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PROGRESS IN MANAGING LATENT INFECTIONS A REVIEW

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Summary

Avocados are infected on the tree during the season by spores of *Colletotrichum gloeosporioides* (the most important anthracnose pathogen) which germinate, form appressoria (round protective structures adhering to the fruit surface), then short infection pegs. Growth of the fungus then ceases until fruit is harvested and begins to ripen. An antifungal chemical (l-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15 diene) was isolated from the skin and the flesh of avocado fruit and shown to degrade as the fruit ripen, and is thought to be primarily responsible for latency of infections of immature fruit. Infection of peeled unripe avocado fruit was not prevented, despite high levels of antifungal dienes, because of compartmentalisation of the antifungal diene in the oil cells in the flesh rendering it unavailable to inhibit the invading fungus. No oil cells are present in the skin, and infection of intact avocado fruit was prevented by freely available antifungal diene in the skin. An intact skin is thus important to prevent infection by *Colletotrichum gloeosporioides.* Degradation of the diene was shown to be by the enzyme lipoxygenase, which was regulated by the naturally occurring antioxidant epicatechin. Epicatechin was also shown to inhibit two cell-macerating enzymes (endopolygalacturonase and pectate lyase) produced by the fungus *Colletotrichum gloeosporioides,* so epicatechin may have several roles in inhibition of the fungus during latency.

Application of a number of exogenous antioxidants and CO_2 treatment were shown to inhibit breakdown of the antifungal diene during ripening, and thus provide some disease control. However, in New Zealand none of these treatments was effective in preventing disease. Studies on the infection of 'Hass' by *Colletotrichum acutatum,* another anthracnose pathogen, have indicated that latency may not be important in infection with this fungus. Copper and benlate sprays are the currently recommended controls for anthracnose followed by postharvest dipping in prochloraz. Biological control is a more environmentally sustainable solution, but is currently an experimental tool only.

Introduction

Avocados are susceptible to two postharvest diseases; stem end rot and anthracnose. These diseases are considered to be an important problem world-wide. Anthracnose diseases are thought to be caused by fungi which latently infect throughout the season. Symptoms are only expressed following ripening under conditions favourable to the fungus. The causal organisms vary slightly from country to country, but the pathogen most consistently reported to be the cause of anthracnose is *Colletotrichum gloeosporioides.* Studies on latent infections of avocados to date have focused on the infection of 'Fuerte' avocado by *Colletotrichum gloeosporioides* (Binyamini and Schiffmann-Nadel, 1972; Coates *et al,* 1993a; 1993b; Prusky and Keen, 1993). In New Zealand the infection of 'Hass' avocados by *Colletotrichum acutatum* has been studied (Everett and Hallett, 1994; 1996). This review considers research findings concerning latent infections of avocados, and how these are controlled. Postharvest temperature management will not be reviewed.

When and how does infection occur?

Anthracnose rots on avocados develop only when the fruit are ripe. It was found that infections of 'Fuerte' avocados occurred throughout the season, but remained latent until fruit ripened, when the fungus investigated, *Colletotrichum gloeosporioides,* resumed growth and invaded the ripening fruit to cause anthracnose rots. (Binyamini and Schiffmann-Nadel, 1972; Peterson, 1978; Coates *et* a/.,1993b). Spores were shown to germinate after landing on the avocado fruit to form a round protective structure (an appressorium) which adheres tightly to the fruit surface. A peg is then produced by the appressorium, which penetrates a short distance into the skin of the avocado and then stops growing (Coates *et al,* 1993a; Prusky *et al,* 1991b; Prusky *et al,* 1991c). Following harvest, symptoms still do not develop, until fruit have softened.

Where do spores come from?

Spores of *Colletotrichum gloeosporioides* were released from dead leaves entangled in the main canopy (Fitzell, 1987). However, removal of dead leaves and twigs from the canopy was not consistently able to reduce the numbers of postharvest rots in avocados (Hartill *et al,* 1991; 1992). The principle means of spread to avocado fruit was by rain-borne inoculum. Increased rainfall resulted in increased levels of rots in harvested avocados in Australia (Peterson, 1978), and more quiescent infections became established in South Africa during the rainy part of the year than in the dry winter months (Darvas and Kotze, 1987).

Why are infections latent?

