Postharvest efficacy and phytotoxicity of fludioxonil on Australian citrus

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IMPORTANT

This report details the result of trials commissioned by EE.Muir and Sons. The conclusions are provisional and may change with further work.

NANCY CUNNINGHAM AND NOLA LUCAS

February 2007

Final Report

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Statement of purpose of report:

This report details the research and development undertaken in Project CT06026 on reviewing the chemical fludioxonil for postharvest use on Australian citrus. Main findings, industry outcomes and recommendations to industry along with suggested areas of future research are discussed.

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Background

Australia exports fresh citrus fruits to many regions including North America, Asia, Europe and the Pacific. Keeping these important export markets open depends upon the ability to provide fruit that is sound and free of pest and diseases. Australian citrus packers have issues with three main citrus pathogens green mould (*Penicillium digitatum*), blue mould (*Penicillium italicum*) and sour rot (*Geotrichum citri-aurantii*). In the Australian domestic market, citrus packers can use a guanidine-based fungicide to control all three pathogens but its use is restricted for some export markets. A consequence of this is that the citrus industry has had to rely on benzimidazole and imidazole based fungicides to prevent decay. Both of these chemistries are very effective in controlling penicillium moulds but reliance on only two postharvest chemistries could greatly increase the possibility of penicillium resistant mould strains occurring.

The citrus industry in the USA has severe issues with resistant mould – primarily to thiabendazole. Surveys of Australian packingsheds have identified some isolated cases of resistant mould spores. Although not widespread, it is of concern to the industry that no alternative chemistries are available. The active constituent group phenylpyrrole has been recognised outside Australia as effective at controlling a variety of fungal diseases. The program outlined in this report was proposed to investigate fludioxonil, a member of the phenylpyrrole group as a possible third chemistry for control of penicillium moulds of citrus. To fully determine the potential for fludioxonil in the Australian citrus market, two main criteria were investigated. They were:

1. Efficacy of fludioxonil against the two main penicillium mould pathogens; green mould (*Penicillium digitatum*) and blue mould (*Penicillium italicum*).
2. Phytotoxicity caused by the product on the main export fruit types (oranges, lemons and mandarins)

This study was commissioned by E.E. Muir and Sons to evaluate the chemical fludioxonil, as a postharvest fungicide for citrus in Australia. The treatments and rates were pre-determined by E.E.Muir and Sons, the manufacturers of the chemical and the South Australian Research and Development Institute. The program aims to investigate the suitability of fludioxonil as a postharvest treatment of Australian fruit under Australian conditions.

If successful, fludioxonil could benefit the industry by providing a rotational fungicide for use in fungicide resistance management.

Media Summary

The Australian citrus industry has an export potential of over 160 million dollars annually, mostly in fresh packed navel oranges, but also easy peel mandarins and lemons. The maintenance of export markets is of vital importance to the survival of an industry that has had to overcome a number of obstacles to increase its market share. The main issues surrounding continued export of fruit is the expectation that fruit will be free of pests and disease. There is also an expectation that fruit treatments should be 'greener'. The last few years has seen an increase in decay levels at markets end. In an attempt to address this the industry has focussed on overall 'best practice' strategies such as sanitation and improving technologies for fungicide application. The issue with this is that the industry, despite improving its overall strategies for decay control, has been hindered by the lack in availability of registered fungicides (currently limited to benzimidazole and imidazole based fungicides). An over reliance on limited number of fungicides raises the potential for increased resistance to these established postharvest fungicides and places valuable export markets at risk.

The main aim of this project is to review a possible third chemistry - fludioxonil, for use by the Australian citrus industry. A new chemistry would fit into packingshed systems that are continually improving packingshed postharvest processes with little disruption to current practices. The main benefit to industry if a new chemistry becomes available is a further decrease in the amount of decayed fruit reaching export destinations.

The aims were addressed by the following activities:

- Efficacy evaluation of fludioxonil on the main citrus pathogens on three cultivars, navel orange, mandarin and lemon.
- Examination of any phytotoxic effects of fludioxonil on navel oranges, mandarins and lemons.
- Comparing efficacy and phytotoxicity of fludioxonil with current citrus postharvest fungicides and fungicide exposure times.

Technical Summary

The main aim of this project was to evaluate a possible third chemistry for postharvest use on Australian citrus. A new 'reduced risk' pesticide, such as fludioxonil, would be more acceptable to consumers, overseas markets and improve fungicide resistance management. Fludioxonil was tested under laboratory protocols simulating commercial packing practices and compared with two common citrus postharvest fungicides containing the actives thiabendazole and imazalil.

Efficacy

Evaluation of the efficacy of fludioxonil against *Penicillium digitatum*, *P. italicum* and a benzimidazole resistant strain of *P. digitatum* was carried out on navel oranges, mandarins and lemon fruit.

The results with susceptible mould strains have shown that fludioxonil was less efficacious than currently registered fungicide treatments. The fungicide (at rates up to 1200ppm active ingredient) did not provide commercially acceptable levels of mould control on navel oranges and mandarins. However, fludioxonil was effective against both Penicillium pathogens on lemons.

The efficacy of fludioxonil on a benzimidazole resistant strain of *P. digitatum* on all three cultivars showed similar levels of infection as that of a benzimidazole sensitive strain of *P. digitatum* indicating that the benzimidazole resistant strain is susceptible to fludioxonil.

