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Preharvest application of fungicides for postharvest disease control on early season tangerine hybrids in Florida

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Abstract

Postharvest anthracnose and stem-end rot, caused by *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*, respectively, can be severe problems to certain ethylene-degreened early season citrus cultivars in Florida. Preharvest application of fungicides can be an effective approach for control of these diseases. The potential of five fungicides, benomyl, thiophanate methyl, azoxystrobin, fludioxonil and pyraclostrobin applied 2, 14, 21, and 28 d before harvest for control of postharvest anthracnose, stem-end rot, and green mould (*Penicillium digitatum*) was evaluated on early season Florida Fallglo and Sunburst tangerine hybrids in 2003 and 2004. Most fungicides significantly reduced anthracnose incidence on Fallglo when applied 2 d before harvest in both years and at 14 and 21 d before harvest in 2003. At other application dates, none of the fungicides was effective. On Fallglo fruit in 2004, the five tested fungicides reduced postharvest anthracnose by 37.4–62.6% when sprayed 2 d before harvest. Little anthracnose was observed on Sunburst fruit in either year. On both cultivars and in both years, benomyl and thiophanate methyl consistently and significantly reduced stem-end rot incidence. Other fungicides were less effective or ineffective in controlling stem-end rot. Benomyl and thiophanate methyl also reduced green mould incidence by 58.9–100% on Sunburst tangerines in 2004, but were ineffective in 2003 due to the presence of resistant strains. Thiophanate methyl appears to be an excellent alternative to benomyl for citrus postharvest disease control since benomyl, the only registered fungicide for preharvest application for postharvest decay control, is no longer being produced in the USA.

Keywords: Citrus fruit; Fungicide; Anthracnose; Green mould; Stem-end rot; *Colletotrichum gloeosporioides*; *Penicillium digitatum*; *Lasiodiplodia theobromae*; Benomyl; Azoxystrobin; Fludioxonil; Thiophanate methyl; Pyraclostrobin

1. Introduction

Postharvest diseases are an important constraint affecting citrus fruit quality, shelf life, and market values. Several fungal pathogens are capable of causing postharvest diseases on Florida citrus fruits. These include green mould [*Penicillium digitatum* (Pers.:Fr.) Sacc], blue mould (*Penicillium italicum* Wehmer), Diplodia stem-end rot caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., Phomopsis stem-end rot caused by *Diaporthe citri* F.A. Wolf, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., sour rot caused by *Geotrichum citri-aurantii* E.E. Bulter, brown rot caused by *Phytophthora palmivora* (E.J. Butler) E.J. Butler or *Phytophthora nicotianae* Breda de Haan, and Alternaria stem-end rot (black rot) caused by *Alternaria* sp., formerly considered to be caused by *Alternaria citri* Ellis & N. Pierce in N. Pierce (Brown and Miller, 1999; Ismail and Zhang, 2004), but now designated as *Alternaria alternata* (Fr.:Fr.) Keissl. (Peever et al., 2005). Green mould, Diplodia stem-end rot, Phompsis stem-end rot, and sour rot are most common postharvest decays on Florida citrus, and other decays do not frequently cause commercially significant losses (Ritenour et al., 2003). Fruits harvested early in the season (September–November) in Florida are often subjected to ethylene degreening treatment (exposure to 2–5 $\mu g\,ml^{-1}$ ethylene at 28–30 °C with about 95% relative humidity (RH)) to improve fruit colour for marketing purposes. This practice can greatly enhance the occurrence

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of Diplodia stem-end rot and anthracnose (Brown, 1975a, b; Barmore and Brown, 1985; Zhang, 2004). The effects of ethylene treatment on other postharvest diseases are not known. The decay incidence and severity of Diplodia stem-end rot is positively correlated with the length of degreening treatment and ethylene concentrations (Brown, 1975a, b; Barmore and Brown, 1985; Zhang, 2004). Diplodia stem-end rot can develop on all types of citrus fruits (Ismail and Zhang, 2004). However, anthracnose can be an important decay on Robinson tangerine and certain other tangerine hybrids such as Fallglo and Sunburst that are harvested in early autumn in Florida when long periods of ethylene degreening are required to give the fruit an attractive appearance (Eckert and Brown, 1986). Anthracnose is a minor problem on oranges, grapefruit and lemons (Eckert and Brown, 1986).

