al.* 1998). Although plant signalling responses and possible cross-talk between different signalling cascades are increasingly well-understood, the mode of action of H_2O_2 remains unknown.

Quiescence of *C. gloeosporioides* appears to be avoidance of a response before gene recognition occurs, although at ripening when the pathogen resumes growth, a compatible interaction seems to take place. Pepper spot isolates, on the other hand, seem to promote a host incompatible response. It was thought that the pepper spot isolates may have had corresponding *Avr* genes to the *R* genes of avocado, which were possibly absent in anthracnose isolates. However, this did not account for the fact that all the isolates were able to produce pepper spot symptoms to varying degrees, including many of the mango isolates. This would imply that a gene mutation is not the evolutionary cause of the recent pepper spot symptom. It suggests that the response is a host mechanism and may involve elicitors other than specific AVR proteins. Wang *et al.* (2004) believe that quiescence of *C. gloeosporioides* in unripe avocado is probably induced by production of ROS by both the pathogen and the host. This indirectly induces fatty acids and synthesis of epicatechin which both result in higher diene levels. Factors such as ethylene, cold stress, fungal inoculation, which induce diene synthesis in avocado fruit have been shown to stimulate production of H_2O_2 in other systems. H_2O_2 acts as a signal molecule in induction of resistance in avocado fruit.

The histopathology of unripe 'Hass' fruit skin inoculated with a *C. gloeosporioides* pepper spot isolate suggested that the infection peg did not penetrate quite as far as previous studies of anthracnose in 'Fuerte' fruit even after 7 days. Coates (1991) demonstrated that the infection peg penetrated 1.5 µm into the cuticle where it became quiescent. In 'Hass' fruit, the infection peg penetrated about 1µm into the cuticle and no further growth was seen.

There were no signs of a host response at a cellular level at this stage. The pepper spot symptom does not become visible until at least two weeks after inoculation, therefore it is possible that there is a short period of quiescence before a host response is initiated.

Most plants are resistant to the majority of pathogenic fungi (Westerink *et al*. 2004). The primary defence of plants to invading pathogens is the preformed presence of thick cell walls made up of cellulose, pectin, lignin and so on, which provide a physical barrier (Jayaraj *et al*. 2004, Stuible and Kombrink 2004). The secondary defence mechanism consists of preformed chemical defence and involves the constitutive expression of plant metabolites such as phenolics, saponins and alkaloids (Jayaraj *et al*. 2004). The most complex defence, however, comprises the triggering of genes involved in plant pathogen interactions. Early defence responses are initiated immediately with signals leading to HR cell death. Further inducible responses result in numerous biochemical and metabolic plant reactions such as the production of ROS, phytoalexins, cell wall components and eventually pathogenesis-related (PR) proteins (Jayaraj *et al*. 2004).

Signal exchange following recognition events determines whether the interaction will be compatible or incompatible (Dickman *et al.* 2003). It is assumed that the interaction between avocado and *C. gloeosporioides* is compatible when anthracnose is the resulting disease. Penetration is usually successful when fungi attempt to penetrate the cells of their host species, as opposed to the failure rate of non-host interactions (Mellersh and Heath 2004). If the plant resistance (*R*) gene is present in the host and the avirulence (*Avr*) gene is present in the pathogen, then an incompatible reaction will ensue. Incompatible reactions are rapid and effective and often associated with a localised death of plants cells known as the HR. It has been ascertained that pepper spot symptoms are a result of this type of interaction. Because there are two symptom types (anthracnose and pepper spot) on the one avocado fruit caused by the same species of fungus (*Colletotrichum gloeosporioides*), it makes it difficult to explain the differences in pathogen invasion and host defences. Turner and Novacky (1974) inoculated tobacco leaves with the incompatible pathogen *Pseudomonas pisi* at low concentration and found dead plant cells in symptomless leaves with many characteristics of HR induced by high concentrations. In past research, it was thought that HR was an artefact produced by the unnaturally high concentrations of bacteria used. Cell death occurred quickly and rose to a maximum in 6 hrs. They inoculated with the compatible pathogen *P. tabaci* and cell death was only detected after 18hrs. Likewise, Kumudini *et al.* (2001) found that for *Sclerospora* downy mildew inoculated onto highly resistant millet (host), HR occurred in 20% of seedlings 2 hrs after inoculation, whereas in highly susceptible millet HR took 8 hrs. In the *Phaseolus vulgaris* (bean*)-C. lindemuthianum* system, incompatibility is characterised by early host cell death at the point of pathogen penetration, inhibition of pathogen growth in the penetrated tissue, and lesion limitation. This early HR does not occur in compatible interactions, thereby indicating a high correlation between the HR and race-cultivar specificity (Esquerré-Tugayé *et al*. 1992). If avocado fruit was not climacteric and did not contain antifungal diene, it is conceivable that establishment of compatible and incompatible disease symptoms could occur on the tree.