Several workers have sought to explain why *Colletotrichum gloeosporioides* ceases growth on immature avocados, only to resume growth after harvest as fruit begin to ripen. A number of antimicrobial compounds have been isolated from the skin, flesh and seed of avocado (Jensen, 1951; Valeri and Gimeno, 1954; Neeman *et al,* 1970;

Adikaram *et al,* 1992; Sivanathan and Adikaram, 1989; Prusky *et al.,* 1982) of which the most important has been identified as 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15 diene (Prusky *et al.,* 1991c) This antifungal diene inhibited spore germination and appressorial formation of *Colletotrichum gloeosporioides* and was found in the skin and flesh of 'Fuerte' and 'Hass' avocados. This compound degraded during ripening, and only when the concentration was low did decay symptoms appear.

Of interest to New Zealand growers was the observation that the antifungal diene decayed more rapidly in the skin of 'Fuerte' avocados than in 'Hass', and subsequently 'Hass' was deemed to be a variety more resistant to infection by *Colletotrichum gloeosporioides* (Prusky *et al.,* 1982; 1988). Prusky *et al.* (1982) also made the observation that levels of antifungal dienes were lower in 'Hass' fruit that was harvested when over mature (fruit harvested about a year after set) compared to fruit harvested in the winter (fruit harvested about 10 months after set).

How is the amount of the antifungal diene regulated?

During ripening the antifungal diene was shown to breakdown (Prusky *et al.,* 1982). The mechanism of this breakdown was investigated and lipoxygenase was identified as the enzyme responsible (Prusky *et al.,* 1983). The regulator of the enzyme activity was identified as the naturally occurring antioxidant epicatechin (Prusky, 1988) (Figure 1).

Kami *et al.* (1989) confirmed the role of epicatechin as the regulator of the breakdown of the antifungal diene. Decrease was related to changes in epicatechin concentrations, not to changes in the amount of lipoxygenase. The amount of the enzyme remained constant, but activity increased 1 hour after harvest, concomitant with a decrease in the amount of diene and epicatechin.

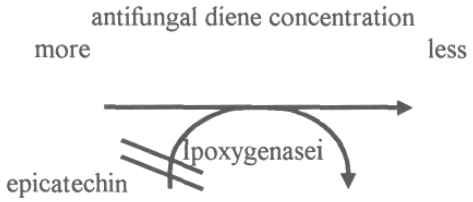

Figure 1 **The degradation of the antifungal compound, 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15 diene is catalysed by the enzyme lipoxygenase, which is in turn inhibited by the antioxidant epicatechin. Diagram adapted from Prusky *et al.* (1988)**

When and where does the amount of antifungal diene vary?

Prusky *et* a/.(1991b) demonstrated that the amount of the antifungal diene in the skin and flesh of avocado fruit decreased to subtoxic levels in the first 1-2 days after harvest,

then regained preharvest levels before gradually declining again during ripening over the next 7-9 days. Hot water treatment was shown to prolong the period of low concentration of antifungal diene in the peel following the initial decline (Plumbley *et* a/., 1993). The decline in levels of the antifungal diene immediately after harvest was presumed to be a stress response which can also be induced by heat shock and by exposure to CO_2, so could probably be induced by a number of stressors. They suggested that the initial rapid decline in concentration of the diene was due to the shock of harvest.

When spores were placed on peeled avocado fruit, the flesh was able to be penetrated even when unripe, despite the presence of high levels of antifungal dienes (Prusky *et al,* 1991b). Further investigation showed that the dienes were compartmentalised in the oil cells in the flesh (Kobiler *et al,* 1993). Oil cells are only found in the flesh, and not in the skin of avocados (Cummings and Schroeder, 1942; Scott *et al,* 1963). Thus the levels of antifungal dienes available to inhibit the growth of *Colletotrichum gloeosporioides* were higher in the skin than in the flesh. Levels of epicatechin in the flesh were very low, concentrations in the skin were 1000 times higher (Prusky *et al.,* 1985). No significant lipoxygenase activity could be detected in the oil cells. Thus an intact skin is essential in the defence of immature avocado fruit against invasion by latent fungi.