Both *C. gloeosporioides* and *L. theobromae* complete their life cycles on citrus trees, and cause quiescent infections on citrus fruit in groves. After harvest, these infections on fruit become active and cause anthracnose and Diplodia stem-end rot, respectively, when fruit and environmental conditions are conducive. Since ethylene degreening of citrus fruit can greatly enhance the occurrence of these two decays, an appropriate treatment for the control of anthracnose and Diplodia stem-end rot should be applied before fruit degreening rather than after. Preharvest application of fungicides on the trees and postharvest fruit drenching with fungicides before fruit degreening treatment are the only two suitable procedures to control stem-end rot and anthracnose during commercial Florida fruit packing process. Commercial drenching of citrus fruit with thiabendazole (TBZ) or imazalil before degreening has been used commonly in Florida to control stem-end rot and other decays. In some cases, the commercial drenching process can cause fruit peel disorders such as 'green ring' (Ritenour and Dou, 2000). In addition, TBZ and imazalil used for drenching fruit are only somewhat effective for anthracnose control (Zhang, unpublished data). Therefore, preharvest application of an effective fungicide can be a good approach to control both stem-end rot and anthracnose before fruit degreening treatment.

Benomyl (Benlate®) which is effective for stem-end rot and anthracnose control (Brown, 1974; Ritenour et al., 2004) was the only registered commercial fungicide that could be used in groves shortly before harvest for postharvest disease control. However, the US EPA has proposed cancellation of benomyl for citrus use on January 1, 2008, and the manufacturer discontinued all sales of benomyl at the end of 2002 (Ritenour et al., 2003). Therefore, an alternative preharvest chemical to replace benomyl for postharvest anthracnose and stem-end rot control on early-season citrus fruit in Florida is needed.

Thiophanate methyl (Topsin-M®) has been shown to be effective for postharvest stem-end rot control (Ritenour et al., 2004), but there were no data to show whether thiophanate-methyl was effective to control postharvest anthracnose and other decays when applied on citrus trees prior to harvest. Smilanick et al. (2006) reported that thiophanate methyl was effective for postharvest green mould control on Navel oranges by the preharvest application of this product in California. Azoxystrobin (Abound®), pyraclostrobin (Headline®) have been registered and used on citrus to control some preharvest diseases (Rogers and Timmer, 2006). Fludioxonil (Graduate®) is a newly registered fungicide for citrus postharvest decay control, but the formulation for preharvest application is known as Scholar. It is still not clear whether the above chemicals are effective for postharvest decay control when they are applied on citrus trees in groves before harvest.

Green mould is normally not a major problem on degreened early season fruit in Florida (Hopkins and Loucks, 1948). This may be largely due to the degreening treatment [28–30 °C and 90–96% RH] (Wardowski, 1996), which exerts curative effects on wounds and prevents green mould development (Hopkins and Loucks, 1948; Zhang and Swingle, 2005). Green mould is commercially controlled through postharvest handling processes and applications of fungicides (Ismail and Zhang, 2004). However, it will be beneficial if a fungicide applied on citrus trees before harvest can also reduce postharvest green mould incidence and severity.

The objectives of this study were to (i) evaluate the potential of four fungicides, thiophanate methyl, fludioxonil, azoxystrobin, and pyraclostrobin, and compare them to benomyl for postharvest disease control, especially of anthracnose and Diplodia stem-end rot, on early season tangerine hybrids by preharvest application in groves; (ii) determine the most appropriate time prior to harvest for postharvest decay control.

2. Materials and methods

2.1. Fungicides

Fungicides, formulations, manufacturers and application rates used in the trials are listed in Table 1. Benomyl was used as the standard preharvest fungicide.

2.2. Citrus

Experimental plots were established in plantings of Fallglo and Sunburst tangerine hybrids at the University of Florida, Citrus Research and Education Centre (CREC), Lake Alfred, FL, in 2003. The same planting of Fallglo was used in 2004, but the Sunburst planting used was in a commercial citrus field in Polk County, FL. Fallglo and Sunburst trees were about 15-year old. The trees in groves were maintained following commercial practices.