It is possible that some of the current practices in citrus packingsheds could increase the efficacy of fludioxonil. These include heating the fungicide and/or combining with GRAS (generally regarded as safe) compounds such as sodium carbonate/bicarbonate.

Phytotoxicity

No phytotoxic effects were observed on fruit when navel oranges, mandarins and lemons were treated with fludioxonil. This included fruit that was treated with high concentrations (up to 1500ppm active ingredient) and held at a low temperature of 3°C for 6 weeks. Fruit then held for a further 2 weeks at room temperature (to simulate temperatures fruit might be held at by supermarkets and consumers) did not show any differences between treatments.

A subset of the stored fruit was weighed during the storage time to determine if differences occurred between fludioxonil treated fruit and fruit treated with a standard application of imazalil (Fungaflor 500EC). Fruit was also observed for any gross physical symptoms such as severe blemish or increased dehydration. Low carriage temperatures can exacerbate gross phytotoxic effects. Results showed that there was no difference in weight loss or blemish incidence between fludioxonil treated fruit and fruit treated with Fungaflor, even when concentrations of fludioxonil were at 1500ppm.

Further work

This study indicated that the efficacy of fludioxonil against mould varied with different cultivars. Efficacy studies using other cultivars, such as Valencia oranges or Tangelos, could provide useful comparisons. Future work could focus on enhancing the efficacy of fludioxonil by heating, in combination with GRAS compounds, such as carbonate salts, or combined with other fungicides, such as imazalil or thiabendazole. Efficacy studies using lower inoculum concentrations may provide a greater understanding of the efficacy of this product. Fludioxonil alone may provide limited control of moulds, but still have a role in fungicide resistance management.

Efficacy and Phytotoxicity of fludioxonil on Australian Citrus

Introduction

The Australian citrus industry currently uses several postharvest chemistries to control the major pathogens; *Penicillium digitatum*, causing green mould, and *Penicillium italicum*, causing blue mould. These chemistries are benzimidazole and imidazole based fungicides that have different modes of action. Widespread resistance to benzimidazole fungicides in many overseas markets made imazalil based fungicides the only alternative in many instances and its continued use along with potential resistance issues could also put Australia's valuable export markets at risk. In Australia we also have a third chemistry to rely upon, a guanidine based fungicide (containing the active guazatine). This chemistry is active not only against penicillium moulds but is also effective against the pathogen causing sour rot (*Geotrichum citri-aurantii*). However, its use is limited as it is not a registered treatment in many export countries. New fungicides classified as 'reduced risk' pesticides are now coming onto the market and have lower toxicology levels than other synthetic fungicides. The active constituent group phenylpyrrole is one group considered as a reduced risk, and its mode of action is as a 'natural mimetic' (Schirra et al, 2005). Fludioxonil, a member of this phenylpyrrole group is known to inhibit spore germination and mycelial growth of a variety of fungi (Hewitt, 2000) including *Botrytis cinerea* in grapes and boysenberry and *Penicillium expansum* in apples (Errampalli et al, 2006 Schirra et al, 2005). Fludioxonil has also been shown not to induce noticeable phytotoxicity symptoms in fruit such as pears (Drake et al 2006). It has also been shown to be effective against thiabendazole sensitive and resistant isolates of *Penicillium expansum* (Errampalli, 2004). Although resistance issues in Australian citrus packingsheds is not common some levels of resistance have been observed and the capacity to respond to serious resistance issues is limited by the amount of available fungicide chemistries. Fludioxonil, if found to be efficacious against penicillium moulds could be useful when managing resistance and preserving current fungicide effectiveness.

Efficacy

Efficacy experimental work was carried out on oranges ('Washington' navels), mandarins ('Honey murcott') and lemons ('Lisbon') with the postharvest disease causing fungi *Penicillium digitatum*, *Penicillium italicum* and a resistant strain of *Penicillium digitatum*. The range of fungicides, along with the concentrations used, were as specified by EE. Muir and Sons in consultation with the manufacturers of the chemical (Syngenta) and SARDI.

Efficacy - Materials and Methods

Fruit

Navel oranges (variety 'Washington'), mandarins (variety 'Honey Murcott') and lemons (variety 'Lisbon') were sourced from commercial orchards in the Riverland, South Australia. Fruit was selected by hand from trees or field bins after harvest and used in experiments before any postharvest fungicides were applied. The fruit were stored for up to 2 weeks at 5°C and 75-90% Relative humidity before use. Before each experiment, fruit were randomised, washed with FruitKleen® (Decco) at 5% (50mL/L), surface sterilised for 3 minutes in a chlorinated sanitiser (sodium hypochlorite 500ppm available chlorine) and allowed to dry at room temperature. After drying, each fruit was marked with 10 small circles around the equator using a permanent marker.

Fungal diseases and inoculum

Efficacy tests were conducted in separate trials for each of the following postharvest diseases.