Prusky *et al.* (1990) showed that antifungal diene concentration was almost doubled in the skin and flesh of avocado fruit on the tree when inoculated with *Colletotrichum gloeosporioides,* both with and without wounding. They also showed that more mature fruit (harvested one year after set) had lesser quantities of antifungal dienes than less mature fruit (harvested 10 months after set) after harvest. Prusky was concerned about the mechanism of stimulation of antifungal dienes especially in unwounded fruit, and hypothesised that ethylene may be involved. However, ethylene has not been found to be involved in the biochemical pathway of the antifungal diene (Ebel, 1986). Prusky instead suggested that the signal may be due to an unknown interaction between the fungal cell wall components and the skin of the avocado fruit.

Other mechanisms for latency are they important?

Although the presence of the antifungal diene at fungitoxic levels in immature fruit looks promising as a mechanism to explain latency, several other factors are known to be important in determining latency of infections in other crops. Amongst these factors are availability of nutrients to the germinating fungus on the surface of immature avocado fruit, and the simple mechanical effect, that the fungus may be physically unable to penetrate hard, immature avocados. However, Prusky *et al.* (1983; 1984) demonstrated clearly that neither of these factors were important.

A further hypothesis to explain latency is inhibition of the fruit rotting enzymes produced by the fungus by immature avocado fruit. Indeed, Prusky *et al.* (1989) showed that a cell macerating enzyme (endopolygalacturonase) from *Colletotrichum gloeosporioides* was inhibited by the antioxidant epicatechin. Wattad *et al.* (1994) showed that epicatechin also inhibited the activity of pectate lyase produced by *Colletotrichum gloeosporioides,* and therefore could have a direct effect on inhibiting this pathogen. Epicatechin has been shown to decrease in concentration during ripening (Prusky *et al,* 1982), but

previously the effect was thought to be restricted to regulation of the lipoxygenase enzyme in breaking down the antifungal diene (Prusky, 1988). It appears from these results that epicatechin may have a more direct effect on inhibiting infection of immature avocado fruit by *Colletotrichum gloeosporioides.*

Other workers (Bateman and Basham, 1976) have shown that a number of fruit-rotting enzymes can be produced by pathogens. When one enzyme is blocked using molecular techniques, then another enzyme system takes over and rotting continues unaffected (Bowen *et al.,* 1995). It is unlikely that blocking one, or even two, enzymes is the sole mechanism for latency. It is more likely that the avocado fruit has more than one mechanism to promote latency of germinating spores of *Colletotrichum gloeosporioides.*

Controlling latent infections by manipulating antifungal diene levels

The hypothesis that decay of avocados can be retarded by addition of an exogenous antioxidant to inhibit the amount of lipoxygenase produced by the avocado was tested by Prusky *et al.* (1988). He found symptom expression was delayed by 1-2 days by addition of the antioxidants alpha-tocopherol, BHT, TBHQ, gum guaiac and epicatechin. However, the delay of 1-2 days of diene degradation was not sufficient to reduce disease caused by anthracnose fungi. Subsequently Prusky *et al.* (1995) demonstrated control of avocado diseases in 'Hass' and 'Fuerte' in semi-commercial experiments by application of the antioxidant butylated hydroxyanisole (BHA) or BHA + prochloraz. The concentrations of antioxidants found to be most efficient were used by White *et* a*l*. (1993) on 'Hass' avocados in New Zealand, but no reduction in rots was found. This may be due to the maturity of New Zealand fruit when it was harvested, as Prusky did find control was less pronounced in fruit that was more mature (one year after fruit set). New Zealand fruit is routinely harvested at an even later date (usually between 13 to 17 months after fruit set).

Some concentrations of CO_2 have been shown to increase the diene level in harvested avocado fruit after the initial decline, and thus inhibit rot development (Prusky *et al,* 1991a), but some concentrations can also increase disease (Prusky *et al.,* 1993). It was shown that the effect was on the fruit, not the fungus (Prusky *et al.,* 1991a; Spalding and Reeder, 1991). Epicatechin levels were also enhanced by CO_2 treatment. The effect was most pronounced when 30% CO_2 was applied for 24 hours immediately after harvest on young fruit (harvested 10 months after fruit set). There was no control of rots of very mature fruit (harvested one year after fruit set) in a small laboratory experiment, but when repeated on a semi-commercial scale control was effective. However, when applied to 'Hass' avocado fruit in New Zealand, where fruit are normally harvested when more mature, no reduction in disease was able to be induced by CO_2 treatment (White *et al,* 1993).