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Table 1
Listing of fungicides tested and their common names, trade names, manufacturers and field application rates

Common name	Trade name	Formulation	Active ingredient (%)	Manufacturer	Rate applied
Benomyl	Benlate	50 WP	50	DuPont	2.2 kg ha^{-1}
Azoxystrobin	Abound	2.08 EC	25	Syngenta	1.2 l ha^{-1}
Fludioxonil	Scholar	1.67 SC	20	Syngenta	1.2 l ha^{-1}
Thiophanate-methyl	Topsin-M	70 WP	70	Cerexagri, Inc.	2.2 kg ha^{-1}
Pyraclostrobin	Headline	2.09 EC	25	BASF Inc.	1.2 l ha^{-1}

2.3. Fungicide applications

All fungicide sprays were applied on trees using a high-pressure handgun sprayer with a 750-l tank (Chemical Container Corp., Lake Wales, FL). The desired rate per hectare for each of the tested chemicals (Table 1) was applied in 1200 l ha^{-1}, and the trees were sprayed to run-off using approximately 5 l/tree. Three to four trees were sprayed for each chemical. Control trees were sprayed with water. In 2003, Fallglo and Sunburst tangerine trees were sprayed on September 24, and October 20, respectively. In 2004, Fallglo trees at the CREC and Sunburst trees in the commercial grove were sprayed on September 13 and October 4, respectively.

2.4. Fruit harvest and postharvest handling

In 2003, Fallglo and Sunburst tangerines were harvested 2, 14, 21, and 28 d after the fungicide application. In 2004, Fallglo fruits were harvested 2, 21, and 28 d after the chemicals were sprayed. Sunburst fruit were harvested 2, 14, 21, and 28 d after spraying fungicides. One hundred and fifty fruit were harvested arbitrarily from the treated trees at each harvest time. Fifty fruit were placed in each of three replicate plastic crates ($59 \times 39 \times 29\,cm^3$). Harvested fruit were brought to the CREC packing facility, and subjected to ethylene degreening treatment the same day. After fruit were degreened with ethylene at 10 μg ml^{-1} at 28–30 °C and 94–96% RH for 48 h, the fruit were washed with a cleaner (Sooty mould Kleen 278, DECCO, Monrovia, CA), rinsed with water, dried at 50–54 °C for about 2 min, and then packed in cartons ($43 \times 27 \times 25\,cm^3$). Packed fruit were incubated at 21 °C at about 95% RH for up to 2 weeks for disease observations.

2.5. Disease evaluation

The incidences of different decays were recorded separately at weeks 1 and 2 during fruit storage. Decayed fruit were removed after the first recording. Cumulative disease incidences were calculated after the second recording.

2.6. Data analysis

Analysis of variance of data was performed using the SAS statistical package (SAS Institute Inc., Cary, NC). Data were transformed to arc sine square root values to normalize variances prior to analysis. Treatment means were separated using the Waller–Duncan k ratio t test at $P<0.05$. Actual disease incidences were presented in all cases.

3. Results

3.1. Anthracnose

In tests on Fallglo tangerine, both fungicide and time of application significantly affected the anthracnose incidence in both years (Table 2). There was a significant fungicide × time of application interaction in both years, indicating that the response to fungicide treatment differed at the various times of application. There were few significant differences in effectiveness among fungicide treatments. Most fungicides significantly reduced anthracnose incidence when applied 2 d before harvest in both years and at 14 and 21 d before harvest in 2003. At other application dates, none of the fungicides was effective.

In tests on Sunburst tangerine, there was no overall significant effect of fungicide treatment in either year (Table 2). However, there was a significant effect of time of application and a significant fungicide × time interaction. At most harvest times, the anthracnose incidence on Sunburst fruit was low and differences between fungicide-treated fruit and nontreated controls were not significant.

3.2. Diplodia stem-end rot

Fungicide treatment and time of application had highly significant effects on the incidence of stem-end rot on both cultivars in both years (Table 3). There was a significant fungicide × time of application interaction in all cases except for Sunburst in 2004, indicating that the time of application affected the performance of the fungicide treatments. Benomyl and thiophanate methyl reduced the incidence of stem-end rot more than most other fungicides on all application dates. Azoxystrobin, pyraclostrobin, and fludioxonil reduced the incidence of stem-end rot on some application dates, but were generally less effective than the benzimidazole products. When applied 21 or 28 d before harvest, thiophanate methyl and benomyl were the only products that were highly effective.