Scientific name	Disease
Penicillium digitatum	Green mould, thiabendazole sensitive (TBZ^S)
Penicillium italicum	Blue mould
Penicillium digitatum	Green mould, thiabendazole resistant (TBZ^R)

Penicillium italicum and *Penicillium digitatum* isolates were obtained from decayed oranges from Riverland and Waite orchards. A benzimidazole-resistant (strongly thiabendazole resistant, weakly imazalil resistant) isolate (USA-1) obtained from Dr Brian Wild, Gosford, NSW, and recovered from oranges imported from the United States of America. Petri dishes were inoculated with these strains

of *Penicillium* and incubated at 25°C for 7-14 days. A conidial suspension was prepared in water and Triton X-100 (0.01%). The suspension was adjusted to 10^6 spores per ml after counting spores with a haemocytometer. A 50:50 isolate blend was achieved by mixing equal parts of benzimidazole-resistant isolate (USA-1) spore suspension with a benzimidazole-sensitive isolate spore suspension. About 1-2hr before treatment, navel oranges, lemons or mandarin fruit were dipped into the *P. italicum* or *P. digitatum* or a 50:50 blend of benzimidazole-resistant and benzimidazole-sensitive isolates spore solution and punctured using a small nail. Oranges and lemons were wounded to a depth of 3-5mm, mandarins to a depth of 1-2mm. Each fruit was punctured in the centre of the circles marked on the equator (i.e. 10 wounds per fruit). The wounds penetrated the albedo tissue, but not the juice sacs. The method used was adapted from inoculation methods described by Eckert and Brown (1986) with all fruit uniformly wounded. The different pathogens and the 50:50 mix were assayed separately to avoid cross contamination. The inoculated fruit were then held at room temperature.

Efficacy testing and assessment

After 1-2 hours the following fungicide treatments and dip times were evaluated.

Treatment (a.i.)	Supplier	Dip time
Control (water dipped)		30 s
600 ppm fludioxonil	Syngenta, E.E. Muir and Sons.	30 s
900 ppm fludioxonil	Syngenta, E.E. Muir and Sons.	30 s
1200 ppm fludioxonil	Syngenta, E.E. Muir and Sons.	30 s
600 ppm fludioxonil	Syngenta, E.E. Muir and Sons.	60 s
500 ppm imazalil	Fungaflor® 500 EC; Janssen-Cilag	30 s
1000 ppm thiabendazole	Tecto 500SC; Syngenta	30 s

Each treatment was applied to 5 fruit of 10 wounds each. Treated fruit were placed in plastic bags to induce high humidity and held at 20°C for 3 days, it which time the incidence of mould was determined. The fruit were then reassessed after 7 days. The treatments were replicated 3 times.

Statistical analysis

Statistical analysis was carried out on the arcsine square root of percentage wounds infected 3 and 7 days after inoculation. A randomised complete block one-way analysis of variance (ANOVA) was employed using Statistix (Analytical Software© 2000) and a mean separation was performed using the Tukey's honestly significant difference (HSD) method at probability level of $p<0.05$. All data was back transformed to the original raw data in the tables and means labelled with similar letters in columns are considered not significantly different from each other.

Efficacy Results *Penicillium digitatum* (TBZ^S)

At the 3 day assessment period, all oranges (Table 1) and lemons (Table 3) treated with fludioxonil, Fungaflor and Tecto showed similar levels of efficacy. However, the result for mandarins (Table 2) shows that both Fungaflor and Tecto were more efficacious than fludioxonil. After 7 days, the test chemical did not perform as well as the two registered chemicals for oranges or mandarins.
Fludioxonil was as efficacious as Fungaflor and Tecto when lemons were treated, except the higher rate of fludioxonil, which had slightly more decay.

Table 1 A comparison of the infection rate of oranges inoculated with *P. digitatum*, and then dipped in different fungicide solutions.

Fungicides	*Rate (ppm)*	*% Infection (SEM)*			
		*3 day**		*7 day**	
Control	0	94.11	a	100	a
Fludioxonil	600	1.15	b	50.32	ab
	900	1.87	b	38.43	ab
	1200	3.96	b	53.51	ab
	600 (60 s)	2.12	b	70.01	ab
Fungaflor 500EC	500	0	b	0.22	b
Tecto 500SC	1000	0	b	1.63	b

Means are value of 3 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, ANOVA on arcsine square root transformed data. $F_{3\ day}= 34.17$, $p<0.05$. $F_{7\ day}=7.2$, $p=0.002$.

Table 2 A comparison of the infection rate of mandarins inoculated with *P. digitatum*, and then dipped in different fungicide solutions.

Fungicides	*Rate (ppm)*	*% Infection (SEM)*			
		3 day		*7 day*	
Control	0	93.99	a	100	a
Fludioxonil	600	10.31	b	71.96	b
	900	5.29	b	67.61	b
	1200	11.39	b	79.01	b
	600 (60 s)	9.23	b	56.72	b
Fungaflor 500EC	500	0	c	0.22	c
Tecto 500SC	1000	0	c	0	c

Means are value of 3 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, ANOVA on arcsine square root transformed data. $F_{3\ day}= 98.16$, $p<0.05$. $F_{7\ day}=74.86$, $p<0.05$.

Table 3 A comparison of the infection rate of lemons inoculated with *P. digitatum*, and then dipped in different fungicide solutions.

