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Postharvest dip, drench and wax coating application of pyrimethanil on citrus fruit: Residue loading and green mould control

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ABSTRACT

Residue loading of pyrimethanil (PYR) with application methods typically used in South African packhouses and green mould control was studied. PYR was applied curatively (after inoculation) and protectively (before inoculation) in dip, drench and wax coating treatments and fruit were inoculated with an imazalil (IMZ)-sensitive or an IMZ-resistant isolate of *Penicillium digitatum*, the causal agent of citrus green mould. The dip treatments consisted of PYR concentrations 0, 50, 100, 250, 500 and 1000 mg L^{-1}; fruit were dipped for 60 s at 18 °C at pH 7. Residues loaded differed between fruit type and batch. At the registered concentration of 1000 mg L^{-1}, an average of 1.96 mg kg^{-1} PYR (range 1.40–2.95 mg kg^{-1}) was loaded. Increased residue levels generally resulted in improved control, but better curative than protective control was observed with effective residue levels of 0.27 and 0.91 mg kg^{-1} for 50% and 75% curative control of green mould, respectively. Effective residue levels for 50% protective control were 0.97–1.43 mg kg^{-1}. Drench treatments consisted of exposure times of 30, 60 and 90 s with 1000 mg L^{-1} PYR. PYR residues on Valencia oranges were 2.46–4.22 mg kg^{-1} PYR, and on navel oranges were 2.09–7.64 mg kg^{-1}. Green mould control on Valencia orange fruit was moderate (57.3–69.8% for curative treatment after 6 h) to relatively poor (4.9–14.6% control for curative treatments after 24 h and 18.1–26.4% for protective treatments). Control on navel oranges following these treatments was poor (0–7%). Drench applications with different combinations of PYR, IMZ, thiabendazole (TBZ), guazatine (GZT) and didecyl dimethyl ammonium chloride were applied to navel orange fruit with 60 s exposure time. In general, fungicide mixtures performed better than single fungicides (44.4–64.6% for mixtures *vs.* 24.3, 39.6 and 46.5% for PYR, GZT and TBZ respectively). Drench applications where fruit were inoculated with spore suspensions containing 10^6, 10^5, 10^4 or 10^3 spores mL^{-1} showed that infections from lower inoculum loads were better controlled (90.6–98.6% *vs.* 32.6–62.2% for 10^3 *vs.* 10^6 spores mL^{-1}). Wax with 2000 or 4000 mg L^{-1} PYR was applied at 0.6, 1.2 and 1.8 L wax ton^{-1} of fruit. Residues loaded on navel oranges were 1.75–6.33 mg kg^{-1}, and on Valencia oranges were 1.05–9.91 mg kg^{-1}. Green mould control on navel oranges was poor and were 0–12.2%. On Valencia oranges, higher levels of protective control (28.8–54.0%) than curative treatments (10.5–27.3%) was observed. The IMZ-resistant isolate was successfully controlled by all PYR treatments, in some cases better than the sensitive isolate. In conclusion, PYR provided very good curative control in dip applications, but gave poor results in drench and wax applications. Green mould control following PYR drench treatment improved on fruit inoculated with smaller spore loads, or when PYR was mixed with other fungicides.

1. Introduction

South Africa is the second largest exporting country of fresh citrus in the world, exceeded only by Spain (CGA, 2016). Green mould, caused by *Penicillium digitatum* (Pers.:Fr) Sacc., is the most common postharvest decay problem (Lesar, 2013), as was also observed in other temperate climates (Kavanagh and Wood, 1967; Eckert and Eaks, 1989). *Penicillium digitatum* is exclusively a wound pathogen and wounds made on mature fruit by insects or during harvest serve as an entry point (Smilanick et al., 2005). Practices including sanitation and careful handling of fruit during harvesting are essential to control this disease. In addition, fungicides are used for postharvest disease control (Eckert, 1995). Thiabendazole (TBZ), imazalil (IMZ) and guazatine (GZT) have been used for decades to control green mould in South Africa (Erasmus

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et al., 2011, 2013, 2015a,b; Njombolwana et al., 2013a,b; Kellerman et al., 2014, 2016). More recently, pyrimethanil (4,6-Dimethyl-*N*-phenylpyrimidin-2-amine; PYR) has also been registered for this use, since resistance against older fungicides are a problem (Kinay et al., 2007; Erasmus et al., 2015b). It has an MRL of 10 mg kg^{-1} in most markets (Hattingh and Hardman, 2015), although buyers may demand even lower levels. Although Kanetis et al. (2010) showed that natural resistance against PYR occurred at very low frequencies in a natural population of *P. digitatum*, making PYR a good alternative for the use against isolates resistant to more commonly used fungicides (Erasmus et al., 2015b). Mixing and rotating fungicides with different modes of action can reduce chances of decay control failure due to a build-up of resistance in *P. digitatum* populations (Kanetis et al., 2008, 2010).