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Table 2
Effect of fungicide applications to Fallglo and Sunburst tangerines at various times prior to harvest for control of anthracnose caused by *Colletotrichum gloeosporioides*

Cultivar	Year	Fungicide	Anthracnose incidence (%)			
			2 d[a]	14 d	21 d	28 d
Fallglo	2003	Control	49.8 a[b]	35.8 a	28.6 a	19.8
		Benomyl	32.3 b	19.3 b	11.1 b	22.3
		Azoxystrobin	33.9 b	16.1 b	14.3 b	14.2
		Fludioxonil	32.2 b	8.6 b	18.8 ab	22.2
		Thiophanate methyl	28.3 b	20.1 b	16.5 b	22.4
						ns
Fallglo	2004	Control	30.5 a	—	16.3	14.8
		Benomyl	11.4 c	—	15.7	12.1
		Azoxystrobin	14.0 bc	—	10.3	19.1
		Fludioxonil	19.1 b	—	11.8	20.2
		Thiophanate methyl	16.4 bc	—	11.3	19.0
		Pyraclostrobin	15.0 bc	—	15.5	12.1
					ns[c]	ns
Sunburst	2003	Control	16.3	13.0 a	6.6	12.6 ab
		Benomyl	2.7	9.8 a	11.6	14.2 a
		Azoxystrobin	3.0	10.8 a	17.9	6.4 b
		Fludioxonil	6.6	5.4 ab	11.4	19.1 a
		Thiophanate methyl	14.0	11.8 a	9.1	20.2 a
		Pyraclostrobin	8.8	0 b	9.4	16.4 a
			ns		ns	
Sunburst	2004	Control	9.3	0 b	4.0	0
		Benomyl	10.4	10.5 a	4.8	0
		Azoxystrobin	10.3	3.0 ab	2.1	2.0
		Fludioxonil	14.7	6.5 ab	2.1	0
		Thiophanate methyl	12.8	5.8 ab	6.7	0
		Pyraclostrobin	7.7	0 b	9.3	0
			ns		ns	ns

	ANOVA (*P* values)			
	Fallglo		Sunburst	
	2003	2004	2003	2004
Fungicide (F)	<0.0001	0.03	0.35	0.33
Time (T)	<0.0001	0.031	0.01	<0.0001
Replication (R)	0.36	0.01	0.51	0.29
F × T	0.006	0.009	0.037	0.07
F × R	0.023	0.06	0.19	0.39
T × R	0.71	0.28	0.40	0.07

[a]Days of fruit harvest after fungicide applications.
[b]Means with the same letter in the same column and test year are not significantly different based on the Waller–Duncan *k* ratio *t*-test ($P<0.05$).
[c]ns = not significant at $P<0.05$.

3.3. Green mould

There was a significant effect of fungicide treatment and time of application on green mould incidence in both years and on both cultivars (Table 4). Again there was a significant fungicide × time of application interaction in most cases indicating that fungicides performed differently at various times of application. On Fallglo, fungicide treatment provided effective control of green mould only when applied 2 d before harvest in 2003, and green mould was higher on treated than nontreated fruit when fungicides were applied 28 d before harvest. Some fungicides controlled green mould when applied 21 d before harvest in 2004. On Sunburst, fungicide application provided some control of green mould in 2003. The strobilurin fungicides, azoxystrobin and pyraclostrobin, were somewhat effective when applied shortly before harvest, but the benzimidazoles did not appear to be effective. However, benomyl and thiophanate methyl provided good control in 2004 and the other fungicides were less effective.

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Table 3
Effect of fungicide applications to Fallglo and Sunburst tangerines at various times prior to harvest for control of stem-end rot caused by *Lasiodiplodia theobromae*

Cultivar	Year	Fungicide	Stem-end rot incidence (%)			
			2 d[a]	14 d	2 d	28 d
Fallglo	2003	Control	21.2 a[b]	46.7 a	52.3 a	64.7 a
		Benomyl	8.1 b	6.0 c	2.9 c	3.6 d
		Azoxystrobin	13.7 b	27.2 b	41.6 b	17.5 c
		Fludioxonil	5.4 cd	0 c	3.9 c	21.2 bc
		Thiophanate methyl	0 d	8.0 c	0 c	24.9 b
Fallglo	2004	Control	36.7 a	—	30.4 a	42.9 a
		Benomyl	2.7 c	—	12.1 b	9.8 d
		Azoxystrobin	22.9 b	—	39.7 a	33.7 ab
		Fludioxonil	19.5 b	—	35.7 a	36.9 ab
		Thiophanate methyl	7.1 c	—	17.9 b	22.4 c
		Pyraclostrobin	16.2 b	—	31.3 a	31.5 bc
Sunburst	2003	Control	35.6 a	30.3 bc	43.8 a	27.8 ab
		Benomyl	0 d	2.7 d	4.7 d	0 d
		Azoxystrobin	18.8 b	37.5 a	36.9 ab	19.6 c
		Fludioxonil	19.0 b	24.6 c	17.5 c	23.8 bc
		Thiophanate methyl	8.2 c	5.6 d	0 d	2.8 d
		Pyraclostrobin	28.9 a	35.3 ab	27.6 bc	31.9 a
Sunburst	2004	Control	29.7 a	30.3 a	23.5 a	29.9 a
		Benomyl	0 c	0 b	3.0 b	2.1 b
		Azoxystrobin	21.6 ab	32.3 a	32.3 a	24.1 a
		Fludioxonil	20.9 b	32.7 a	21.0 a	20.9 a
		Thiophanate methyl	3.0 c	3.0 b	2.1 b	6.5 b
		Pyraclostrobin	16.0 b	26.3 a	23.2 a	16.2 a

