TESTING EFFICACY OF FUNGICIDES AGAINST POSTHARVEST PATHOGENS OF AVOCADO (*PERSEA AMERICANA* CV. HASS)

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ABSTRACT

Currently there are only three fungicides registered for use on avocado. Two of these (copper and benomyl) are for orchard use and one (prochloraz) is for postharvest use. Benomyl has now been withdrawn from sale. To test whether on-orchard control of rots could be improved, some fungicides not currently registered for avocados were screened for inhibition of spore germination and mycelial growth in the laboratory. Fungicides tested were azoxystrobin, benomyl, captan, copper hydroxide, fluazinam, kresoxim-methyl, prochloraz, pyrimethanil and trifloxystrobin. On the basis of these results, azoxystrobin, benomyl, copper hydroxide, fluazinam, kresoxim-methyl and prochloraz were selected for further efficacy testing on the orchard. Carbendazim was also tested in the field as a replacement for benomyl. Copper hydroxide gave best rot control followed by azoxystrobin and carbendazim.

Keywords: avocado, fungicide, orchard, spray, efficacy.

INTRODUCTION

Replicated spray trials in Australia, South Africa and New Zealand have demonstrated that regular application of copper fungicides in the orchard can reduce postharvest rots of avocado (*Persea americana* Mill.) (Peterson & Inch 1980; Darvas 1981; Everett 2003). On the basis of these results at least eight orchard applications of copper fungicides per season have been recommended for New Zealand fruit exported to USA (Anon. 2000).

Currently the only fungicides registered for use on avocados in New Zealand are benomyl, copper in its various formulations (e.g. copper hydroxide, copper oxychloride and copper sulphate) and prochloraz. However, only copper can be used on orchards because benomyl has recently been withdrawn from sale, and prochloraz is only registered for postharvest use. An alternative to benomyl is its metabolite carbendazim (Rouchaud et al. 1977), which is not currently registered for avocados, but is commercially available. Because copper fungicides are a heavy metal and can accumulate in the soil to undesirably high levels their use is not necessarily sustainable.

Five fungi have been identified as being important pathogens of avocado in New Zealand (Hartill 1991). These are *Colletotrichum acutatum*, *C. gloeosporioides*, *Botryosphaeria parva*, *B. dothidea* and *Phomopsis* sp. Each of these fungi can cause postharvest diseases of avocado, infecting either through the side of the fruit (body rots) or through the picking wound (stem-end rots). Field isolates of these fungi were used to test nine fungicides *in vitro* for their effect on spore germination and mycelial growth. On the basis of these results six fungicides were selected for field-testing as orchard sprays against body rot and stem-end rot diseases of avocado. Carbendazim was also tested. The rationale for this study was two-fold: to improve control by testing alternatives to currently registered fungicides and to provide a more environmentally friendly alternative to copper for rot control.

METHODS

In vitro fungicide testing

A total of nine fungicides were selected for testing. These were azoxystrobin, benomyl, captan, copper hydroxide, fluazinam, kresoxim-methyl, prochloraz, pyrimethanil and trifloxystrobin. When this trial began benomyl was still available, which is the reason it, and not carbendazim, was tested *in vitro*. Fungicide groups and active ingredients (ai) are listed in Table 1.

Colletotrichum acutatum, *C. gloeosporioides*, *B. parva*, *B. dothidea* and *Phomopsis* sp. were grown on Difco® potato dextrose agar for 3-6 weeks under lights (12:12 h light:dark). Spores were harvested in sterile distilled water and spore concentration was adjusted to 10^5 spores/ml with the aid of a haemocytometer. Fungicide stock solutions were made in sterile deionised water to a concentration of 1000 and 10,000 µg ai/ml, by w/v for solid formulations and v/v for liquid formulations. The appropriate amount of fungicide stock solutions were added to 10 ml of 1.5% w/v water agar in a 5 cm diameter sterile plastic Petri plate to achieve final concentrations of 0, 1, 10, 100 and 1000 µg/ml (three replicate plates per concentration). A volume of 100 µl of spores (10^5 spores/ml) was placed on the surface of each Petri plate. After 24 h at 20°C, the total number of spores in three microscope fields at a magnification of x100 were counted and the number of germinating spores was recorded (ca 20 spores per microscope field of view). Percent spore germination per plate was calculated using the mean of three fields of view. At high fungicide concentrations, spores were stained with cotton blue to facilitate visualisation.

Fungal cultures were grown as for the spore germination tests, but mycelial plugs were removed from the edge of actively growing colonies (1-2 weeks growth) with a sterilised 5 mm diameter cork borer and placed on Difco® potato dextrose agar (PDA) amended with the test fungicides. Media were not pH adjusted. A preliminary test established that the concentrations most appropriate for this test were 0, 1, 10, 100 and 1000 µg ai/ml. Mycelial diameter was measured every 1-2 days for 10 days and plotted against time. Comparisons were made on the basis of mycelial growth rate, in mm/day, for the linear phase of the fungal growth curve. There were three replicate plates per treatment.

