FIELD AND POSTHARVEST MANAGEMENT OF AVOCADO FRUIT DISEASES

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Introduction

The sale of high quality fruit to consumers is an important challenge for the Australian avocado industry. Unfortunately surveys have consistently identified a number of quality defects in avocados displayed for retail sale, with postharvest diseases being one of the major quality problems (Hofman and Ledger, 2001). Anthracnose and stem-end rot are the most serious diseases of concern after harvest, respectively affecting in the order of 26 and 7% of “Hass” avocados sampled from retail outlets (Story and Rudge, 1997). In the case of both diseases, decay can extend deep into the flesh. In the cultivar “Hass”, these diseases can be difficult to detect externally due to the black skin of ripe fruit. Unfortunately, the realisation often only occurs when the consumer cuts the fruit open at home.

Field diseases of avocado fruit such as sooty blotch and pepper spot can also impact on fruit quality. Although the damage caused by these diseases is only cosmetic, fruit can be downgraded or even rendered unmarketable as a result.

Increasing restrictions on pesticide use due to environmental and food safety concerns is another issue facing the avocado industry, particularly in growing regions in close proximity to residential areas and water supplies. Although the avocado industry has not been greatly affected by fungicide restrictions to date, it must continue to look at ways to reduce fungicide usage in order to meet future requirements. Two areas of concern are: (1) the build-up of copper levels in soil as a result of frequent application of copper-based fungicides (e.g. copper hydroxide and copper oxychloride) during the fruit development phase, and (2) the postharvest application of prochloraz.

The development of new technologies for the control of avocado fruit diseases must take into account the changing face of the industry. With reduced returns becoming an unfortunate reality for many producers at the present time, disease management strategies must be able to demonstrate a favourable cost-benefit relationship. The trend towards larger scale production also needs to be taken into account, as this may impact on the application of disease management strategies.

The aim of this paper is to present the latest information on the biology and management of avocado fruit diseases based on an integration of field and postharvest approaches, including cultural practices, fungicide usage, maintenance of natural host resistance, postharvest temperature management and controlled ripening.

The causal agents of avocado fruit diseases

The most serious diseases of avocado fruit are all caused by fungi. In Australia, anthracnose is predominantly caused by the fungus *Colletotrichum gloeosporioides*, although *C. acutatum* is a minor causal agent of the disease. Although symptoms of anthracnose do not normally appear in “Hass” fruit until ripening, the initial stages of infection by the pathogen actually occur in the field on developing fruit. Once the fungus penetrates the outer skin layer of fruit, it remains there in a dormant or 'quiescent' state until changes occur in the fruit skin which allow infection to proceed. The biochemical changes which occur during fruit ripening are thought to be the primary trigger for allowing infection to proceed. In particular, changes in the concentration of antifungal compounds called 'dienes' are thought to be important in the regulation of anthracnose quiescence in avocado (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. Ways in which these diene levels can be manipulated to delay anthracnose development will be discussed later in this paper.

Stem-end rot is another important postharvest disease of avocado which can be caused by a number of different fungi including *Dothiorella* spp., *Lasiodiplodia theobromae* and *Phomopsis perseae*. The anthracnose pathogen *C. gloeosporioides* can also cause a stem-end rot of avocado fruit. The relative importance of these different stem-end rot pathogens varies with different orchards and/or growing regions. For example, in some orchards *Dothiorella* spp. is the predominant cause of stem-end rot, whereas in others *C. gloeosporioides* may be the most important causal agent. The way in which the stem-end rot fungi infect avocado fruit has not been clearly established. One theory is that the fungi occur as "endophytes" of avocado stem tissue, gradually colonising inflorescences, fruit pedicels and then fruit stem-end tissue. Evidence for this mode of infection has been found in the case of mango stem-end rot (Johnson *et al*., 1992), however further studies are required to establish this in avocado. Another theory is that spores of the stem-end rot fungi infect avocado at flowering, eventually leading to colonisation of the fruit stem-end tissue. In either case, stem-end infections remain in a quiescent state until fruit ripening, and in that sense are similar to anthracnose. A third possible mode of infection for the stem-end rot fungi is at harvest time via the freshly cut surface of the fruit pedicel (Everett, 1999).