Fungicides	*Rate (ppm)*	*% Infection (SEM)*			
		3 day		*7 day*	
Control	0	100	a	100	a
Fludioxonil	600	0	b	0	c
	900	0	b	0	c
	1200	0	b	7.76	b
	600 (60 s)	0	b	0	c
Fungaflor 500EC	500	0	b	0	c
Tecto 500SC	1000	0	b	0	c

Means are value of 3 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, ANOVA on arcsine square root transformed data. $F_{3\ day}= 0.0192$, $p<0.05$. $F_{7\ day}=117.21$, $p<0.05$.

Efficacy Results *Penicillium italicum*

Fludioxonil was effective at reducing decay on oranges (at all rates and dip times) and was as efficacious as both Fungaflor and Tecto 3 days after treatment. All treatments were also significantly different from the water treated controls (Table 4). After 7 days, decay levels were much higher than at 3 days. The percentages of wounds infected for fungicide-treated fruit were similar and significantly lower than control treated fruit, except for one treatment (fludioxonil at 600ppm dipped for 60 s).

Result for mandarins (Table 5) shows that fludioxonil treated fruit at both the 3 and 7 day assessment had significantly lower decay than control treated fruit. At the 3 day assessment period, only fruit treated with fludioxonil at 1200ppm and 600ppm (60 s dip) had comparable decay levels with Fungaflor and Tecto treated fruit. After 7 days, all fludioxonil treated fruit had significantly higher levels of decay than fruit treated with Fungaflor and Tecto.

Fludioxonil treated lemons had low decay levels 3 and 7 days after treatment. Decay was not significantly different from Fungaflor and Tecto treated fruit (Table 6). All fungicide treatments were significantly different from the controls.

Table 4 A comparison of the infection rate of oranges inoculated with *P. italicum*, and then dipped in different fungicide solutions.

Fungicides	*Rate (ppm)*	*% Infection (SEM)*			
		3 day		*7 day*	
Control	0	93.78	a	100	a
Fludioxonil	600	1.15	b	18.78	b
	900	2.37	b	42.54	b
	1200	0.68	b	32.94	b
	600 (60 s)	0.45	b	46.37	ab
Fungaflor 500EC	500	0	b	3.96	b
Tecto 500SC	1000	0	b	0	b

Means are value of 3 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, ANOVA on arcsine square root transformed data. $F_{3\ day}= 62.45$, $p<0.05$. $F_{7\ day}=8.87$, $p=0.0008$

Table 5 A comparison of the infection rate of mandarins inoculated with *P. italicum*, and then dipped in different fungicide solutions.

Fungicides	*Rate (ppm)*	*% Infection (SEM)*			
		3 day		*7 day*	
Control	0	88.61	a	99.78	a
Fludioxonil	600	9.92	b	69.26	b
	900	9.94	b	58.04	b
	1200	3.83	bc	62.08	b
	600 (60 s)	2.22	bc	57.37	b
Fungaflor 500EC	500	0	c	0	c
Tecto 500SC	1000	0	c	0	c

Means are value of 3 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, ANOVA on arcsine square root transformed data. $F_{3\ day}$=43.76, $p<0.05$. $F_{7\ day}$=176.69, $p<0.05$.

Table 6 A comparison of the infection rate of lemons inoculated with *P. italicum*, and then dipped in different fungicide solutions

Fungicides	*Rate (ppm)*	*% Infection (SEM)*			
		3 day		*7 day*	
Control	0	100	a	100	a
Fludioxonil	600	0.89	b	8.33	b
	900	0.45	b	5.13	b
	1200	1.68	b	6.93	b
	600 (60 s)	0	b	1.31	b
Fungaflor 500EC	500	0	b	0	b
Tecto 500SC	1000	0	b	0	b

Means are value of 3 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, ANOVA on arcsine square root transformed data. $F_{3\ day}$= 230.55, $p<0.05$. $F_{7\ day}$=55.34, $p<0.05$.

Efficacy Results *Penicillium digitatum* (TBZ^S + TBZ^R)

Oranges inoculated with a mixture of TBZ^r/TBZ^s strains of *P. digitatum* and treated with fludioxonil (at all rates and dip times) had similar decay levels as fruit treated with Fungaflor at the 3 day assessment (Table 7). Fruit treated with Tecto had higher rates of decay than either fludioxonil or Fungaflor treated fruit, but was still effective enough to be significantly different from the control treated fruit. When fruit were assessed, 7 days after treatment, fludioxonil treated fruit had significantly higher rates of decay than Fungaflor treated fruit but significantly lower decay when compared with either control treated or Tecto treated fruit.

There was no significant difference in the amount of decay in mandarins for all fungicide treatments at the 3 day assessment (Table 8). After 7 days, decay in Tecto treated fruit was not significantly different from the controls or fludioxonil treated fruit at 600ppm (30 s and 60 s dip) and 1200ppm. Fungaflor was the only treatment that had no decay in mandarins at the later assessment period.

Lemons inoculated with a mixture of TBZ^r/TBZ^s strains of *P. digitatum* and treated with fludioxonil (at all rates and dip times) had similar decay levels as fruit treated with Fungaflor 3 and 7 days after treatment (Table 9). However, all fungicide treated fruit has some level of decay after 7 days. Decay levels in Tecto treated fruit were very high in lemons and not significantly different from decay levels in control treated fruit.

Table 7 A comparison of the infection rate of oranges inoculated with a 50:50 blend of Benzimidazole-sensitive and –resistant (TBZ^r/TBZ^s) isolates of *P. digitatum*, and then dipped in different fungicide solutions.