Antifungal dienestoxicity to humans

No information is available concerning the toxicity of the antifungal compounds to animal and human consumers (Prusky and Keen, 1993). However, similar compounds have been shown to be highly toxic. For example high levels of the plasma

cholinesterase inhibitors alpha-solanine and alpha-chaconine resulted in withdrawal of the potato variety Lenape from the market (Beier, 1990). Caution concerning the use of dienes as fungicides must be exercised, whether enhanced in the fruit by some other treatment, or applied directly to fruit.

Chemical control

Latent diseases of avocados are currently controlled using a preharvest spray regime of copper and benlate, and a postharvest dip in Sportak^{R} (prochloraz). Current recommendations for New Zealand growers are for 7 sprays with copper and three with Benlate for a 12 month growing season (AEC, 1994). Postharvest dipping with Sportak^{R} appears to be, at the current level of knowledge, unreliable. It appears that there may be a curing effect, or alternatively an infection period immediately after harvest that is not halted by a delayed application of prochloraz. Hartill (1988) found no difference in rots if prochloraz was applied within 24 hours of picking. However, recent results (Everett, unpublished) suggest that coolstorage for 24 hours before application of prochloraz may reduce the effectiveness of this fungicide. Current practices of harvesting and coolstoring may indeed result in avocado fruit being coolstored for up to three days before prochloraz is applied. It is important to investigate the effect of timing of application of prochloraz on its effectiveness, in combination with coolstorage. However, chemicals are becoming increasingly less acceptable in the marketplace. Furthermore, fruit treated with prochloraz, the only chemical available for use as a postharvest dip, cannot be exported to Asia, the USA and countries in Europe (AEC, 1994).

Biological control

Biological control of insects has attained a certain degree of respectability and there are a large number of successful insect biological control programmes, based on release of predators, pheromones, sterile males, and application of *Bacillus thuringiensis* spores as a pesticide. There are also examples of biological control agents used to successfully control plant pathogens. One of the most successful is the biological control of crown gall, *Agrobacterium tumefaciens,* by a closely related bacterium, *Agrobacterium radiobacter* (Moore and Warren, 1979). This biocontrol agent was isolated by an Australian researcher, Alan Kerr, and released worldwide, including New Zealand. A change in cultural practices of rose growing has reduced the importance of this biocontrol agent, but nonetheless it remains a commercial success story. Of equal success is the control of *Heterobasidion annosum* in British forests by spraying tree stumps with a species of *Peniophora* (Rishbeth, 1963). This method of biological control has been used with almost 100% success for three decades. Currently there are over 40 biocontrol agents (BCAs) of fungal or bacterial diseases in commercial use worldwide. BCAs have the advantage that they are naturally occurring microorganisms, and their presence in a higher than natural concentration on the exterior of fruit such as avocados, in which the skin is not consumed, is unlikely to be harmful.

The avocado industry in South Africa has been committed to biological control research

over the past nine years, and has been trail-blazing for the rest of the world (Korsten *et al.,* 1995). The most efficient use of BCAs is as a preharvest spray. This precludes the necessity for postharvest treatments of the fruit. Commercialisation is envisaged as soon as registration is successful.

Coates *et al.* (1995) also isolated antibiotic producing BCAs for testing. These workers were able to demonstrate efficacy of the BCAs when applied as preharvest sprays. In New Zealand (Everett, 1996) two BCAs tested as postharvest dips significantly reduced anthracnose (Figure 2).

