PREHARVEST SANITISERS AND FUNGICIDES FOR REDUCING *PENICILLIUM DIGITATUM* INOCULUM ON CV. SATSUMA MANDARIN

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ABSTRACT

The efficacy of three sanitisers (didecyldimethyl ammonium chloride, sodium hypochlorite and chlorobromohydantoin) and two fungicides (kresoxim methyl, potassium phosphite), applied as whole-tree sprays prior to harvest, was compared for reducing the number of colony forming units (cfu) of *Penicillium digitatum* on the surface of cv. Satsuma mandarin fruit. The effects on the cfu of the saprophytic fungi *Cladosporium* spp. and *Epicoccum purpurascens* were also examined. The efficacy of the sanitisers and fungicides differed according to the species of fungi. Didecyldimethyl ammonium chloride gave approximately a 75% reduction in numbers of cfu of *P. digitatum*, *Cladosporium* spp. and *E. purpurascens*. Sodium hypochlorite significantly reduced cfu of *P. digitatum* but did not affect cfu of *Cladosporium* spp. and *E. purpurascens*. Kresoxim methyl, potassium phosphite and chlorobromohydantoin exhibited no effect on cfu of *P. digitatum* and *E. purpurascens*. However, cfu of *Cladosporium* spp. was significantly reduced when fruit were treated with potassium phosphite.

Keywords: *Penicillium digitatum, Cladosporium* spp., *Epicoccum purpurascens*, fruit surface, green mould, preharvest treatment.

INTRODUCTION

Green mould caused by *Penicillium digitatum* (Pers.) Sacc. causes significant losses of cv. Satsuma mandarin (*Citrus reticulata* Blanco) after harvest in New Zealand. Traditionally, green mould has been controlled by postharvest application of fungicides. In New Zealand, carbendazim, thiophanate methyl and imazalil are registered for the control of green mould in citrus. All the registered fungicides are acceptable to the USA market but only fruit treated with benzimidazoles are allowed for export to Japan. However, there is a market advantage in exporting fungicide-free fruit due to consumer demands for pesticide-free food and the industry consequently seeks to reduce the use of postharvest fungicides. Sanitisers, particularly sodium and calcium hypochlorites, have been used for many years in packhouse flotation tanks as an alternative treatment for postharvest rots (Eckert & Eaks 1989; Brown & Wardowski 1984; Taverner 2001). A particular advantage of using such sanitisers is that market access is not restricted because of fungicide residues.

In New Zealand, a large proportion of postharvest citrus rots originate from minor skin wounds sustained during harvest (R.A. Fullerton, unpubl. data). Minimising spore numbers on fruit prior to harvest could therefore reduce the subsequent incidence of postharvest rots. This study investigated whether the use of sanitisers as a tree spray prior to harvest could reduce the load of spores on cv. Satsuma mandarin. Specifically, the efficacy of the sanitisers, sodium hypochlorite, chlorobromohydantoin and didecyldimethyl ammonium chloride, was compared with that of two fungicides, kresoxim methyl and potassium phosphite. The latter is registered for *Phytophthora* spp. in citrus (Young 2007) and was included in the study to test its effectiveness against other fungi. The efficacy of the five chemicals was examined by comparing the number of colony forming units (cfu) of *P. digitatum* on the fruit before and after spray application. The common saprophytes, *Cladosporium* spp. and *Epicoccum purpurascens* Ehrenb., were included in the study to investigate the general efficacy of the tested sanitisers and fungicides.

METHODS

The preharvest spray trial was undertaken in a cv. Satsuma mandarin orchard at harvest maturity in the Matakana area, north of Auckland, New Zealand. The trial comprised six treatments: three sanitisers, two fungicides and a water control. Details of the chemicals and application concentrations used are listed in Table 1. The adjuvant, Nu-film-17® (1 ml/litre), was used in all treatments (including water control) to assist coverage and retention of the chemicals on the plant surfaces. The sodium hypochlorite solution was buffered to pH 6.9 using sodium bicarbonate (1%) and citric acid. At this pH, approximately 85% of available chlorine will be in the form of hypochlorous acid, maximising its disinfection capacity (Eckert & Eaks 1989; Smilanick et al. 2002). Sodium bicarbonate was added to the chlorobromohydantoin solution based on a pilot study (P.A. Rheinländer & R.A. Fullerton, unpubl. data) that showed an increased activity against *P. digitatum* at a higher pH (unamended Bromicide® Gel solution has a pH of 7.3).

TABLE 1: Sanitisers and fungicides, and their concentrations, applied as preharvest sprays to cv. Satsuma mandarin. ai = active ingredient.

Chemical	Trade name	Type	Concentration applied (ai)
Nil (water control)	–	–	–
sodium hypochlorite + 1% sodium bicarbonate + citric acid to pH 6.9	sodium hypochlorite solution	sanitiser	0.02%
didecyl dimethyl ammonium chloride	Sporekill™	sanitiser	0.012%
chlorobromohydantoin + sodium bicarbonate to pH 8.4	Bromicide® Gel	sanitiser	0.0053%
potassium phosphite	Foli-R-Fos® 400	fungicide	0.12%
kresoxim methyl	Stroby® WG	fungicide	0.005%

The treatments were applied to single tree plots separated by guard trees in a randomised block design with three replicate blocks. Prior to treatment, 10 fruit were sampled at random from each datum tree for assessing pre-treatment spore numbers. Chemicals were applied to individual trees to the point of runoff using a spray wand attached to a motorised power sprayer. Applications were made on 13 July 2005. The following morning a second sample of 10 fruit per tree was taken for post-treatment examination of viable spore numbers.