	ANOVA (*P* values)			
	Fallglo		Sunburst	
	2003	2004	2003	2004
Fungicide (F)	<0.0001	<0.0001	<0.0001	<0.001
Time (T)	<0.0001	<0.0001	0.0057	0.02
Replication (R)	0.52	0.14	0.49	0.001
F × T	<0.0001	0.04	0.0002	0.28
F × R	0.23	0.31	0.58	0.49
T × R	0.63	0.67	0.46	0.82

[a] Days of fruit harvest after fungicide applications.
[b] Means with the same letter in the same column and test year are not significantly different based on the Waller–Duncan *k* ratio *t*-test ($P<0.05$).

4. Discussion

In Florida, early harvested citrus fruit are often subjected to ethylene degreening treatment to improve fruit colour for marketing purposes, and this practice can significantly increase the occurrence of anthracnose and Diplodia stem-end rot (Brown, 1975b; Brown and Barmore, 1977; Barmore and Brown, 1985; Zhang, 2004). The colonization of citrus fruit by *C. gloeosporioides* and *L. theobromae* occurs on the trees in groves where the fungi produce quiescent infections (Ismail and Zhang, 2004). Therefore, control measures for these two diseases applied before degreening, such as preharvest fungicide applications, could be more effective compared to post-degreening treatment.

In our current study, five fungicides were evaluated for their efficacy for anthracnose, Diplodia stem-end rot, and green mould control in two consecutive years. There was considerable variation of the data between years, citrus cultivars, fungicides, and harvest times. For anthracnose control on Fallglo fruit, all tested fungicides were effective when applications were made at 2 d before harvest in both years, and at 14 d prior to harvest in 2003. In 2004, a scheduled harvest at 14 d after application was not made due to hurricane Jeanne. Ritenour et al. (2004) also conducted preharvest application of fungicides for postharvest anthracnose control. They tested benomyl, pyraclostrobin, phosphorous acid and thiophanate methyl for anthracnose control on Sunburst fruit, and found that only benomyl and phosphorous acid significantly reduced

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Table 4
Effect of fungicide applications to Fallglo and Sunburst tangerines at various times prior to harvest for control of green mould, *Penicillium digitatum*

Cultivar	Year	Fungicide	Green mold incidence (%)			
			2 d[a]	14 d	21 d	28 d
Fallglo	2003	Control	42.3 a[b]	12.4	8.5	13.0 c
		Benomyl	38.8 ab	23.1	0	26.7 a
		Azoxystrobin	13.6 d	8.9	0	14.8 bc
		Fludioxonil	27.5 bc	9.6	2.8	21.2 abc
		Thiophanate methyl	24.1 cd	21.0	4.1	24.5 ab
				ns[c]	ns	
Fallglo	2004	Control	0	—	18.6 a	12.2
		Benomyl	0	—	9.4 b	9.0
		Azoxystrobin	0	—	13.0 ab	7.8
		Fludioxonil	0	—	11.8 ab	14.0
		Thiophanate methyl	0	—	6.1 b	4.3
		Pyraclostrobin	0	—	8.5 b	6.5
			ns			ns
Sunburst	2003	Control	29.8 a	35.9 ab	42.3 b	25.6 ab
		Benomyl	22.7 ab	44.5 a	55.9 a	15.6 b
		Azoxystrobin	13.3 c	30.0 bc	21.8 c	21.8 b
		Fludioxonil	24. 0 a	37.3 ab	52.7 a	25.5 ab
		Thiophanate methyl	23.6 ab	41.6 a	41.9 b	33.5 a
		Pyraclostrobin	15.7 bc	23.6 c	39.1 b	24.7 ab
Sunburst	2004	Control	16.4 a	21.8 ab	6.4 bc	25.3 bc
		Benomyl	0 b	3.7 d	0 c	2.0 d
		Azoxystrobin	11.7 a	21.0 ab	5.0 bc	21.0 c
		Fludioxonil	17.0 a	27.9 a	18.1 a	29.6 b
		Thiophanate methyl	0 b	6.1 cd	0 c	10.4 d
		Pyraclostrobin	9.1 ab	14.4 bc	9.2 b	39.8 a

