The effect of longer spray intervals, reduced rates, or mixtures of three fungicides on fruit rots in strawberry

W.S. Washington[A], **S. Engleitner**[B] and **N. Shanmuganathan**[C]
[A] Department of Natural Resources and Environment, Agriculture Victoria, Institute for Horticultural Development, Knoxfield, Private Bag 15, South Eastern Mail Centre, Victoria 3176, Australia.
[B] Present address: State Chemistry Laboratory, Sneydes Road, Werribee, Victoria 3030, Australia.
[C] Retired.

Summary

Three field experiments tested the effect of thiram, iprodione and dichlofluanid for the control of fruit rots in strawberry crops in Victoria, Australia. Each fungicide was applied at full label rate at 7 day or 14 day intervals, or at half label rate at 7 day intervals. In addition, half label rate mixtures of thiram and iprodione or dichlofluanid and iprodione were tested at 7 day intervals. Grey mould, caused by *Botrytis cinerea*, was the most common fruit rot in two of the three experiments (31.7 and 22.5% of fruit rotted in unsprayed plots) while anthracnose, caused by *Colletotrichum acutatum*, was the most common rot in one experiment (28.3% of fruit rotted in unsprayed plots). The most consistent control when all rots were combined was achieved by half rate mixtures of either dichlofluanid with iprodione (reducing all rots from 39.1 to 4.9% in one trial) or thiram with iprodione (reducing all rots from 39.1 to 7.4% in the same trial), each applied at 7 day intervals. Half rates and 14 day spray intervals of most other treatments gave significant control of rots in most cases with the exception of iprodione in two of the experiments. Iprodione when sprayed alone at these sites failed to significantly reduce grey mould or total fruit rots, providing further evidence of the presence of dicarboximide resistant strains of *B. cinerea* in Victorian strawberry crops. Residue testing of fruit from one trial showed that residues of iprodione and dichlofluanid, but not thiram, were within the current approved maximum residue limits.

Introduction

The most common diseases affecting strawberry fruit in Victoria are grey mould caused by *Botrytis cinerea* Pers.:Fr., anthracnose or blackspot caused by *Colletotrichum acutatum* Simmonds, leather rot caused by *Phytophthora cactorum* (Leb. & Cohn) Schroet., leak caused by *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. and other Mucoraceous fungi, and stem end rot caused by *Gnomonia comari* Karst (Washington *et al.* 1992, Washington and Shanmuganathan 1993). In Victoria, as in most other strawberry production areas, these diseases are largely controlled by intensive spraying of fungicides during the cropping season (Maas 1984). In recent years, however, the public perception of fungicides is that they have excessively adverse effects on human health and the environment (Ragsdale and Sisler 1994). As a result, there is considerable pressure to reduce fungicide use in horticulture.

Fungicides have been registered at rates which provide reliable efficacy even under conditions of high disease pressure. As a result, under lower disease pressure, scope may exist for use of lower rates (Meland 1988) or longer spray intervals as a way to minimize fungicide use while maintaining disease control. This study investigated the use of lower rates, longer spray intervals or reduced rate mixtures of fungicides as a way to optimize fungicide use in strawberry crops in Victoria, Australia.

Methods

Three experiments were established on commercial strawberry farms, at Wandin (about 40 km east of Melbourne), Main Ridge (about 70 km south of Melbourne) and Coldstream (about 40 km east of Melbourne and 10 km north of the Wandin experiment).

The fungicides tested were thiram (Agchem Thiram 800, 800 g kg^{-1} a.i., Incitec Ltd.), iprodione (Rovral, 500 g kg^{-1} a.i., Rhône-Poulenc Rural Australia Pty. Ltd.) and dichlofluanid (Euparen, 500 g kg^{-1} a.i., Bayer Australia Ltd.). Each fungicide was sprayed at full rate (as found on the registered product label) at a 7 day interval, at full rate at a 14 day interval and at half rate at a 7 day interval. Half rate mixtures of thiram and iprodione and of dichlofluanid and iprodione, each at a 7 day interval, were also tested.