Numbers of viable spores on fruit surfaces were inferred from the number of colony forming units (cfu) that developed on potato dextrose agar (PDA). Each fruit was agitated for 20 seconds with 5 ml sterile water in a zip-lock plastic bag. Aliquots of 0.1 ml from each washing were plated directly onto single plates of PDA containing ampicillin (100 µg/ml) + streptomycin sulphate (100 µg/ml) to inhibit bacterial growth. The plates were incubated at room temperature for 48 h and numbers of colonies of *P. digitatum, Cladosporium* spp. and *E. purpurascens* on each plate were counted. The number of cfu of each species was calculated for each fruit and the numbers averaged for each tree (30 fruit per treatment).

The effects of the chemicals on cfu/fruit for each fungal species were tested using a two-way ANOVA. Data were log-transformed to meet test assumptions, i.e. homogenous variances.

RESULTS AND DISCUSSION

The efficacy of the tested compounds differed according to the species of fungi (Fig. 1). Didecyldimethyl ammonium chloride was the most effective chemical, significantly decreasing cfu by approximately 75% for all three species (*P. digitatum*

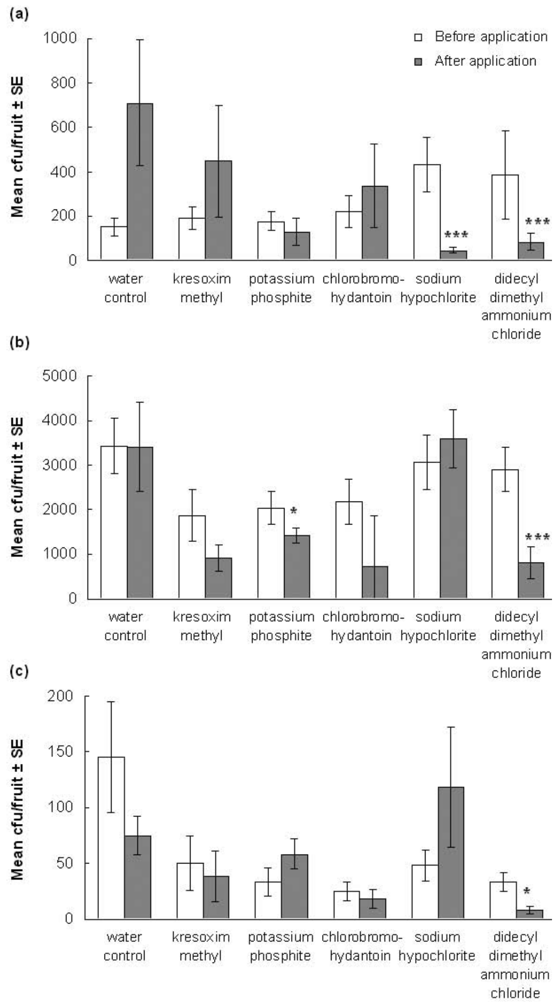

FIGURE 1: Number of cfu per fruit of (a) *Penicillium digitatum*, (b) *Cladosporium* spp. and (c) *Epicoccum purpurascens* recorded from washings of the fruit surface of cv. Satsuma mandarins before and after application of test chemicals. Values are the mean for 30 fruit per treatment (10 fruit sampled from each of three replicate trees). Error bars are standard errors (SE). Significant differences between before and after test chemical application are indicated by * (P<0.001) or * (P<0.05).**

P<0.001; *Cladosporium* spp. *P*<0.001; *E. purpurascens P*<0.05). Sodium hypochlorite significantly reduced cfu of *P. digitatum* (*P*<0.001), but no effect (*P*>0.05) was observed for *Cladosporium* spp. and *E. purpurascens*. Kresoxim methyl, potassium phosphite and chlorobromohydantoin exhibited no effect on cfu of *P. digitatum* and *E. purpurascens*. However, cfu of *Cladosporium* spp. were significantly reduced when fruit were treated with potassium phosphite (*P*<0.05). This finding suggests that potassium phosphite, which is normally considered to be specific to species of *Phytophthora* and *Pythium*, does express some activity against other fungal species, including *Cladosporium* spp.

The number of cfu was subject to high variability between individual fruit from the same treatment (Fig. 1). This was particularly apparent in the water control, where the number of *P. digitatum* colonies was five times higher on the fruit sampled after spray application. No competition was observed between colonies of the three species on the PDA plates and the variability in cfu was likely to be a result of 'nests' of spores on the surface of some fruit.

Although sanitisers are widely used for reducing spore contamination of packhouse equipment and fruit washing systems there is no recent record of them being used as preharvest orchard sprays. While chlorine is highly effective at killing spores of *P. digitatum* in water (Smilinak et al. 2002), it is considerably more difficult to kill spores on the surface of fruit (Eckert & Eaks 1989). Nevertheless, in a laboratory experiment, Brown & Wardowski (1984) reported a 98% kill of spores of *P. digitatum* on oranges after 15 seconds spray of 200 ppm chlorine. The present study achieved a ~90% reduction of cfu of *P. digitatum* on cv. Satsuma mandarin after application with sodium hypochlorite in the field. This is an encouraging result considering the difficulty of obtaining complete fruit coverage in the field.

The present trial has demonstrated the feasibility of applying sanitisers as a preharvest treatment to reduce the spore numbers on the surface of cv. Satsuma mandarin. The sanitisers sodium hypochlorite (buffered to pH 6.9) and didecyldimethyl ammonium chloride were the most effective at reducing cfu of *P. digitatum.* Didecyldimethyl ammonium chloride also effectively reduced cfu of the other test species indicating a broad efficacy range. Further storage trials are required to determine whether the reduction of cfu observed in the field would result in a similar reduction in storage rots.

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