	ANOVA (*P* values)			
	Fallglo		Sunburst	
	2003	2004	2003	2004
Fungicide (F)	0.0005	0.01	<0.0001	<0.0001
Time (T)	<0.0001	<0.0001	<0.0001	<0.001
Replication (R)	0.066	0. 64	0.28	0.18
F × T	0.005	0.22	<0.0001	0.001
F × R	0.076	0.16	0.73	0.16
T × R	0.56	0.97	0.047	0.32

[a]Days of fruit harvest after fungicide applications.
[b]Means with the same letter in the same column and test year are not significantly different based on the Waller–Duncan *k* ratio *t*-test ($P<0.05$).
[c]ns = not significant at $P<0.05$.

anthracnose for one of the two harvests after grove application of the fungicides. Brown and Barmore (1976) reported that benomyl reduced the anthracnose incidence of Robinson tangerine fruit when it was applied by postharvest drenching before degreening treatment.

The lack of control of anthracnose on Sunburst tangerines by preharvest application of fungicide was largely attributable to low disease levels and to the state of maturity of the fruit. In both years, Sunburst fruit had already developed a natural orange colour when the applications were made. Increased natural orange colour development on citrus fruit increases their natural resistance to anthracnose (Brown and Barmore, 1977; Brown, 1978). This natural resistance was observed on Fallglo fruit during our tests in 2003. Anthracnose incidence gradually decreased from the 2- to the 28-d harvests on control Fallglo fruit in 2003. Additional study is still needed to make conclusions for the efficacy of tested fungicides for postharvest anthracnose control by preharvest chemical applications, but thiophanate methyl performed as well as benomyl that was recommended previously (Ritenour et al., 2003).

Benomyl and thiophanate methyl consistently reduced Diplodia stem-end rot incidence on Fallglo and on Sunburst fruits harvested on all dates after the fungicides were applied and are consistent with those reported by others (Ritenour et al., 2004). Our tests also indicated that the period of time that benomyl and thiophanate methyl

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were effective for Diplodia stem-end rot control was at least 28 d after they were sprayed on trees in the grove. Other researchers (Brown and Albrigo, 1972; Smilanick et al., 2006) also showed that effective residue levels of benomyl and thiophanate methyl in citrus fruit peel after spraying the chemicals were maintained for more than a month. Brown and Albrigo (1972) reported that benomyl residue (methyl 2-benzimidazolecarbamate) in peel of Hamlin oranges at 70 d, and Valencia oranges at 84 d after grove applications was still sufficient to reduce the incidence of green mould. Smilanick et al. (2006) showed that thiophanate methyl residues persisted in peel of Navel oranges and significantly reduced the incidence of green mould up to 7 weeks after field application.

Benomyl and thiophanate methyl showed a similar spectrum and properties for stem-end rot control in our tests. These may be due to the formation of carbendazim by both benomyl and thiophanate methyl after they were applied to citrus trees. Carbendazim is the principal degradation product of benomyl and thiophanate methyl in plants and soil environments (Anastassiades and Schwack, 1998; Tomlin, 2000). Carbendazim is also an effective fungicide for stem-end rot and green mould control (Eckert and Brown, 1986).

Among other tested fungicides in this study, azoxystrobin, fludioxonil and pyraclostrobin only significantly reduced Diplodia stem-end rot incidence in some harvests in the 2 years of tests. Both azoxystrobin and fludioxonil significantly reduced Diplodia stem-end rot incidence on Fallglo fruit in 2003, but they were not effective on Fallglo fruit in 2004 nor on Sunburst fruit in either year.

Green mould caused by *P. digitatum* is one of the most important postharvest diseases on Florida citrus (Ismail and Zhang, 2004; Zhang and Swingle, 2005). Preharvest fungicide application is one of the effective approaches for postharvest green mould control. Normally, green mould is not a severe problem on early season citrus in Florida since the degreening treatment uses high temperatures (28–30 °C) which have a curing effect on wounds and prevent green mould development (Hopkins and Loucks, 1948; Brown, 1973; Brown and Barmore, 1983; Zhang and Swingle, 2005). However, a considerable amount of green mould was observed on both Fallglo and Sunburst fruits of some harvests in the current study. The current tests showed that only benomyl and thiophanate methyl consistently and significantly reduced green mould on Sunburst fruit in 2004 and reduced green mould incidence on Fallglo harvested 21 d after application in 2004. Both benomyl and thiophanate methyl were reported to be effective for postharvest green mould control by preharvest applications (Brown and Albrigo, 1972; Smilanick et al., 2006). However, neither benomyl nor thiophanate methyl reduced green mould incidence on either cultivar in 2003 in the current study.