Experiment 1

This experiment was conducted at Wandin in autumn 1990 on three-year-old strawberry plants, cv. Red Gauntlet. Plants were grown in double rows at 60 cm spacing on raised beds covered with black polyethylene. Irrigation was by trickle and overhead sprinklers.

Sprays were applied with a hand operated knapsack sprayer at 7 or 14 day intervals using approximately 1700 L ha^{-1}. Treatments were arranged in a randomized block design with plots of 12 plants, each replicated five times. A total of 4 (at 14 day intervals) or 8 (at 7 day intervals) sprays were applied between 12 April and 1 June 1990. Fifty mature fruit were sampled at random from each treatment (10 per plot) on 10 May, 18 May, 25 May and 8 June. Fruit were placed in plastic ice-block trays, one per compartment, inside sealed plastic bins lined with moist blotting paper, and incubated for three days at room temperature. The number and type of rotted fruit was recorded and the incidence of fruit rots was calculated by combining data from all sampling dates.

Experiment 2

This experiment was conducted at Main Ridge in the same way as experiment 1 except that plants were two-year-old, fungicides were sprayed 6 (at 14 day intervals) or 11 (at 7 day intervals) times between 16 March and 23 May 1990, and fruit were sampled on 18 April, 2 May, 9 May, 16 May, 23 May and 30 May.

Experiment 3

This experiment was conducted at Coldstream in the same way as experiment 1 except that plants were three-year-old cv. Tioga. Fungicides were sprayed 5 (at 14 day intervals) or 9 (at 7 day intervals) times between 1 October and 27 November 1990, and fruit were sampled on 5 November, 13 November, 15 November, 19 November, 23 November, 27 November and 4 December. Samples of 20 fruit per plot were harvested from all plots of each treatment on 27 November (before the next spray was applied and eight days after the previous spray), frozen at -20°C before being analysed at the State Chemistry Laboratories, Melbourne. Thiram residues were determined by reducing the dithiocarbamate present by heating the sample with hydrochloric acid/stannous chloride in a sealed container. Carbon disulphide liberated from dithiocarbamate was measured by headspace gas-liquid chromatography using a flame photometric detector in the sulphur mode. Iprodione residues were determined by extraction of the sample in acetone, filtering the extract, diluting with aqueous sodium sulphate solution and partitioning the analytes into dichloromethane. Analytes were cleaned up on an activated florisil column before determination by gas chromatography using a nitrogen selective detector. Dichlofluanid residues were determined by similar methods as for iprodione (above) except that after partitioning, the dichloromethane was then dried and inverted to hexane. Determination was by gas chromatography using a nitrogen selective detector (G. Roberts, personal communication).

Results

In the untreated plots of experiment 1, *B. cinerea* was the most common cause of fruit rot (22.5%), followed by Mucoraceous fungi (2.5%). Small numbers of rots caused by other fungi made up a total of 33.0% rotted fruit (Table 1). All treatments except two thiram treatments alone and one iprodione treatment alone reduced the incidence of total fruit rots. All treatments except two of the three thiram treatments applied alone reduced the level of grey mould. No trends were apparent with the control of leak. Thiram at the 14 day spray interval was less effective against grey mould than at the 7 day interval. Half rate mixtures of thiram and iprodione gave similar control of grey mould as half rate iprodione but were better than the full rate iprodione schedules and all schedules of thiram alone. Half rate mixtures of dichlofluanid and iprodione were more effective against grey mould than all thiram schedules and the two full rate iprodione schedules, but were not more effective than all the dichlofluanid schedules. The best control of all rots was achieved by a half rate mixture of dichlofluanid and iprodione, followed by the full rate dichlofluanid schedules.

In the untreated plots of experiment 2, *B. cinerea* was the most common cause of fruit rot (31.7%), followed by Mucoraceous fungi (7.3%). Small numbers of rots caused by other fungi made up a total of 48.3% rotted fruit (Table 2). Both fungicide combinations, two of the three thiram and two of the three dichlofluanid treatments reduced the incidence of total fruit rots. Similarly both fungicide combinations, all thiram treatments and two of the three dichlofluanid treatments reduced grey mould. None of the iprodione treatments alone reduced grey mould. By contrast, only the iprodione treatments when applied alone or in combination with other fungicides, and the highest rate of dichlofluanid reduced levels of rot caused by Mucoraceous fungi. All thiram treatments either alone or in mixture gave the best control of grey mould. The two half rate mixture schedules gave the lowest overall rot levels, but were not significantly better than all other treatments except iprodione at a 14 day schedule.