Fungicides	*Rate (ppm)*	*% Infection (SEM)*			
		3 day		*7 day*	
Control	0	82.25	a	100	a
Fludioxonil	600	0	c	62.33	b
	900	0	c	58.49	b
	1200	0.22	c	70.53	b
	600 (60 s)	0.22	c	60.79	b
Fungaflor 500EC	500	0	c	0.45	c
Tecto 500SC	1000	13.94	b	99.78	a

Means are value of 3 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, ANOVA on arcsine square root transformed data. $F_{3\ day}$= 41.93, $p<0.05$. $F_{7\ day}$=38.03, $p<0.05$.

Table 8 A comparison of the infection rate of mandarins inoculated with a 50:50 blend of TBZ^r/TBZ^s isolates of *P. digitatum*, and then dipped in different fungicide solutions.

Fungicides	*Rate (ppm)*	*% Infection (SEM)*			
		3 day		*7 day*	
Control	0	29.4	a	95.94	a
Fludioxonil	600	0.68	b	23.22	bcd
	900	0	b	15.64	cd
	1200	0.91	b	25.93	bcd
	600 (60 s)	0.45	b	42.18	abc
Fungaflor 500EC	500	0	b	0	d
Tecto 500SC	1000	0.68	b	80.02	ab

Means are value of 3 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, ANOVA on arcsine square root transformed data. $F_{3\ day}= 10.55$, $p=0.0003$. $F_{7\ day}=10.75$, $p=0.0003$.

Table 9 A comparison of the infection rate of lemons inoculated with a 50:50 blend of TBZ^r/TBZ^s isolates of *P. digitatum*, and then dipped in different fungicide solutions.

Fungicides	*Rate (ppm)*	*% Infection (SEM)*			
		3 day		*7 day*	
Control	0	100	a	100	a
Fludioxonil	600	2.37	b	3.96	b
	900	0	b	9.25	b
	1200	0	b	9.88	b
	600 (60 s)	0.07	b	10.67	b
Fungaflor 500EC	500	0	b	2.37	b
Tecto 500SC	1000	91.9	a	100	a

Means are value of 3 replicates. Means labelled with similar letters in columns are not significantly different from each other using Tukey's honestly significant difference test, ANOVA on arcsine square root transformed data. $F_{3\ day}= 70.26$, $p<0.05$. $F_{7\ day}=20.85$, $p<0.05$.

Efficacy - Discussion

Fludioxonil gave better control of *P. italicum* and *P. digitatum* (TBZ^S and TBZ^S + TBZ^R strains*)* on lemons than when the same fungi occurred on navel oranges or mandarins. Although fludioxonil did not control decay to commercially acceptable levels on oranges and mandarins it did provide some measure of control above a water only treatment. Trials with a TBZ^S + TBZ^R blend of *P. digitatum* indicated a strong resistance to thiabendazole (Tecto 500SC). The efficacy of fludioxonil did not change significantly in any of the cultivars tested with an introduction of the TBZ^R strain, indicating that it could have some potential for treating resistant mould. In lemons, where fludioxonil was the most efficacious, both fludioxonil and Fungaflor 500EC treated lemons had a low rate of decay when a TBZ^R + TBZ^S isolate blend of *P. digitatum* was used as inoculum. This was similar to results when a TBZ^S only isolate was used. This suggests that both Fungaflor and fludioxonil controlled both TBZ^R and TBZ^S strains in lemons when compared with fruit treated with water alone or with Tecto 500EC. This trend was also seen in oranges and mandarins, but in these cultivars fludioxonil did not reduce decay to commercially acceptable levels when either TBZ^S or a combination of TBZ^S + TBZ^R blend was used. Lower rates of an imazalil or fludioxonil based product would be required to establish the relative efficacy of Fungaflor 500EC and fludioxonil using a resistant isolate in lemons. In the USA, green and silver lemons are stored for much longer periods than other types of citrus fruit and after long storage is often rerun over packinglines, this promotes the spread of TBZ^R fungi in packing facilities. Although the practice of running already processed fruit through the packingline process is discouraged in Australia, it still occurs, and the potential threat of resistant strains occurring in lemons could be repeated here. Fludioxonil in rotation with other postharvest fungicides could potentially play a role in reducing resistance in similar circumstances.

Phytotoxicity

Phytotoxicity work was carried out using the same varieties as used in efficacy work using standard packingshed cleaner and waxes. The range of fungicides, along with the concentrations used, were as specified by EE Muir and Sons in consultation with manufacturers of the chemical (Syngenta) and SARDI.