The significant differences in performance of benomyl and thiophanate methyl in green mould control between 2003 and 2004 might be due to fungicide resistance development in our test facility. We found that TBZ-resistant isolates had developed in our storage room, and that those isolates also were resistant to benomyl and thiophanate methyl (data not shown). After the fungicide resistance problem was detected in the spring of 2003, the storage room was completely sanitized in the summer of 2003, which largely eliminated TBZ-resistant *P. digitatum* isolates. Both benomyl and thiophanate methyl performed well for green mould control in 2004 tests.

Resistance of *P. digitatum* isolates to postharvest fungicide TBZ in citrus packinghouses has been reported in California and Florida (Brown, 1982; Eckert, 1990; Holmes and Eckert, 1999). Minimizing the fungicide resistance development is a necessary strategy for the effective control of citrus postharvest diseases. Many approaches could be used to prevent and reduce the development of fungicide resistance by fungal pathogens. For example, chemicals used should have multiple modes of action. Since TBZ, benomyl and thiophanate methyl have the same mode of action, care should be taken for appropriate use of these fungicides to prevent and reduce resistance development. We suggest that imazalil or sodium *o*-phenylphenate (SOPP) or both, but not TBZ, might be used in postharvest stage in packinghouse in order to reduce the potential development of benzimidazole resistance if thiophanate methyl is used in preharvest application. Fruit drenching with TBZ or imazalil would be not necessary if thiophanate methyl is used in groves.

During the current study, little sour rot and Phomopsis stem-end rot were observed. Sour rot and Phomopsis stem-end rot are normally more prevalent on mature to over mature fruit or on fruit held in cold storage for long periods (Ismail and Zhang, 2004). TBZ and benomyl have been reported to be effective for Phomopsis stem-end rot control, but not for sour rot control (Brown and Miller, 1999). It is expected that thiophanate methyl should also be effective for Phomopsis stem-end rot control since benomyl, thiophanate methyl and TBZ have the same mode of action. Although mid- to late-season harvested fruit cultivars do not need ethylene degreening for fruit peel colour improvement, it appears that preharvest applications of benomyl or thiophanate methyl could also be applied on mid- and late-season fruits to reduce the Diplodia stem-end rot, Phomopsis stem-end rot and Penicillium moulds. Preharvest application of fungicides appears to be a valuable component for an effective integrated management of citrus postharvest diseases.

The major purpose of this study was to search for alternative preharvest fungicides to benomyl for the control of postharvest anthracnose and Diplodia stem-end rot on Florida early season citrus fruit. Benomyl was the only registered commercial compound for preharvest application to control postharvest diseases, but is no longer available since the manufacturer has terminated production. Based on our study in two consecutive years, although considerable variations among data were observed, the overall results indicate that benomyl and thiophanate methyl performed similarly and effectively for the control of postharvest anthracnose, Diplodia stem-end rot, and green mould on Florida early harvested citrus fruits after they were applied on citrus trees in groves. Thiophanate methyl, but not azoxystrobin, fludioxonil and pyraclostrobin, appears to be an excellent alternative to benomyl. It currently has an emergency registration under Section 18 for preharvest use on citrus and full registration is in progress.

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References

Anastassiades, M., Schwack, W., 1998. Analysis of carbendazim, benomyl, thiophanate methyl and 2,4-dichlorophenoxyacetic acid in fruits and vegetables after supercritical fluid extraction. J. Chromatogr. A 825, 45–54.

Barmore, C.R., Brown, G.E., 1985. Influence of ethylene on increased susceptibility of oranges to *Diplodia natalensis*. Plant Dis. 69, 228–230.

Brown, G.E., 1973. Development of green mold in degreened oranges. Phytopathology 63, 1104–1107.

Brown, G.E., 1974. Postharvest citrus decay as affected by Benlate applications in grove. Proc. Florida State Hortic. Soc. 87, 237–240.

Brown, G.E., 1975a. Anthracnose, a serious decay of degreened Robinson tangerines. Proc. Florida State Hortic. Soc. 88, 308–311.