Pepper spot is a field disease of avocado fruit that has been increasing in importance over the past decade. It is particularly common on the cultivar "Hass", and affects fruit and stem tissue (e.g. leaf petioles, twigs and fruit pedicels). Symptoms appear as small, circular, shiny black lesions which are superficial and raised. Lesions initially appear on twigs, then develop on fruit pedicels and finally on the fruit skin. On "Hass" avocado fruit, lesions increase in number from around March onwards, such that large areas of the fruit surface may be covered by harvest time. The symptoms are often most severe on the upper sun-exposed side of the fruit. The disease appears to be more common on trees affected by Phytophthora root rot. *Colletotrichum gloeosporioides*, the causal agent of anthracnose, has been shown to also cause pepper spot (Willingham *et al.* 2000).

Sooty blotch is a field disease that is generally adequately controlled by the copper fungicide spray program used for anthracnose control. Although symptoms of the disease are only superficial, they can reduce the market value of fruit. Recently, the causal agent of this disease in Australia was shown to be *Stomiopeltis* sp. (K.G. Pegg, personal communication, 2001).

Management of avocado fruit diseases

Maintenance of healthy avocado trees

The physiological state of avocado trees has a major influence on the development of fruit diseases, which is not surprising since infection by the principal fruit pathogens of avocado is at least initiated in the orchard. Manipulating various physiological parameters therefore provides an ideal opportunity for managing these diseases. Previous studies have demonstrated that there is a high degree of variability between anthracnose levels on fruit harvested from individual "Hass" avocado trees in a single block (Coates *et al.*, 1996; Vuthapanich, 2001). Determining the causes of this variability will help us to develop effective control measures for avocado fruit diseases, particularly anthracnose. Whiley *et al.* (1997) proposed that rootstocks and mineral nutrition may be a factor in the observed variability in anthracnose susceptibility as well as other aspects of fruit quality. Certainly, links between calcium nutrition and anthracnose have been demonstrated. Vuthapanich (2001) reported that low calcium levels in "Hass" avocado fruit could be correlated with high anthracnose levels. Unfortunately, improving calcium levels in fruit is not as simple as applying more fertiliser. Calcium uptake into avocado fruit is poor and further studies are needed to investigate the factors which influence this.

The objective of our recent studies has been to investigate the link between rootstocks, tree nutrition, antifungal compounds and anthracnose susceptibility in "Hass" avocado. While the details of this work are presented elsewhere in these proceedings (Willingham *et al*), the main finding to date has been that rootstocks can have a major impact on anthracnose development, and that this can be related to differences in antifungal diene and mineral nutrient concentrations (Willingham *et al*., 2001). Over two seasons, anthracnose levels were significantly lower in fruit harvested from "Hass" grafted to "Velvick" rootstock than in those harvested from "Hass" grafted to "Duke 6" rootstock. Diene levels were lower and nitrogen/calcium ratios higher in leaves from the "Hass" / "Duke 6" combination than in those from the "Hass" / "Velvick" combination. Further work is currently underway to determine the influence of nitrogen fertiliser applications on anthracnose susceptibility and diene levels in "Hass" avocado grown on the two different rootstocks.

While not a new approach, maintenance of tree hygiene is another important strategy in the management of avocado fruit diseases. Spores of the fungi which cause avocado fruit diseases build-up in the canopies of avocado trees. As a warm, humid environment is most conducive to the build-up of these spores, it is beneficial to improve tree ventilation by reducing canopy density through pruning. All dead branches and leaves, as well as any infected fruit, should be regularly removed from tree canopies, as these can harbour large numbers of spores.

Fungicide application

Current control of avocado fruit diseases is heavily reliant on the application of fungicides, both in the field and after harvest. Copper-based fungicides such as copper hydroxide and copper oxychloride are applied in the field from fruit set to harvest for the control of anthracnose. This program will also give good control of pepper spot and sooty blotch as well as some control of stem-end rot.

While copper fungicide sprays generally give good disease control, there are some disadvantages and limitations. Because copper-based fungicides are protectant in their activity, they must be applied every 28 days (or every 14 days during wet weather) from fruit set to harvest in order to be effective for anthracnose control, making it a very labour intensive and costly practice. Furthermore, it may be difficult to maintain this protective cover adequately during prolonged wet weather. This may explain why copper sprays are not always effective in disease control.