An aspect of this work that has been difficult to explain is the lack of evidence for plant cell penetration by the infection peg or hyphae of *C. gloeosporioides*. Plants have evolved recognition systems for many fungal surface-derived compounds, which initiate signalling cascades (Scheel and Nuernberger 2004) and the concept has recently been put forth that indirect perception of AVR proteins can occur to trigger responses without direct interaction (Westerink *et al*. 2004, Yarden *et al.* 2003). In some cases *Avr* gene products are proteins

that are either known to be directly secreted into the apoplasm or thought to be secreted directly into the plant cell (Greenberg 1997). This would help to explain the development of pepper spot lesions prior to cell penetration. Indeed, the relationship between anthracnose *C. gloeosporioides* and avocado may in fact be incompatible, but as the signals may only be noticed once the fruit is ripening and diene levels decreasing, it is too late for a resistance response to have an effect.

The nature of such host signals and how they are perceived, and how genes responsible for pathogenic development (including infection structure differentiation and host penetration) and subsequent colonisation are activated are beginning to be understood (Dickman *et al.* 2003). Molecular communication begins as soon as the fungal conidium lands on the plant surface (Kolattukudy *et al.* 2000). The first contact of *C. gloeosporioides* is on the plant cuticle through which inhibitors can diffuse, followed by host surface contact-induced events, including expression of early genes. Conidia respond to host signals that induce germination and appressoria formation by transcriptional activation of another set of genes. Yakoby *et al*. (2002) suggest that the initiation of early signalling events affecting fruit resistance is determined by the capability of the pathogen to interact with the fruit during appressorium formation. Elicitors are released from both pathogens and hosts and are probably specific (Esquerré-Tugayé *et al*. 1992). Stimulation of many defence responses by elicitors, wounding, or infection involves transcriptional activation of the corresponding defence response genes (Ebel and Cosio 1994). Prior to establishment of infection, fungi must overcome the external barrier of the plant, the cuticle, which is made up of at least 50% cutin. *C. gloeosporioides* secretes cutinase (Bailey *et al.* 1992, Kolattukudy *et al.* 2000) which assists appressorium penetration, as well as endopolygalacturonase, pectin lyase A, pectin methyl esterase, and pectate lyase B during colonisation of infected tissue (Yakoby *et al.* 2001). It has been suggested that pectic fragments released by the enzyme endopolygalacturonase act as fungal elicitors of defence (Esquerré-Tugayé *et al*. 1992). Once a signal has been prompted by the penetration of fruit skin by *C. gloeosporioides*, only then can the HR resulting in pepper spot be initiated.

Cross-Protection Studies

The potential for using non-pathogenic strains of *Colletotrichum* for cross-protection against anthracnose was explored in this project by attempting to introduce non-pathogenic strains of *Colletotrichum gloeosporioides* by a root dipping or seedling transmission technique. Unfortunately, both of these techniques failed to show sufficient transfer of the non-pathogenic strains to seedling plants. Problems were also encountered in the assaying procedure and highlighted the need to refine the triple sterilisation procedure. These limited studies demonstrated potential new areas of study on cross-protection which if successful could have a huge impact on integrated disease management strategies.

5. TECHNOLOGY TRANSFER

During the project the avocado industry were informed of our activities via articles in grower newsletters:

Willingham, S., Pegg, K., O'Brien, R., Coates, L., Cooke, T. and Dean, J. (2002). Which copper fungicide should I use? *Sunshine Coast Avocado Growers Association Newsletter*, **72,** 15-16.

Willingham, S. (2003). The role of rootstocks, nutrition and antifungal compounds in resistance of avocado to anthracnose. *Talking Avocados,* Autumn edition, **14,** 23-27.

Anderson, J.M., Cooke, A.W., Willingham, S.L., Coates, L.M., Pegg, K.G., Langdon, P., Dean, J.R. and Beasley, D. (2003) Amistar – Now registered for use on avocado. *Talking Avocados*, Spring edition, p 31-33

Anderson, J. (2003) Effect of harvesting methods and maturity on stem-end rot in 'Hass' avocado. *Talking Avocados*, Winter edition, p.22-24.

Giblin, F. (2003). Incidence of pepper spot on 'Hass' avocado fruit. *Talking Avocados*, Winter edition, p.20-21.

Whiley, T., Allen, A. and Anderson, J. (2004) Fifth World Avocado Congress Report. *Talking Avocados*, Autumn edition, p.18-21.

Anderson, J., Pegg, K., Coates, L., Dann, E., Cooke, T., Smith, L. and Dean, J. (2004) Silicon and disease management in avocados. *Talking Avocados*, Spring edition, p.23-25.

Pegg, G., Giblin, F. and Pegg, K. (2004) Brown root rot caused by *Phellinus noxius* can lead to losses in avocado orchards. *Talking Avocados*, Spring edition, p.21-22.