Phytotoxicity - Materials and Methods

Fungicide treatments

Navel oranges (variety 'Washington'), lemons (variety 'Lisbon') and mandarins (variety 'Honey Murcott') were sourced from commercial orchards in the Riverland, South Australia. Fruit was selected by hand from trees or field bins after harvest and used in experiments before any postharvest fungicides were applied. The fruit were stored for up to 2 weeks at 5°C and 75-90% Relative humidity before use. Before each experiment, fruit were randomised, washed with FruitKleen® (Decco) at 5% (50mL/L), surface sterilised for 3 minutes in a chlorinated sanitiser (sodium hypochlorite 100ppm available chlorine) and then dipped in a fungicide for 30s or 60s, and allowed to dry at room temperature. Fruit dipped in 500ppm imazalil (Fungaflor) was considered the 'control' or standard treatment for commercially treated fruit. The fungicide treatments were:

Treatment	Manufacturer	Dip time
600 ppm fludioxonil	Syngenta, E.E. Muir and Sons.	30 s
900 ppm fludioxonil	Syngenta, E.E. Muir and Sons.	30 s
1200 ppm fludioxonil	Syngenta, E.E. Muir and Sons.	30 s
1500 ppm fludioxonil	Syngenta, E.E. Muir and Sons.	30 s
600 ppm fludioxonil	Syngenta, E.E. Muir and Sons.	60 s
500 ppm imazalil (control)	Fungaflor® 500 EC; Janssen-Cilag	30 s

After fruit was dipped in fungicide it was allowed to dry on racks before being waxed (Decco Citrus Lustre 402A) at a rate of 150µL/100gm of fruit. Fruit were then placed in cold storage at 3°C (µ 1°C) and 90% RH for 6 weeks. After cold storage, fruit were moved into room temperature (20°C, relative humidity 65-70%) for two weeks to simulate conditions retailers and consumers are likely to store fruit. The higher temperature and lower humidity conditions after extended cold storage are also likely to exacerbate any postharvest disorders. All fruit were weighed weekly and assessed for any blemishes.

Statistical analysis

Analysis of variance was applied to fruit weight loss using Statistix7 (Analytical Software, 2000).

Phytotoxicity Results – fruit weight

Weight loss was used as an indicator of phytotoxicity. In this trial, fruit treated with fludioxonil at various concentrations and Fungaflor 500EC (control or standard fruit), had similar weight reductions when stored for at least 6 weeks in cold storage as shown by the average percentage weight loss seen in Table 10.

Table 10 Average percentage weight loss of fruit after 6 weeks at 3°C(±1°C)

Treat	***Rate***	***Average percentage weight loss (± SEM)[a]***		
		Oranges	***Mandarins***	***Lemons***
Fludioxonil	600	5.08 ± 0.14	5.42 ± 0.14	4.08 ± 0.48
	900	5.29 ± 0.11	5.31 ± 0.12	3.61 ± 0.25
	1200	5.33 ± 0.41	5.43 ± 0.24	4.14 ± 0.58
	1500	5.33 ± 0.41	5.13 ± 0.21	4.05 ± 0.32
	600 (60 s dip)	4.75 ± 0.3	5.35 ± 0.17	4.1 ± 0.33
Fungaflor 500EC	500	4.66 ± 0.27	5.68 ± 0.21	3.63 ± 0.25

[a] Mean of five replicates.

Oranges

Weight loss of fungicide-treated navel oranges were similar when measured during week 1 (F=1.34, df=5,35, p=0.28), week 2 (F=1.41, df=5,35, p=0.25), week 3 (F=1.24, df=5,35, p=0.31), week 4 (F=1.37, df=5,35, p=0.26), week 5 (F=1.55, df=5,35, p=0.20), and week 6 (F=1.46, df=5,35, p=0.23). At ambient temperature weight loss was not significantly higher in treated vs control treated fruit during week 7 (F=1.03, df=5,35, p=0.42), and week 8 (F=0.93, df=5,35, p=0.47).

Mandarins

Weight loss of fungicide-treated mandarins were similar when measured during week 1 (F=0.44, df=5,47, p=0.81), week 2 (F=0.90, df=5,47, p=0.49), week 3 (F=0.82, df=5,47, p=0.55), week 4 (F=0.70, df=5,47, p=0.62), week 5 (F=0.73, df=5,47, p=0.61), and week 6 (F=0.69, df=5,47, p=0.63). At ambient temperature weight loss was not significantly higher in treated vs control treated fruit during week 7 (F=0.60, df=5,47, p=0.70), and week 8 (F=0.83, df=5,46, p=0.54).

Lemons

Weight loss of fungicide-treated lemons were similar when measured during week 1 (F=0.21, df=5,35, p=0.95), week 2 (F=0.34, df=5,35, p=0.89), week 3 (F=0.21, df=5,35, p=0.96), week 4 (F=0.16, df=5,35, p=0.98), week 5 (F=0.22, df=5,35, p=0.95), and week 6 (F=0.13, df=5,35, p=0.98). At ambient temperature weight loss was not significantly higher in treated vs control treated fruit during week 7 (F=0.18, df=5,35, p=0.97), and week 8 (F=0.13, df=5,35, p=0.98).

Figure 1 Weight loss of navel oranges kept at 3°C for 6 weeks and 20°C for a further 2 weeks.