In the untreated plots of experiment 3, *C. acutatum* was the most common cause of fruit rot (28.3%), followed by *B. cinerea* (5.7%), *P. cactorum* (3.1%) and Mucoraceous fungi (0.6%). Small numbers of rots caused by other fungi made up a total of 39.1% rotted fruit (Table 3). Each fungicide and fungicide combination significantly reduced the incidence of total fruit rots and grey mould, compared with the untreated control, with the exception of all

Table 1. Effect of different rates and spray intervals of three fungicides on the incidence of fruit rot of strawberry at Wandin in autumn 1990.

Treatment	Timing (days)	a.i. 100 L^{-1} (g)	Grey mould (%)	Leak (%)	Total (%)
Thiram	7	120	9.5 (0.309)[A]	4.0 (0.152)[A]	16.0 (0.403)[A]
Thiram	14	120	19.0 (0.442)	2.0 (0.109)	23.5 (0.497)
Thiram	7	60	14.0 (0.382)	6.5 (0.252)	25.0 (0.522)
Iprodione	7	50	10.5 (0.324)	3.5 (0.146)	22.5 (0.483)
Iprodione	14	50	8.0 (0.278)	5.5 (0.210)	17.5 (0.429)
Iprodione	7	25	4.0 (0.174)	7.0 (0.204)	19.0 (0.449)
Dichlofluanid	7	100	2.5 (0.096)	2.5 (0.119)	9.0 (0.297)
Dichlofluanid	14	100	3.0 (0.154)	3.5 (0.141)	8.5 (0.260)
Dichlofluanid	7	50	2.0 (0.127)	5.5 (0.223)	14.5 (0.379)
Thiram and Iprodione	7	60 and 25	3.0 (0.135)	4.5 (0.209)	12.0 (0.343)
Dichlofluanid and Iprodione	7	50 and 25	1.5 (0.077)	1.0 (0.045)	4.5 (0.186)
Untreated			22.5 (0.490)	2.5 (0.119)	33.0 (0.610)
LSD (P=0.05)			(0.125)	NS	(0.137)

[A] Figures in parentheses are means of the arcsine-transformed proportions.

Table 2. Effect of different rates and spray intervals of three fungicides on the incidence of fruit rot of strawberry at Main Ridge in autumn 1990.

Treatment	Timing (days)	a.i. 100 L^{-1} (g)	Grey mould (%)	Leak (%)	Total (%)
Thiram	7	120	16.2 (0.410)[A]	4.7 (0.193)[A]	29.7 (0.558)[A]
Thiram	14	120	13.7 (0.360)	3.3 (0.164)	28.7 (0.556)
Thiram	7	60	14.3 (0.378)	12.0 (0.326)	36.0 (0.639)
Iprodione	7	50	26.7 (0.522)	1.7 (0.082)	32.3 (0.583)
Iprodione	14	50	46.3 (0.748)	1.0 (0.063)	53.0 (0.816)
Iprodione	7	25	27.3 (0.545)	2.3 (0.116)	37.7 (0.656)
Dichlofluanid	7	100	18.7 (0.440)	1.0 (0.078)	26.7 (0.539)
Dichlofluanid	14	100	21.7 (0.477)	6.0 (0.232)	36.3 (0.640)
Dichlofluanid	7	50	17.7 (0.425)	2.3 (0.134)	27.0 (0.543)
Thiram and Iprodione	7	60 and 25	13.7 (0.374)	1.0 (0.078)	24.0 (0.497)
Dichlofluanid and Iprodione	7	50 and 25	18.7(0.447)	1.0 (0.045)	23.7 (0.493)
Untreated			31.7 (0.597)	7.3 (0.259)	48.3 (0.769)
LSD (P=0.05)			(0.142)	(0.130)	(0.187)

[A] Figures in parentheses are means of the arcsine-transformed proportions.