The concentrations at which 50% of spores failed to germinate (EC_{50}) were calculated by the following method. Logit transformations ($\text{logit} = \ln\{p/(1-p)\}$) of spore germination (as a proportion of germination on unamended agar) and mycelial growth (as a proportion of growth on unamended media) averaged over the three replicate plates were plotted against the logarithmic transformation of fungicide concentration to linearise the response. The slope of the linear portion of the transformed data was calculated by linear regression. The EC_{50} was calculated from each linear regression equation for $Y=0$, that is the logit for $p = 0.5$ or 50%. A constant value of 0.01 was added to non-transformed data to enable 0 and 100% values to be used in the calculations.

Orchard trial

The field trial was carried out on an orchard block in the Bay of Plenty (Walker Road West, Katikati) with fruit-bearing trees (5-6 years old) suitable for individual spray applications. The block was surrounded by shelter and was adjacent to a mature block of avocado trees.

Eight treatments were applied on eight occasions at recommended rates (Table 1), on the 16th of each appropriate month, starting in December 1999 and finishing December 2000. The eight months that fungicides were applied were December, January, February, August, September, October, November and December. Treatments were eight applications of (1) fluazinam, (3) azoxystrobin, (4) low kresoxim-methyl (2.5 g ai/100 litres), (5) high kresoxim-methyl (5 g ai/100 litres), (6) carbendazim, (7) prochloraz, (8) copper hydroxide and (9) an untreated control. Treatment 2 consisted of a combination of several products for the eight applications, as follows: fluazinam was applied first, followed by three applications of fluazinam in combination with prochloraz, then one application of fluazinam, two applications of benomyl with azoxystrobin and finally one application of fluazinam. The trial was in a randomised block design, with five replicate trees per treatment. Sprays were applied with a pressurised hand gun sprayer.

TABLE 1: Fungicides tested and described according to active ingredient, chemical group and application rate when used in field testing.

Fungicide	Active ingredient	% ai	Chemical group	Recommended application rate (µg ai/ml)	Rates used (ai/100 litres)
Shirlan®	fluzinam	50	pyridinamine	500	50 ml
Amistar® SC	azoxystrobin	50	strobilurin	50	5 g
Stroby® WG	kresoxim-methyl	50	strobilurin	25 and 50	2.5 g and 5 g
Benlate®	benomyl	50	benzimidazole	250	25 g
Bavistin® DF	carbendazim	50	benzimidazole	250	25 g
Sportak®	prochloraz	45	imidazole	450	45 ml
Blue Shield® DF	copper hydroxide	50	inorganic copper	2000	200 g
Flint®	trifloxystrobin	50	strobilurin	50	n.f.t. ¹
Scala®	pyrimethanil	40	anilinopyrimidine	300	n.f.t.
Captan®80W	captan	80	cyclic imide	1000	n.f.t.

¹not field tested

Fruit were harvested on 17 January 2001. Twenty fruit were picked and packed into 20 count avocado boxes from each of five replicate trees per treatment, a total of 100 fruit per treatment. Fruit were placed in cool storage (5.5°C) for 28 days then ripened at 20°C.

Ripening fruit was tested for firmness by gentle hand squeezing each day after placement at 20°C. When ripe, fruit were cut into quarters and peeled. Fruit were assessed for body rot symptoms by recording coverage of skin as a percentage of total skin area affected (0–10 scale). Stem end rots were scored on a 0–10 scale. Data are presented as incidence (percentage of fruit affected) and severity (the individual scores of coverage of fruit by rots from 0–10) and as incidence x severity {(incidence/100) x severity}.

All data were analysed using the GLM analysis of variance function of SAS and means were separated by Fisher's protected least significant difference test.

RESULTS

In vitro fungicide testing

The fungicide that consistently and most effectively inhibited spore germination for four of the five fungi tested (*C. acutatum*, *B. parva*, *B. dothidea* and *Phomopsis* sp.) was fluzinam at a concentration of 0.1 µg/ml (Table 2). The strobilurins were the next most effective fungicide group, and of these fungicides, kresoxim-methyl was the most effective, at concentrations ranging from 0.1 to 1.9 µg/ml. Copper hydroxide inhibited spore germination of all the fungi tested but only at high concentrations (2.4 to 11.6 µg/ml). Benomyl did not inhibit spore germination of *Botryosphaeria* spp. Prochloraz and pyrimethanil were only effective against the *Botryosphaeria* spp. at the highest concentration tested (369.6, 913.1, 562.3 and 1147.2 µg/ml, Table 2). Benomyl was more effective against *C. gloeosporioides* than against *C. acutatum*.