Brown, G.E., 1975b. Factors affecting postharvest development of *Colletotrichum gloeosporioides* in citrus fruits. Phytopathology 65, 404–409.

Brown, G.E., 1978. Hypersensitive response of orange-colored Robinson tangerines to *Colletotrichum gloeosporioides* after ethylene treatment. Phytopathology 68, 700–706.

Brown, G.E., 1982. Resistance of decay fungi to benzimidazole fungicides used in Florida citrus packinghouses. Proc. Florida State Hortic. Soc. 95, 239–242.

Brown, G.E., Albrigo, L.G., 1972. Grove application of benomyl and its persistence in orange fruit. Phytopathology 62, 1434–1438.

Brown, G.E., Barmore, C.R., 1976. The effect of ethylene, fruit colour, and fungicides on susceptibility of 'Robinson' tangerines to anthracnose. Proc. Florida State Hortic. Soc. 89, 198–200.

Brown, G.E., Barmore, C.R., 1977. The effect of ethylene on susceptibility of Robinson tangerines to anthracnose. Phytopathology 67, 120–123.

Brown, G.E., Barmore, C.R., 1983. Resistance of healed citrus exocarp to penetration by *Penicillium digitatum*. Phytopathology 73, 691–694.

Brown, G.E., Miller, W.R., 1999. Maintaining fruit health after harvest. In: Timmer, L.W., Duncan, L.W. (Eds.), Citrus Healthy Management. APS Press, Inc., St. Paul, MN, pp. 175–192.

Eckert, J.W., 1990. Impact of fungicide resistance on citrus fruit decay control. In: Proceedings of ACS Symposium Series, American Chemical Society, Washington, DC, pp. 286–302.

Eckert, J.W., Brown, G.E., 1986. Postharvest citrus diseases and their control. In: Wardowski, W.F., Nagy, S., Grierson, W. (Eds.), Fresh Citrus Fruit. Van Nostrand Reinhold Company Inc., New York, pp. 315–360.

Holmes, G.J., Eckert, J.W., 1999. Sensitivity of *Penicillium digitatum* and *P. italicum* to post-harvest citrus fungicides in California. Phytopathology 89, 716–721.

Hopkins, E.F., Loucks, K.W., 1948. A curing procedure for the reduction of mold decay in citrus fruits. Univ. Florida Agric. Expt. Station Bull. 450, 1–26.

Ismail, M., Zhang, J., 2004. Post-harvest citrus diseases and their control. Outlooks Pest Manage. 15, 29–35.

Peever, T.L., Carpenter-Boggs, L., Timmer, L.W., Carris, L.M., Bhatia, A., 2005. Citrus black rot is caused by phylogenetically distinct lineages of *Alternaria*. Phytopathology 95, 512–518.

Ritenour, M.A., Dou, H., 2000. Factors contributing to the 'green ring' disorder of fresh market citrus. Proc. Florida State Hortic. Soc. 113, 297–299.

Ritenour, M.A., Zhang, J. Wardowski, W.F., Brown, G.E., 2003. Postharvest decay control recommendations for Florida citrus fruit. Fla. Coop. Ext. Serv., Univ. Florida, CIR 359-A (Revised), pp. 1–6.

Ritenour, M.A., Pelosi, R.R., Burton, M.S., Stover, E.W., Dou, H., McCollum, T.G., 2004. Assessing the efficacy of preharvest fungicide applications to control postharvest diseases of Florida citrus. HortTechnology 14, 58–62.

Rogers, M.E., Timmer, L.W., 2006. Florida Citrus Pest Management Guide. University of Florida, IFAS Ext. ENY-601.

Smilanick, J.L., Mansour, M.F., Sorensen, D., 2006. Pre- and postharvest treatments to control green mold of citrus fruit during ethylene degreening. Plant Dis. 90, 89–96.

Tomlin, C.D.S., 2000. A world compendium. In: Tomlin, C.D.S. (Ed.), The Pesticide Manual, 12h ed. British Crop Protection Council, UK.

Wardowski, W.F., 1996. Recommendations for degreening Florida fresh citrus fruits. Florida Coop. Ext. Serv. Circular 1170, 1–3.

Zhang, J., 2004. Effect of ethylene on natural resistance of citrus fruit to stem-end rot caused by *Diplodia natalensis* and its relation to postharvest control of this decay. Proc. Florida State Hortic. Soc. 117, 364–367.

Zhang, J., Swingle, P.P., 2005. Effects of curing on green mold and stem-end rot of citrus fruit and its potential application under Florida packing system. Plant Dis. 89, 834–840.

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