Another issue is the build-up of visible copper residues on the skin of fruit, which can be difficult to remove on the packingline, particularly in rough-skinned cultivars such as “Hass”. The black skin of ripe “Hass” fruit also makes the residues more visible to consumers. We are currently addressing this issue in a copper fungicide field trial on “Hass” avocado being conducted on a northern New South Wales orchard. “Hass” avocados sprayed with a range of copper fungicides, including some new formulations (ie. Kocide® Blue, Kocide® Liquid Blue, Liquicop®) will be evaluated for visible fungicide residues on fruit at harvest time. These treatments are also being assessed for phytotoxic effects (in association with foliar phosphonate sprays) as well as disease control efficacy. Fruit will be harvested, ripened and assessed for disease in July/August 2001.

Preharvest applications of copper fungicides alone are generally not sufficient to control anthracnose, even when trees are well maintained. Postharvest application of prochloraz (Sportak®), which has some curative activity, can help control the anthracnose infections that copper sprays failed to prevent from establishing. Prochloraz is not effective, however, against the stem-end rot pathogens *Dothiorella* sp. and *Lasiodiplodia theobromae*.

New compounds for the control of avocado fruit diseases

Given the limitations of the currently recommended fungicide treatments for control of avocado fruit diseases, we have undertaken studies over the past four years to screen new compounds. The objective has not necessarily been to find a replacement for copper fungicides, but to find treatments which can be used in association with copper in a way which will reduce some of the current problems such as high spray frequency, variable disease control efficacy and visible residues. The strobilurins, a new group of fungicides derived from a naturally occurring compound from a mushroom, have been identified in our studies as compounds with considerable potential for the control of anthracnose, stem-end rot, pepper spot and sooty blotch in avocado. Although the strobilurins are fungicides, they have been described as “environmentally benign” as they are active at low concentrations, have a low toxicity to mammals and bees (so can be used in IPM programs), are non-persistent in the environment and break down readily in the soil (Willingham, 2001).

In a field trial conducted during the 1998/99 avocado season, three different strobilurin fungicides (azoxystrobin, trifloxystrobin and kresoxim-methyl) were evaluated for disease control on “Hass” avocado trees. Sprays were applied on a 28 or 14/28 day foliar spray schedule, commencing soon after fruit set and continuing until harvest time (see Table 1 for treatment details). These treatments were compared to the industry standard copper hydroxide spray program as well as to an integrated copper hydroxide + azoxystrobin program. Fruit were harvested at maturity, ripened and assessed for postharvest diseases. Fruit did not receive a postharvest prochloraz treatment. Table 2 shows the results of these postharvest assessments. The azoxystrobin formulation Amistar® (0.2 or 0.4 g/L as a 28 day spray) and the trifloxystrobin formulation Flint® (as a 14/28 day spray) significantly reduced anthracnose incidence compared to the untreated control. Although Amistar® at 0.4 g/L applied as a 14/28 day foliar spray halved the incidence of anthracnose compared to control fruit, the reduction was not statistically significant. Similarly, Kocide® (copper hydroxide) did not significantly reduce anthracnose incidence in this trial, although an integrated treatment of Kocide® + Amistar® was effective. The kresoxy-methyl formulation Stroby® was ineffective for anthracnose control. The only treatment to significantly reduce stem-end rot (which was predominantly caused by *Dothiorella* spp. in this trial) was the integrated Kocide® + Amistar® spray program.

In a separate trial on the cultivar "Fuerte", preharvest spray applications of Amistar® at 0.4 g/L on a 28 day spray schedule gave very good control of anthracnose compared to both untreated control fruit and fruit sprayed with Kocide® on a 28 day schedule (Table 3). Amistar® also gave effective control of sooty blotch on autumn flush branches (Table 3).