As well as conducting grower field days:

3/7/03, AAGF Avocado Roadshow, Atherton, QLD
24/7/03, AAGF Avocado Roadshow, Bundaberg, QLD
29/7/03, AAGF Avocado Roadshow, Gatton, QLD
1/8/03, AAGF Avocado Roadshow, Duranbah, NSW
AAGF Avocado Roadshow, Stuarts Point NSW (14/8/03), Renmark SA (25/11/03) and Pemberton WA (27/11/03)

Other publications arising from this project:

Pegg, K.G., Coates, L.M., Korsten, L. and Harding, R.M. 2002. Foliar, fruit and soilborne diseases, In: The Avocado: Botany, Production and Uses, Whiley et al., eds., CABI International.

Anderson, J.M., Pegg, K.G., Coates, L.M., Willingham, S.L., Cooke, A.W. and Dean, J.R.. 2003. Managing anthracnose in 'Hass' avocado: influence of rootstocks and nutrition. Poster displayed at the International Congress of Plant Pathology held in February 2003 in Christchurch NZ.

Giblin, F.R., Coates, L.M., Bentley, S. and Irwin, J.A.G. 2003. Pepper spot development in 'Hass' avocado fruit: pathogen variability, epidemiology and infection processes. Poster displayed at the International Congress of Plant Pathology held in February 2003 in Christchurch NZ.

Anderson, J.M., Pegg, K.G., Willingham, S.L., Coates, L.M., Cooke, A.W. and Dean, J.R. (2003) The influence of rootstock and mineral nutrition on anthracnose development in 'Hass' avocado. 5th World Avocado Congress, Supplement 1. (Oral presentation)

Anderson, J.M., Pegg, K.G., Coates, L.M., Willingham, S.L., Cooke, A.W. and Dean, J.R. (2003) The integrated management of anthracnose in 'Hass' avocado. 8th International Congress of Plant Pathology, Vol 2. p 135. (Poster presentation)

Willingham, S.L., Coates, L.M., Cooke, A.W. and Dean, J.R. (2004). Tree vigour influences disease susceptibility of 'Hass' avocado fruits. *Australasian Plant Pathology*, **33,** 17-21.

Reports:

HAL Milestone reports (7 in total).

The wider scientific community was informed of our research activities by attendance at the following conferences:

February 2003, 8th International Congress of Plant Pathology, Christchurch NZ
October 2003, 5th World Avocado Congress, Spain
28-30th October 2003, CRC Research Symposium 2003, Brisbane QLD
13th September 2001, APPS Conference, Cairns, QLD

Meetings:

15/11/01 – AAGF R&D Workshop, Maroochy Horticultural Research Station
5/9/02 – AAGF R&D Workshop, Holiday Inn, Brisbane
28/8/03 - AAGF R&D Workshop, Brisbane
22/4/04 – Combined Rural Traders meeting
25/8/04 – AAGF R&D Workshop, Medina, Brisbane

6. RECOMMENDATIONS

Rootstock and nutrition studies

- Rootstock influences tree phenology (vegetative flushing, canopy colour), crop load, mineral accumulation and postharvest (anthracnose) diseases.
- The West Indian 'Velvick' rootstock had a less vegetative canopy, higher crop load, better balance of mineral accumulation (low N/Ca and high Ca+Mg/K ratios) and less anthracnose than Mexican 'Duke 6' rootstock. However, this rootstock effect was lost in the second season, possibly due to very high disease incidences (>75%).
- The Guatemalan ('A8', 'A10', 'Nabal') rootstocks were also found to be superior for disease control compared with the Mexican ('Parida 1') rootstock. However, rootstock effects were not apparent in the last two seasons, possibly due to heavier crop loads on 'Parida 1'.
- Correlations between disease levels and nutrient concentrations were strong across rootstocks with lower disease levels related to more favourable balances of N/Ca and Ca+Mg/K ratios.
- Nitrogen fertiliser form (ammonium vs nitrate) did not significantly affect tree phenology, crop load, mineral concentrations or disease levels.
- Withholding nitrogen fertiliser resulted in less vegetative canopies, a better balance of nutrients and showed a trend of less postharvest anthracnose (although not significant).

New products

- Current industry standard copper products (copper oxychloride and copper hydroxide) as effective as new formulations
- Compatibility tests (with phosphonates) are an important assessment of any new copper product to avoid phytotoxicity problems
- Defence promoting compound, Kasil, a new soluble silicon product, warrants further evaluation for disease control

Harvesting method studies

Snap instead of clip picking should only be considered under the following circumstances:

- Trees are healthy and not stressed
- Fruit is fully mature but not over mature (about 23-29% dry matter)
- Fruit is not picked in wet humid environment
- Growth regulants (such as Sunny®) have not been used (fruit are prone to skin tearing when snap picked)

Pepper spot studies

- Cross-infectivity potential of *Colletotrichum gloeosporioides* from mango to avocado is low
- Pepper spot severity in orchards can be minimised by reducing tree stress (Phytophthora root rot, water deficit, sunburn) and using Guatemalan or West Indian rootstocks ('Velvick', 'A8', 'A10', 'Nabal') instead of Mexican ('Duke 6')

Cross-protection studies

- Cross-protection of avocado seedlings with non-pathogenic strains of *Colletotrichum gloeosporioides* requires further research to refine transmission techniques before a complete assessment of success can be made

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Sunshine Horticultural Services

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