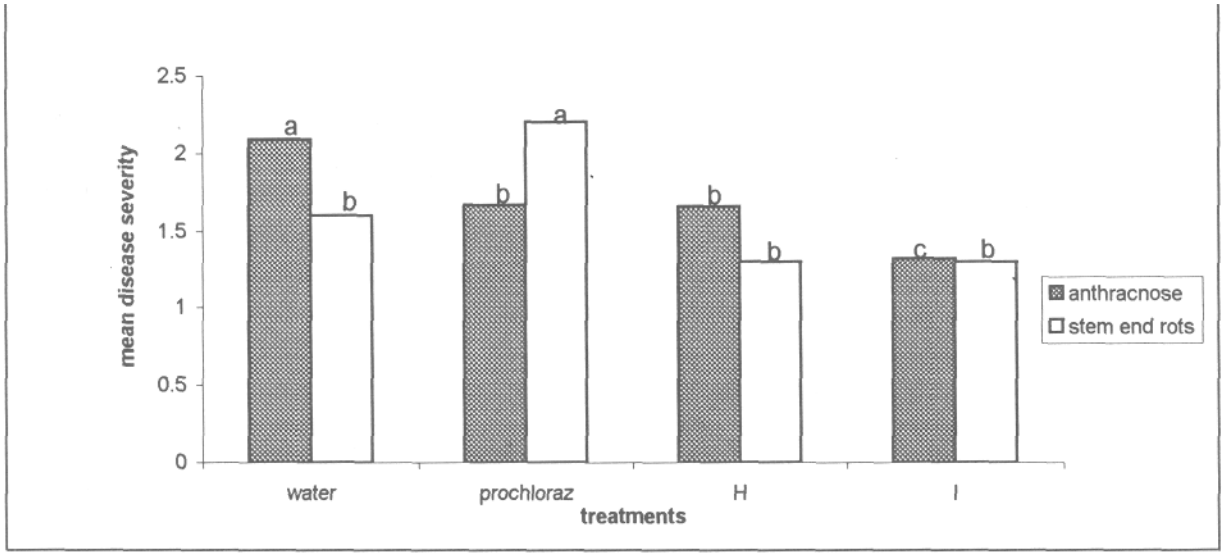

Figure 2 **Application of two biological control agents (H and I) to 'Hass' avocado fruit as a postharvest dip to test efficacy against postharvest rots. Columns with the same letter are not significantly different according to Fisher's protected LSD test (P< 0.01)**

Colletotrichum acutatum: Infection studies in New Zealand

Colletotrichum gloeosporioides is the most important fungus isolated from anthracnose lesions in New Zealand. Two species of *Botryosphaeria (Dothiorella), Colletotrichum acutatum* and *Phomopsis* (Fig. 3) were of lesser importance.

In New Zealand, the infection of 'Hass' avocado by *Colletotrichum acutatum* was studied due to the paucity of data on this pathogen. Results tend to suggest that *Colletotrichum acutatum* may be a wound pathogen, as only a few fruit became infected when unwounded fruit were artificially inoculated throughout the season in the orchard (Everett and Hallett, 1994). Most fruit became infected when inoculated at harvest both through the side of the fruit and through the stem end. If *Colletotrichum acutatum* is a wound parasite, it is likely that the wounded surface provided by the picking wound would facilitate infection. However, it is unclear why more fruit became infected through

the side at harvest. We hypothesised that damage caused by grading equipment may be responsible, but found damage was insufficient to aid infection (Everett and Hallett, 1996).

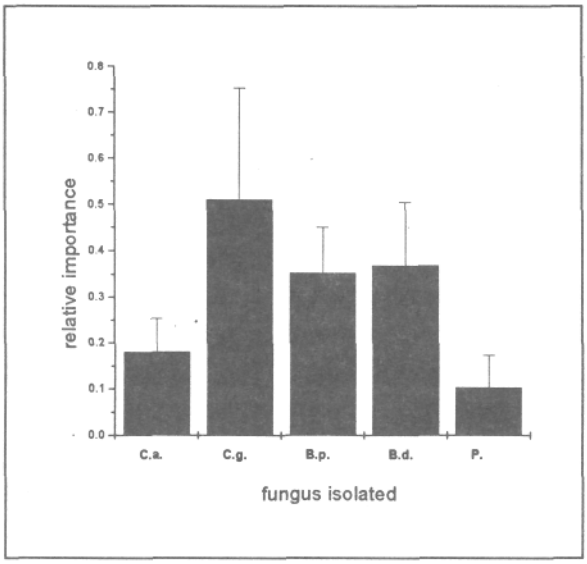

Figure 3 **Relative importance of fungi isolated from anthracnose lesions from 'Hass' avocados in New Zealand. Fruit were sampled from 4 different orchards, over a 5 year period; 1227 fruit were sampled altogether. Bars are standard errors of 8 samples**

C.a.= *Colletotrichum acutatum*, C.g.=*Colletotrichum gloeosporioides*, B.p.=*Botryosphaeria parva*, B.d.=*Botryosphaeria dothidea*, P.=*Phomopsis*

When fruit were inoculated with wounding 7 months and 4 months prior to harvest, infection did not occur. In contrast, almost 100% of fruit wounded in the orchard 1 week prior to harvest became infected, as did fruit wound inoculated postharvest (Fig. 4). This is an unexpected result, as Prusky *et al.* (1993) has shown that antifungal dienes in the flesh of avocado fruit are not available to restrict infection, and when the skin was removed from fruit in the orchard they became infected by *Colletotrichum gloeosporioides* preharvest in his experiments. It is unclear whether the effect noticed in New Zealand is due to the uniqueness of climate, the different variety used, or whether it is specific to the pathogen *Colletotrichum acutatum.* The importance of latency in the infection of 'Hass' avocados by *Colletotrichum acutatum* in New Zealand is currently being further investigated.