Table 3. Effect of different rates and spray intervals of three fungicides on the incidence of fruit rot of strawberry at Coldstream in spring 1990.

Treatment	Timing (days)	a.i. 100 L^{-1} (g)	Grey mould (%)	Black spot (%)	Leather rot (%)	Leak (%)	Total (%)
Thiram	7	120	0.6 (0.048)[A]	2.9 (0.126)[A]	0.0 (0.0)[A]	0.0 (0.0)[A]	4.3 (0.179)[A]
Thiram	14	120	0.9 (0.072)	1.4 (0.090)	0.0 (0.0)	1.1 (0.066)	4.3 (0.204)
Thiram	7	60	0.6 (0.048)	2.9 (0.148)	0.0 (0.0)	1.4 (0.076)	6.0 (0.210)
Iprodione	7	50	3.1 (0.175)	20.0 (0.460)	5.4 (0.201)	0.3 (0.024)	30.0 (0.578)
Iprodione	14	50	7.4 (0.267)	17.1 (0.426)	5.1 (0.199)	0.0 (0.0)	32.3 (0.603)
Iprodione	7	25	4.3 (0.157)	24.3 (0.506)	2.6 (0.124)	0.0 (0.0)	32.9 (0.609)
Dichlofluanid	7	100	1.1 (0.066)	3.7 (0.184)	0.0 (0.0)	2.6 (0.093)	7.7 (0.270)
Dichlofluanid	14	100	0.6 (0.034)	2.0 (0.124)	0.0 (0.0)	1.1 (0.066)	4.9 (0.216)
Dichlofluanid	7	50	1.7 (0.116)	4.0 (0.178)	0.0 (0.0)	0.6 (0.034)	7.1 (0.257)
Thiram and Iprodione	7	60 and 25	1.7 (0.082)	4.6 (0.207)	0.6 (0.048)	0.0 (0.0)	7.4 (0.273)
Dichlofluanid and Iprodione	7	50 and 25	1.4 (0.090)	2.3 (0.088)	0.0 (0.0)	0.0 (0.0)	4.9 (0.197)
Untreated			5.7 (0.239)	28.3 (0.588)	3.1 (0.141)	0.6 (0.034)	39.1 (0.675)
LSD (P=0.05)			(0.105)	(0.123)	NS (0.123)[B]	NS	(0.104)

[A] Figures in parentheses are means of the arcsine-transformed proportions.
[B] Approximate LSD (P=0.05) for comparing non-zero mean with zero mean = LSD (non-zero mean) / $\sqrt{2}$.

Table 4. Fungicide residues (µg g^{-1} fresh weight) in strawberries harvested 7 or 14 days after the previous spray for the 7 or 14 day schedules, respectively, after spraying three fungicides at Coldstream in spring 1990.

Treatment and number of sprays	Number of sprays	Thiram	Iprodione	Dichlofluanid
Full rate[A] / 7 day	8	19.6	6.9	5.6
Full rate / 14day	4	9.8	4.6	6.4
Half rate / 7 day	8	7.5	5.2	2.3
Thiram / iprodione half rate mix / 7 day	8	7.5	5.1	–
Dichlofluanid / iprodione half rate mix / 7 day	8	–	4.5	4.8
LSD (P=0.05)		5.4	NS	NS

[A] See table 1 for a.i. of each fungicide.

treatments with iprodione alone. Leather rot was completely controlled by all thiram and dichlofluanid treatments, with the exception of the mixture of thiram and iprodione, but iprodione alone failed to control this disease. No trends were apparent with the low levels of Mucoraceous rots in this experiment.

Residue analysis showed that thiram residues in fruit were approximately halved in fruit harvested from either the full rate 14 day spray interval (9.8 µg g^{-1}) or half rate at 7 day interval (7.5 µg g^{-1}) when compared with fruit from full rate 7 day spray intervals (19.6 µg g^{-1}) (Table 4). Fungicide residues of iprodione or dichlofluanid varied little irrespective of the rate or timing of sprays. Residues varied between 4.5 to 6.9 µg g^{-1} for iprodione and 2.3 to 6.4 µg g^{-1} for dichlofluanid.