Since it is not feasible to apply strobilurin fungicides on a 28 day spray schedule due to the high risk of developing pathogen resistance, a field trial was conducted during the 1999/00 season to evaluate applying Amistar® or Flint® as part of an integrated spray program with Kocide®. In the first program, two Amistar® applications were made (28 days apart) during flowering and two (28 days apart) were made before harvest. For the remainder of the time, Kocide® was applied on a 14/28 day schedule. In the second program, Kocide® was applied on a 14/28 day schedule, except when three Amistar® or Flint ® applications were made (28 days apart) before harvest. Amistar® was tested at two concentrations (0.2 or 0.4 g/L) and with or without the addition of Bion®, a compound which reduces disease by inducing the fruit's natural defence mechanisms. Autumn flush branches were assessed for sooty blotch, and fruit were assessed for pepper spot prior to harvest. Harvested fruit were ripened and assessed for anthracnose and stem-end rot incidence and severity. Results are presented in Table 4. All of the treatments, except for Program 2 + Bion, had significantly lower incidences of anthracnose than the untreated control treatment. While not significantly different, Program 2 treatments had lower incidences of anthracnose than Program 1 treatments. In this trial, the current industry standard program of Kocide® was equally as effective as Program 1 and 2 treatments. The severity and incidence of stem-end rot (predominantly caused by *Dothiorella* spp.) was reduced by all of the treatments, except for the low rate of Amistar® in Program 2. All treatments significantly reduced the severity of pepper spot and sooty blotch.

Our studies have also shown that Amistar® is effective for the control of anthracnose when applied as a postharvest dip treatment. "Hass" avocado fruit were dipped in Amistar® for 10 minutes at either of two rates (0.125 or 0.25 g/L), with or without the addition of a wetting agent (Pulse®). Treatments consisting of Pulse® alone and the industry standard postharvest Sportak® treatment were included in the experiment for comparison purposes. Following treatment fruit were ripened at 22°C and assessed for anthracnose and stem-end rot. Results are presented in Table 5. The severity of anthracnose was significantly reduced by both concentrations of Amistar® when the wetting agent Pulse® was added to the dip suspension. Sportak® also reduced anthracnose significantly. None of the treatments significantly reduced stem-end rot levels, which were predominantly caused by *Dothiorella* spp.

It should be noted that while the strobilurins show considerable potential for the control of avocado fruit diseases, they are not currently registered for this use and as such should not be applied to avocado in any way.

Management of the postharvest storage environment

Postharvest storage conditions and practices can have a major influence on the development of anthracnose and stem-end rot. Fruit which are held beyond optimum "eating ripe" stage will very quickly develop high levels of disease. This is not an uncommon occurrence, particularly in "Hass" fruit where the thick skin can make it difficult to detect ripening. To avoid this problem, retailers need to rotate and sell fruit as quickly as possible, and where this is not possible, hold fruit at low temperatures (<4°C) once they are near ripe (Hofman and Ledger, 2001).

Disease development is also strongly influenced by ripening temperature. Hopkirk *et al.* (1994) reported that both body and stem rots of "Hass" avocado increased as the ripening temperature increased from 20 to 30°C, with only low levels of disease occurring at 15°C. Hofman (P.J. Hofman, 2000, unpublished results) found similar results, although noted that fruit retained significantly more green skin colour when ripened at 15 or 18°C than at 20°C. Recommending a ripening temperature therefore becomes a trade-off between disease and skin colour. The danger with fruit ripened at the lower temperatures is that retailers and consumers may mistakenly consider fruit to be unripe due to the presence of some green colour, and as a result hold fruit for longer times than they should.

By reducing fruit ripening time, controlled ripening using ethylene is another way to minimise postharvest disease development in avocado. Table 6 shows the effect of ethylene treatment on anthracnose and stem-end rot development in "Fuerte" avocado fruit. The severity of both diseases was significantly reduced by controlled ripening.

Future directions

Effective management of avocado fruit diseases requires an integrated approach incorporating a range of strategies such as appropriate rootstock selection, optimisation of nutrient application, maintenance of tree hygiene, enhancement of natural host resistance mechanisms, application of fungicides, postharvest temperature management and controlled ripening. Our future studies will focus further on the role of rootstock/scion combinations, nutrition, antifungal compounds, enzyme activity and fruit pH in the development of anthracnose, as well as evaluate new compounds (new copper formulations, strobilurins, host-defence promoting compounds, anti-gibberellins) for the control of field and postharvest diseases of avocado fruit. More basic studies will be undertaken on the biology of the anthracnose and pepper spot pathogens, particularly in relation to genetic diversity using molecular markers as well as infection processes.