In contrast to the results of spore germination inhibition, the strobilurin group of fungicides only proved effective against mycelial growth of *C. acutatum* (Table 3). They were either not effective (trifloxystrobin against *B. parva*) or only moderately effective inhibitors of mycelial growth of *B. parva*, *B. dothidea*, *Phomopsis* sp. and *C. gloeosporioides*. All other fungicides tested inhibited mycelial growth of the five fungi included in the study to some degree.

Of the fungicides tested, prochloraz was the most effective inhibitor of mycelial growth of four of the five fungi tested (*C. acutatum*, *C. gloeosporioides*, *B. dothidea* and *Phomopsis*) at rates ranging from 0.29 to 0.36 µg/ml. Fluzinam was next most effective against *C. acutatum* and *C. gloeosporioides* at rates of 7.8 and 1.7 µg/ml, respectively, and benomyl against *B. parva*, *B. dothidea* and *Phomopsis* sp. (0.1 to 1.61 µg/ml).

Orchard trial

Overall the best fungicide for reducing both stem-end rot and body rot symptoms was copper hydroxide (Table 4), resulting in 74% fewer stem-end rots and 49% fewer body rots (calculated as {(untreated-treated/untreated) x 100}). Azoxystrobin was the next most effective fungicide for reducing body rot symptoms, significantly reducing severity and incidence x severity scores, and reducing incidence by 37%. For severity and incidence x severity scores, all fungicides except kresoxim-methyl at the high rate and fluazinam significantly reduced body rot symptoms. Stem end rot incidence x severity score was significantly reduced compared to the untreated control by azoxystrobin, carbendazim and copper hydroxide, and incidence by copper hydroxide.

TABLE 2: EC_{50}^1 required to inhibit spore germination of five avocado postharvest fungal pathogens.

Fungicide	<i>C. acutatum</i>	<i>C. gloeosporioides</i>	<i>B. parva</i>	<i>B. dothidea</i>	<i>Phomopsis</i> sp.
azoxystrobin	0.1	0.2	1.4	2.9	11.9
benomyl	7.9	0.3	n.e. ²	n.e.	15.5
copper hydroxide	3.0	2.4	3.6	4.2	11.6
captan	0.1	9.5	13.2	28.8	3.3
trifloxystrobin	1.3	7.4	1.1	3.5	5.2
pyrimethanil	1.3	3.3	562.3	1147.2	34.1
fluazinam	0.1	1.9	0.1	0.1	0.1
prochloraz	0.5	31.6	369.6	913.1	35.4
kresoxim-methyl	0.6	0.1	1.9	0.3	0.3

¹Effective concentration (µg/ml) at which 50% of spores fail to germinate.

²Not effective.

TABLE 3: EC_{50}^1 required to inhibit mycelial growth of five avocado postharvest fungal pathogens.

Fungicide	<i>C. acutatum</i>	<i>C. gloeosporioides</i>	<i>B. parva</i>	<i>B. dothidea</i>	<i>Phomopsis</i> sp.
azoxystrobin	7.8	100.0	1067.3	165.9	2080.6
benomyl	37.0	1.9	0.4	1.6	0.1
copper hydroxide	1114.2	131.9	587.0	316.2	652.9
captan	121.9	107.2	325.4	259.7	224.7
trifloxystrobin	8.3	150.6	n.e. ²	236.1	757.9
pyrimethanil	1018.7	289.1	804.7	101.8	412.5
fluazinam	7.8	1.7	6.6	4.3	0.5
prochloraz	0.3	0.3	8.3	0.4	0.3
kresoxim-methyl	8.1	46.2	136.3	23.7	39.8

¹Effective concentration (µg/ml) at which 50% of mycelial growth is inhibited.

²Not effective.

TABLE 4: Incidence, severity and incidence x severity scores of body rot and stem-end rot symptoms from ripened avocado fruit treated in the field with different fungicide regimes applied eight times during the season.

Treatment	Body rots			Stem end rots		
	Incidence (%)	Severity (0-10)	(Incidence /100) x severity	Incidence (%)	Severity (0-10)	(Incidence /100) x severity
1. fluazinam	46 bc	0.30 a	0.14 ab	32 abc	0.36	0.11 ab
2. mixture ¹	47 bc	0.16 cd	0.07 cde	26 abc	0.59	0.15 ab
3. azoxystrobin	36 bc	0.09 d	0.03 e	21 bc	0.35	0.07 b
4. low kresoxim-methyl	49 abc	0.16 cd	0.08 cde	36 abc	0.39	0.14 ab
5. high kresoxim-methyl	73 a	0.20 bc	0.15 a	42 ab	0.52	0.22 a
6. carbendazim	52 bc	0.18 c	0.09 bc	20 bc	0.34	0.07 b
7. prochloraz	54 ab	0.15 cd	0.08 cd	47 a	0.47	0.22 a
8. copper hydroxide	29 c	0.14 cd	0.04 de	10 c	0.53	0.05 b
9. untreated	57 ab	0.26 ab	0.15 a	38 ab	0.56	0.21 a
P-value	P=0.06	P<0.0001	P<0.0001	P=0.07		P=0.03
LSD	28.2	0.08	0.05	30.9	n.s.	0.11