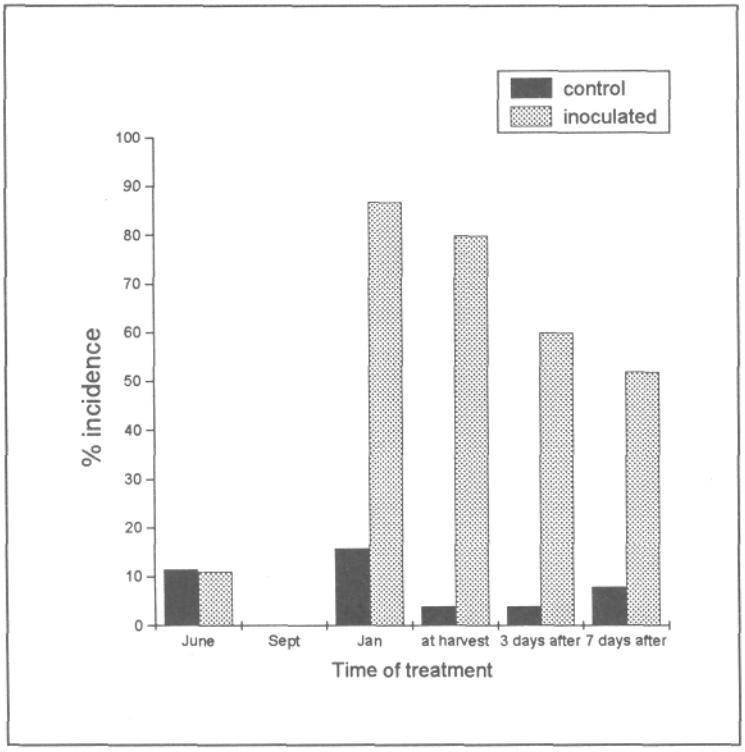

Figure 4 **Results of reisolations from 'Hass' avocado fruit inoculated with spores of *Colletotrichum acutatum* in the orchard (June, Sept. Jan) and postharvest (at harvest, 3 days after harvest and 7 days after harvest) with wounding. Fruit were harvested one week after the Jan. inoculation and rots assessed when fruit were ripe**

Concluding remarks

This review has endeavoured to extensively cover the overseas research on latent infections of avocados, in particular the more detailed studies on the mechanisms of latency and when infection is most likely to take place. Of particular interest are the outcomes that are possible from basic study of mechanisms of latency. From a basic understanding of the processes involved, Prusky and co-workers were able to manipulate the levels of the chemicals regulating degradation of the antifungal dienes in the skin of avocados to attain some control of the postharvest rots. No studies, at this depth, have currently been undertaken on the other anthracnose pathogens. Unfortunately manipulation of the degradation of the antifungal dienes was not found to be successful in New Zealand. A basic difference in evaluation methods for rots may have attributed to this. Prusky *et al.* (1995) did not cut the avocado fruit to examine the effect of their treatments on internal rots, whereas the New Zealand workers did. Control of practical importance requires internal rots to be reduced. As well, New Zealand avocados are routinely harvested when more mature than the fruit used by Prusky in his experiments. These differences highlight the limitations of restricting research to a single model that is infection of 'Fuerte' by *Colletotrichium gloeosporioides,* and the dangers of applying results of overseas research. Anthracnose

of avocados in New Zealand is caused by at least four other pathogens in addition to *Colletotrichium gloeosporioides.* Further basic research concerning the infection of avocados by the other pathogens is urgently required, and indeed our research is aiming to redress these deficiencies in the basic knowledge of the diseases of avocados with relevance to New Zealand growers.

Because of increasing awareness of the benefits of growing 'clean green' products, the use of chemicals in the orchard and postharvest needs to be re-evaluated. Research on environmentally sustainable alternatives such as biological control needs to become a priority.

Acknowledgements

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