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Table 1: Strobilurin and copper fungicide treatments applied as foliar sprays to "Hass" avocado trees during the 1998/99 season.

1	Untreated
2	Kocide® (ai. 500g/kg copper hydroxide, Shell) 2g/L as a 14 or 28 day foliar spray
3	Amistar® WG (ai. 500g/kg azoxystrobin, Zeneca) 0.4 g/L as a 14 or 28 day foliar spray
4	Amistar® WG (ai. 500g/kg azoxystrobin, Zeneca) 0.2 g/L as a 28 day foliar spray
5	Amistar® WG (ai. 500g/kg azoxystrobin, Zeneca) 0.4 g/L as a 28 day foliar spray
6	Stroby® WG (ai. 500g/kg kresoxim-methyl, BASF) 0.1 g/L as a 28 day foliar spray
7	Stroby® WG (ai. 500g/kg kresoxim-methyl, BASF) 0.2 g/L as a 28 day foliar spray
8	Kocide® (ai. 500g/kg copper hydroxide, Shell) 2 g/L blocked with Amistar® WG (ai. 500g/kg azoxystrobin, Zeneca) 0.4 g/L, ie. Kocide® applied commercially as a 14 or 28 day foliar spray and Amistar® blocked twice as a 28 day foliar spray during Dec, Jan and Feb and again in June, July and August.
9	Flint® (ai. CGA 279202) 0.07 g/L as a 14 or 28 day foliar spray

Table 2: Effect of field foliar sprays of strobilurin fungicides as compared to copper fungicides on postharvest disease development in "Hass" avocado fruit ripened at 22°C. All assessments were made at the "eating ripe" stage. Mean values followed by the same letter within columns are not significantly different at P < 0.05.

Treatment [1]	Anthracnose		Stem–end rot	
	severity (% surface area affected)	incidence (% fruit affected)	severity (% surface area affected)	incidence (% fruit affected)
Control	9.4[a]	39.3[a]	2.3[a]	10.8[a]
Kocide (14/28 d)	2.7[a]	25.9[abc]	0.7[a]	7.0[a]
Amistar 0.4 (14/28 d)	1.6[a]	21.2[abc]	2.3[a]	11.3[a]
Amistar 0.2 (28 d)	1.1[a]	14.8[c]	2.9[a]	10.2[a]
Amistar 0.4 (28 d)	1.1[a]	13.5[c]	1.2[a]	4.1[ab]
Stroby 0.1 (28 d)	7.1[a]	34.9[ab]	1.5[a]	6.3[a]
Stroby 0.2 (28 d)	6.1[a]	37.3[a]	0.8[a]	5.4[a]
Kocide+Amistar	0.8[a]	16.9[bc]	0.1[a]	0.9[b]
Flint (14/28 d)	0.4[a]	10.1[c]	1.1[a]	4.2[ab]

[1] see Table 1 for treatment details.

Table 3: Effect of field foliar sprays (28 day schedule) of Amistar® and Kocide® on sooty blotch on the autumn flush branches of "Fuerte" avocado trees and on postharvest diseases in "Fuerte" avocado fruit ripened at 22^{0}C. All fruit assessments were made at the "eating ripe" stage. Mean values followed by the same letter within columns are not significantly different at P < 0.05.

Treatment	Sooty blotch (1-4)[1]	Anthracnose		Stem-end rot	
		severity (% surface area affected	incidence (% fruit affected)	severity (% surface area affected)	incidence (% fruit affected)
Control	3.3^a	38.1^a	94.0^a	0.0^b	0.0^b
Kocide	2.2^b	27.3^a	87.1^a	0.1^b	1.8^a
Amistar	1.4^b	11.8^b	43.3^b	0.0^b	0.0^b

[1] 1-4 scale where 1 = no symptoms, 2 = mild symptoms, 3 = moderate symptoms and 4 = severe symptoms