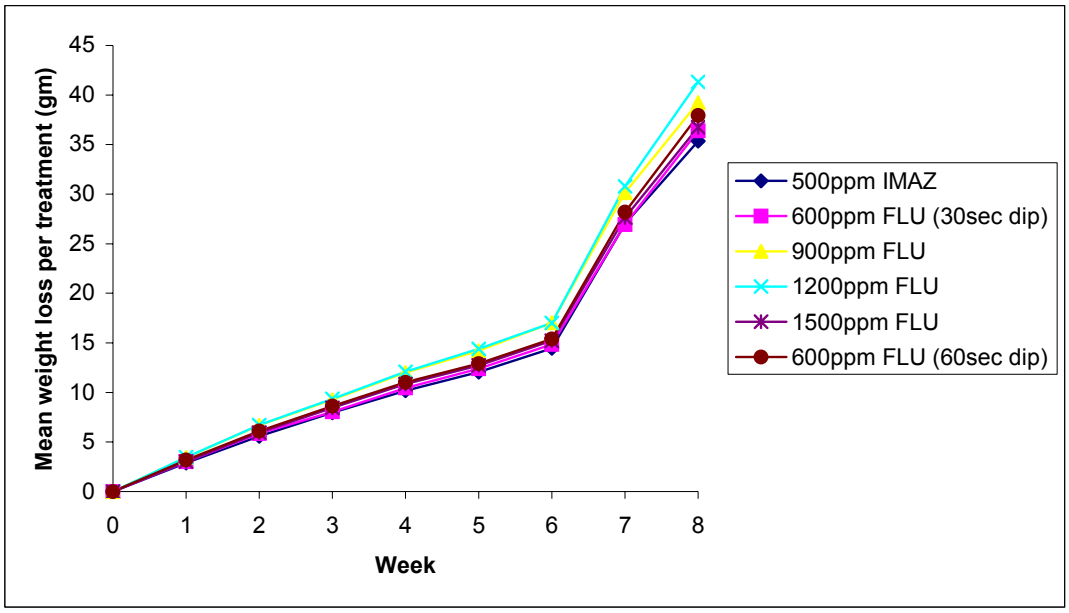


Figure 2 Weight loss of mandarins kept at 3°C for 6 weeks and 20°C for a further 2 weeks.

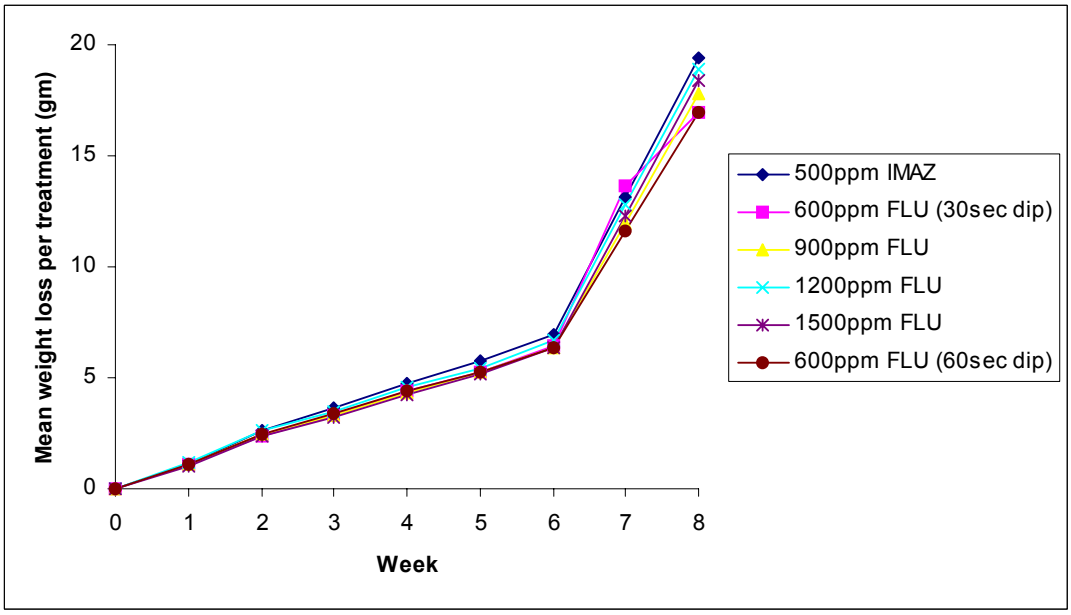
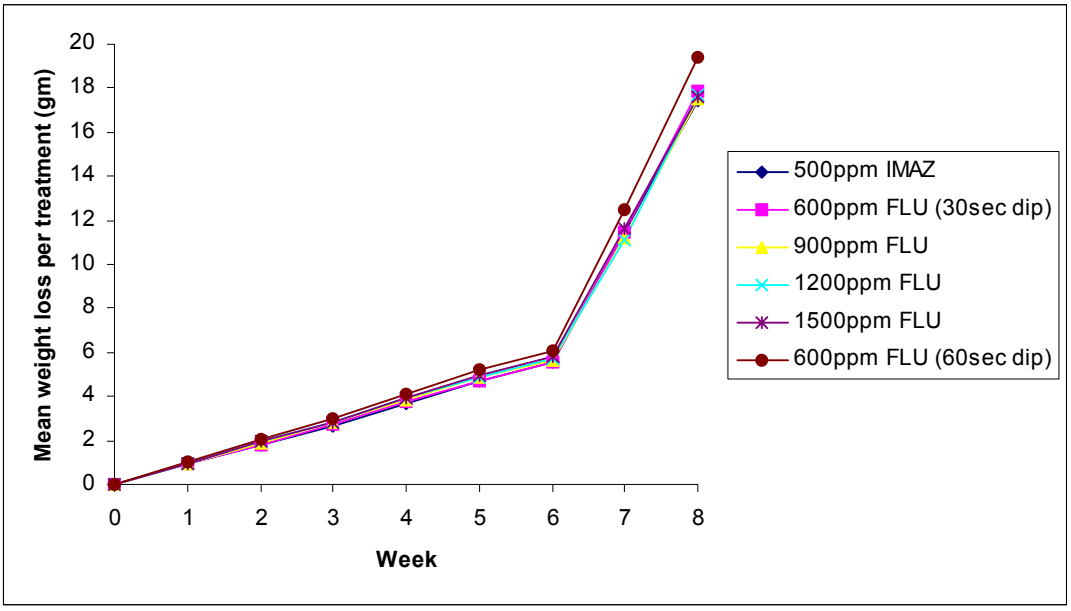


Figure 3 Weight loss of lemons kept at 3°C for 6 weeks and 20°C for a further 2 weeks.