¹The eight applications were: (1) fluazinam, (2, 3 & 4) fluazinam and prochloraz, (5) fluazinam, (6 & 7) benomyl and azoxystrobin and (8) fluazinam.

DISCUSSION

In other studies on fungicide control of avocado rots, copper and azoxystrobin were also the most effective fungicides for orchard control of postharvest rots of avocados. For example, in Queensland, Peterson & Inch (1980) reduced infection by body rots (caused almost exclusively by *C. gloeosporioides*) by 91% by applying copper oxychloride every 2 or 4 weeks throughout the season. Willingham et al. (2001) likewise reduced body rots by 7.3%, 65.6% and 76% following application of copper hydroxide at 14- or 28-day intervals in three field trials in Queensland. Failure of control in one trial (7.3%) was explained by high frequency of rain washing off the copper. The frequency of application in Queensland, Australia, was higher than was used in the orchard trial in New Zealand, and was higher than the eight per season (a season is 12-18 months long) recommended for the New Zealand avocado industry. In two of the three Australian experiments azoxystrobin reduced body rots more effectively than did copper hydroxide, whereas in the present study copper hydroxide was the most effective fungicide tested. Similar to the results of the current study, kresoxim-methyl did not perform as well in the Australian field study as either azoxystrobin or copper hydroxide. Stem end rots were reduced 35.2%, 34.5% and increased, for the three field trials, following application of copper hydroxide in Australia (Willingham et al. 2001). Best control of stem end rots was achieved by a combination of copper hydroxide and azoxystrobin (92%), and of body rots with copper hydroxide (76%). Despite a higher frequency of applications, on one site control was less than was achieved in the present trial. Although these results cannot be applied directly to New Zealand conditions because the relative importance of the pathogens and the climate are different between the two countries (Everett et al. 2000), control of rots in New Zealand may be further improved by increasing the number of fungicide applications and by applying copper hydroxide in combination with azoxystrobin.

Copper hydroxide and the strobilurins did not inhibit mycelial growth of some fungi *in vitro*, and yet performed well in the field compared to some fungicides that did inhibit

mycelial growth of all fungi. In contrast, benomyl did not inhibit spore germination of either *Botryosphaeria* species, and yet its metabolite, carbendazim, significantly reduced both stem-end rots and body rots in the field. From these results it seems that the mechanism of inhibition of fungal infection of avocado fruit is not important. That is, either mycelial growth (benomyl), or spore germination (copper hydroxide and the strobilurins) may be inhibited leading to effective control. However, fluazinam inhibited both mycelial growth and spore germination of all fungi tested, but did not perform well in the field. In a series of comparative tests Everett & Neilson (1996) showed that results of *in vitro* testing could eliminate those fungicides not worthy of further testing, but that *in vivo* testing was required to enable the best fungicide to be selected for application on plants. Testing in the field is important for other factors not tested *in vitro*, such as retention on leaf and fruit surfaces (Gent et al. 2003) and stability to UV light (Boudina et al. 2003), that can also influence fungicide effectiveness. The ineffectiveness of fluazinam in the field may have been because of these factors or due to a compound in the formulation that increased susceptibility of avocado fruit to infections by fungi. Alternatively, this fungicide may have detrimentally affected populations of microbes on the fruit surface that were inhibiting the rot fungi, as has been described for copper fungicides on avocado leaf microflora (Stirling et al. 1999).

Results of the orchard testing show that carbendazim could be used as a substitute for the systemic fungicide benomyl due to its effectiveness for rot control. Carbendazim, as a metabolite of benomyl, also has a systemic mode of action (Leroux & Gred 1974). Thus carbendazim could be applied during flowering by growers instead of benomyl to control *Phomopsis* (Everett et al. 1999). Carbendazim, but not benomyl, is available for purchase.

In summary, copper hydroxide was the best fungicide tested. Continued use of this fungicide is recommended, since it is relative inexpensive, copper is already registered for use on avocado and application is currently recommended practice. Carbendazim, prochloraz, kresoxim-methyl at 5 g/100 litres and azoxystrobin were also effective controls for postharvest rots of avocados and could be considered by growers as alternatives to copper if environmental considerations become a priority. Carbendazim could be used as a substitute for benomyl as that chemical is no longer available for purchase.

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