Table 4: Effect of field foliar sprays of strobilurin fungicides applied in anti resistance blocking strategies with Kocide® on preharvest and postharvest disease development in "Hass" avocado. Sooty blotch was assessed on autumn flush branches, and pepper spot assessed on fruit prior to harvest. All postharvest fruit assessments were made at the "eating ripe" stage. Mean values followed by the same letter within columns are not significantly different at P<0.05. (NB. Program 1 = two amistars/flints at flowering and two before harvest; Program 2 = three amistars/flints before harvest)

Treatment	Pepper spot (1-6)[1]	Sooty blotch (1-4)[2]	Anthracnose		Stem-end rot	
			severity (%)[3]	incidence (%)[4]	severity (%)[3]	incidence (%)[4]
Untreated	3.1^a	3.9^a	3.6^a	22.9^a	2.4^a	8.9^a
2 g/L Kocide®	1.0^d	1.8^{bcd}	0.5^a	5.4^c	0.8^b	2.9^b
Program 1						
0.2 g/L Amistar®	1.7^b	1.8^{bcd}	1.0^a	11.7^{bc}	1.0^{ab}	4.6^b
0.4 g/L Amistar®	1.0^d	1.5^{cd}	0.6^a	10.1^{bc}	0.2^b	2.6^b
Program 2						
0.2 g/L Amistar®	1.1^{cd}	1.9^{bcd}	0.3^a	5.4^c	0.3^b	2.1^b
0.4 g/L Amistar®	1.1^{cd}	1.8^{bcd}	0.3^a	5.8^c	1.1^b	2.1^b
Program 2 + 0.05 g/L Bion						
0.2 g/L Amistar®	1.0^d	1.3^d	1.1^a	12.7^{abc}	0.6^b	2.1^b
0.4 g/L Amistar®	1.1^{cd}	2.0^{bc}	1.2^a	20.5^{ab}	0.4^b	2.0^b
Program 2						
0.07 g/L Flint®	1.6^{bc}	2.3^b	2.6^a	12.8^{bc}	0.2^b	2.3^b

[1] 1-6 scale where 1 = none; 2 = few small lesions scattered; 3 = moderate number of small lesions or few large lesions; 4 = many large spots scattered; 5 = fruit 1/4 covered with joined lesions; and 6 = fruit 50% or more covered with joined lesions.
[2] 1-4 scale where 1 = no symptoms, 2 = mild symptoms, 3 = moderate symptoms and 4 = severe symptoms.
[3] percentage fruit surface area affected. [4] percentage fruit affected.

Table 5: Effect of postharvest dip treatments of Amistar®, Pulse® and Sportak® on postharvest disease development in "Hass" avocado fruit ripened at 22^0C. All assessments were made at the "eating ripe" stage. Mean values followed by the same letter within columns are not significantly different at P < 0.05.

Treatment	Anthracnose		Stem-end rot	
	severity (% area affected)	incidence (% fruit affected)	severity (% area affected)	incidence (% fruit affected)
Control	52.7^{ab}	92.6	3.5^{b}	37.0^{bcd}
Amistar 0.25 g/L	32.6^{bcd}	81.5	2.4^{b}	27.8^{cd}
Amistar 0.25 g/L+Pulse	11.5^{d}	72.2	1.5^{b}	24.1^{d}
Amistar 0.125 g/L	45.3^{abc}	88.8	2.7^{b}	30.3^{cd}
Amistar 0.125 g/L+Pulse	17.9^{d}	84.8	9.2^{a}	45.2^{b}
Pulse	66.8^{a}	98.2	7.6^{a}	63.0^{a}
Sportak	29.4^{cd}	87.0	2.9^{b}	29.6^{cd}
Ethanol 10%[1]	46.4^{abc}	96.3	3.2^{b}	38.9^{bc}

[1] Experimental New Zealand treatment.

Table 6: Postharvest disease levels in "Fuerte" avocado fruit held at 24^0C following ethylene (100 ppm) ripening at 18^0C for 24 hours. Mean values followed by the same letter within columns are not significantly different at P<0.05.

Treatment	Anthracnose severity (% area affected)	Stem-end rot severity (% area affected)	Mean ripening time (days)
Air-ripened	5.1^{a}	3.2^{a}	8.3^{a}
Ethylene-ripened	1.1^{b}	0.2^{b}	6.1^{b}