Results – fruit condition

After 6 weeks at 3°C, navel oranges, mandarins and lemons treated with fludioxonil at all concentrations and dip times did not show significantly different levels of blemish or any other phytotoxicity symptoms compared with a standard (control) treatment of Fungaflor. Figure 1 shows fruit treated with fludioxonil at 600ppm after 6 weeks cold storage and 1 week ambient temperature. After fruit were taken out of cold storage mandarins showed an increased amount of dehydration for all fungicide treatments. Figures 2 and 3 show dehydration in fludioxonil and Fungaflor treated fruit.

Figure 1 (left) shows fruit treated with fludioxonil at 600ppm in good condition after 7 weeks storage

Figure 2 (above right) and Figure 3 (below right) showing severe dehydration symptoms in mandarin treated fruit

Discussion

All treated fruit had low levels of blemish independent of fungicide treatment. This was reflected in weight loss of fruit after extended cold storage with no significant differences seen between fungicide treatments. There was slightly higher decay in mandarins as well as an increase in the amount of blemish after 6 weeks in cold storage. However, mandarins are generally smaller with a higher surface to volume ratio and thinner skin making them more prone to decay and dehydration than oranges or lemons. There were no visual differences between treatments during the storage period. This fludioxonil product (up to 1500ppm) did not induce any major phytotoxic response on oranges, mandarins and lemons. Under circumstances where good quality fruit is treated there appeared to be no differences between fludioxonil and other fungicides used.

Recommendations

Whilst the Australian citrus industry continues to expand its fresh fruit export markets, issues relating to decay control and fungicide use will also persist. Many of the export markets are placing further restrictions on the use of chemical fungicides and citrus packers are looking for alternatives to replace them. Fludioxonil in this instance could provide a possible alternative for some cultivars of citrus. There are several issues that need to be considered before fludioxonil could be used by the citrus industry in Australia. They include the cultivar treated and the importance of a resistance management strategy.

In this study, fludioxonil was effective at controlling moulds on lemons, but not on navel oranges and mandarins at the same concentrations. Fludioxonil could have a range of effectiveness with other citrus cultivars not presented in this study. For instance, fludioxonil appears to be more efficacious on Valencia oranges when compared to navel oranges (data not presented). Further work with different cultivars, and higher concentrations for navel oranges and mandarins, may be warranted. The results with oranges and mandarins indicated that mould development was suppressed at 3 days, but developed by the 7 day assessment. This suggests that the fungicide may more fungistatic, than fungicidal, under the selected conditions. Unfortunately, doubling the concentration for 600ppm to 1200ppm did not result in a significant reduction in decay. This suggests that considerably higher concentration may be necessary to control mould on oranges and mandarins.

Under the conditions used in this study, fludioxonil appears to be limited as a 'stand alone' treatment for citrus fruit. However, there is potential for fludioxonil to be enhanced and/or used in combination with current citrus postharvest fungicides. The strategy would be to rotate fungicide groups to improve fungicide resistance management.

Many citrus packers in Australia are seeking to enhance their fungicides by heating and/or combining with chemicals that are considered to have low environmental toxicology. Heating fungicides increases the residue of fungicide remaining on fruit. Schirra et al (2005) showed that efficacy against Penicillium species could be enhanced significantly by heating fludioxonil to 50°C, and it was effective at much lower concentrations than what would be needed if the fungicide were at room temperature. There are many studies that demonstrate the effectiveness of combining fungicides with low toxic compounds, or generally regarded as safe compounds (GRAS), such as carbonate salts, to control mould (Smilanick et al 1999, 2005, Palou et al 2001, 2002). Integrated treatments are becoming more wide spread in Australia and further examination of heated fludioxonil and combinations of fludioxonil with GRAS compounds is warranted. Supplementary work looking into

these practises will establish if these methods can be used to enhance fludioxonil efficacy on Australian citrus.

Finally, the methods used in this study are based on well-established procedures for evaluating postharvest synthetic fungicides in citrus (Eckert and Brown 1986). In the first instance, it was appropriate to compare fludioxonil using the same evaluation criteria as applied to existing synthetic fungicides. However, 'reduced risk' chemicals are being increasing evaluated under more favourable conditions. For instance, fludioxonil is effective in controlling mould on oranges and mandarins where they are wounded only (not inoculated) and then dipped for 3 minutes (Schirra, 2005). This approach is more acceptable where fungicide resistance is widespread and/or there are concerns with chemical residues in food. If these other factors are of importance to Australia, or countries importing Australian citrus, then further study is needed to determine the effect of inoculum load and dip time on the efficacy of fludioxonil.

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