Discussion

These results show that half label rate mixtures of either thiram and iprodione or dichlofluanid and iprodione sprayed at 7 day intervals gave the most consistent rot control. They also show that for some specific disease combinations, reduced rates and or increased spray intervals of thiram or dichlofluanid can give effective fruit rot control, equal to that obtained with label rates. Meland (1988) showed that half rates of dichlofluanid, tolylfluanid or vinclozolin (a dicarboximide fungicide) could control grey mould of strawberries, except in the case of dichlofluanid or tolylfluanid where resistance to these fungicides was present.

The poor control of grey mould by all treatments with iprodione in two of the three experiments indicates the presence of dicarboximide resistance of *B. cinerea* at these sites. Previous studies have confirmed resistance at the Main Ridge property (Washington *et al.* 1992), and dicarboximide resistance in *B. cinerea* has been recorded frequently overseas (see numerous reports in Pommer and Lorenz, 1995). Creemers (1992) showed that full rate mixtures of dicarboximide fungicides with thiram or tolylfluanid gave good control of grey mould, even where dicarboximide resistance was present. Our results show that half rate mixtures of iprodione with thiram give good control of grey mould even in the presence of dicarboximide resistance. Grey mould control with this mixture in experiments where dicarboximide resistance was present was no different to that obtained with half rate thiram alone, indicating that iprodione contributed little to the control obtained with the mixture. In the experiment where no dicarboximide resistance was present (i.e. iprodione was effective), this effect was reversed, and thiram at half rate appeared to give little control .

Results also show poor control by dichlofluanid at the Main Ridge site, which is consistent with the findings of resistance of *B. cinerea* to dichlofluanid in the earlier study by Washington *et al.* (1992). The resistance of *B. cinerea* to dichlofluanid has been reported less frequently (Gjaerum and Munthe 1985, 1987, Hunter *et al.* 1987, Malathrakis 1989, Pappas and Elena 1992, Sansiviero *et al.* 1995) and whether it really occurs has been the subject of some debate. However, recent studies by Pollastro *et al.* (1996) have shown that a number of classes of resistance exist and that at least two genes appear to be responsible for the resistance of isolates to dichlofluanid or the closely related chemical tolylfluanid.

The experiment at Coldstream also highlighted the inadequate control that iprodione gives against black spot and leather rot, confirming the narrow spectrum of activity shown by this fungicide. This result also highlights the difficulty of controlling the complex of diseases which can occur on strawberry.

The poor control in two of the three thiram treatments in the Wandin experiment is difficult to explain. As these treatments were the longer spray interval or the lower rate treatments, it is possible that the weather conditions at a crucial time during this experiment resulted in more rapid degradation of the already lower residues of thiram, allowing significant infection of fruit by *B. cinerea*.

The currently approved maximum residue level or MRL for thiram on strawberry in Australia is T3 µg g^{-1} (temporary), for iprodione 12 µg g^{-1}, and for dichlofluanid 10 µg g^{-1} (Anon 1996). Thus as used in these experiments, thiram residues exceed the temporary MRL which has been set for strawberry in Australia, when applied at the label rate. The residue levels found for iprodione and dichlofluanid are within those approved for strawberry.

These results have shown that the combination of half rates of a broad spectrum fungicide (one which has activity against a wide range of different pathogens e.g. thiram or dichlofluanid) with a narrow spectrum fungicide (one which has activity against a few, usually related pathogens e.g. iprodione) give the most consistent control of all the fruit rots found in this study. Of the three fungicides tested in the present work, dichlofluanid mixed with iprodione would be the only combination which would give good rot control and not leave residues on fruit in excess of current MRLs for strawberry. Care would be needed to ensure that fungicide resistant strains of rot pathogens did not become dominant and cause failure of disease control measures. The appropriate use of fungicides from new fungicide groups unrelated to dicarboximides in schedules with older fungicides active against rot pathogens, and management practices to minimize disease should be integrated to reduce the risk from the development of fungicide resistant strains.

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