Previous work has shown that the method of application greatly influences the efficacy of postharvest fungicides (Smilanick et al., 2006; Kanetis et al., 2008; Erasmus et al., 2011, 2013, 2015a; Njombolwana et al., 2013a,b; Kellerman et al., 2014, 2016). This is also true for efficacy of PYR as applied as dip, wax coating and drench applications (D'Aquino et al., 2006; Smilanick et al., 2006; Kanetis et al., 2007, 2008). Elevated temperature and pH was found to positively influence PYR efficacy (D'Aquino et al., 2006; Kanetis et al., 2008). Dip and drench application was much more successful than wax coating of PYR (Smilanick et al., 2006; Kanetis et al., 2007).

In South Africa, common application methods for postharvest fungicides include dip, wax coating and drench application. A survey was done by Erasmus et al. (2011) to determine the most common parameters for these methods. Whilst specific parameters varied between packhouses, the most common application methods used in citrus packhouses were dip, wax coating and drench application. Drenching fruit after harvest is a common way to reduce decay development during degreening (Smilanick et al., 2006; Erasmus et al., 2011; Kellerman et al., 2014). In South Africa, drench application is most commonly applied at 250 L bin^{-1} for 760 L bins (Erasmus et al., 2011; Kellerman et al., 2014). This is an important step in the postharvest treatment regime, and poor control will lead to the selection of resistant fungal strains that will be carried into the packhouse after degreening. For TBZ and IMZ, it was found that each fungicide on its own had better curative action as a dip application, and better protective action as a wax coating application under these conditions (Erasmus et al., 2011, 2013, 2015a; Njombolwana et al., 2013a; Kellerman et al., 2014). Collectively, these projects have defined the relationship between residue loading and green mould control and modelled effective residue levels indicative of green mould control as well as the application parameters required to load the requisite IMZ or TBZ residue levels on fruit.

The aim of this study was to determine effective residue values for PYR as a curative and protective dip application, and to investigate the efficacy of PYR in a wax coating and drench application to control green mould on citrus, on its own as well as in combination with other fungicides.

2. Materials and methods

2.1. Fruit

For dip application, four different batches of citrus fruit were used: Satsuma mandarin (*Citrus unshiu*) and 'Nules' Clementine mandarin fruit (*C. reticulata*), and two *C. sinensis* batches, both 'Australian' navel orange fruit. For the drench trials, 'Washington' navel orange fruit (*Citrus sinensis* (L.) Osbeck), 'Eureka' lemon and Satsuma mandarin fruit were obtained from packhouses in Citrusdal. Fruit was washed over rotating brushes using a 1 mL L^{-1} quaternary ammonium compound solution (Sporekill, 120 g L^{-1} didecyl dimethyl ammonium chloride, Hygrotech, Pretoria, South Africa), dried in a drying tunnel and stored at 4 °C for 5 days at the most. One day before the trial, the fruit was transferred from cold storage to ambient temperature (22 °C).

2.2. Fungal cultures and spore suspensions

The isolates of *P. digitatum* used in the trials were STE-U 6560, which is sensitive to TBZ and IMZ, and STE-U 6590, which is resistant to GZT, TBZ and IMZ. The IMZ EC_{50} and EC_{95} values for the sensitive isolate (STE-U6560) were 0.07 and 0.10_g mL^{-1} and for the resistant isolate (STE-U6590) they were 1.83 and 4.52_g mL^{-1}. (Erasmus et al., 2011, 2015b). The isolates were plated out from −80 °C storage cultures onto PDA (DIFCO, Becton, Dickinson and Company, USA and Le Pont de Claix, France) and grown for 7–14 days at 25 °C before each trial. Spore suspensions were freshly prepared each day of inoculation by filtering the cultures grown on PDA through two layers of cheesecloth with distilled water amended with Tween 20 (Sigma-Aldrich, St Louis, MO, USA) at a concentration of 0.01 mL L^{-1}. Spores were counted with a haemocytometer and the final spore concentration adjusted to 10^6 spores mL^{-1}. Viability of spores was verified after each trial by plating out the spore suspension on PDA.

2.3. Fruit inoculation

For dip application trials, fruit destined for curative treatment was inoculated 24 h before treatment. Fruit was wounded and inoculated simultaneously with a triple wound inducer, which consisted of three insect needles placed in a needle clamp to create three small wounds of 0.5 mm wide and 2 mm deep; the wound inducer was dipped into the spore suspension directly before wounding. Four wounds were made at equal distances around the stem end. Fruit destined for protective treatment was first treated, left to dry and then inoculated. Three replications of 12 fruit each was inoculated per treatment combination. Both the sensitive and resistant isolate were used in separate treatments in the dip application trials.

For drench and wax application, fruit were wound-inoculated in a similar manner, but using a wound inducer that consisted of a 7 mm diameter cylindrical rod with a protruding tip 2 mm long and 1 mm in diameter. This wound inducer made a 2-mm deep wound into the peel, piercing the flavedo into the albedo. Four wounds were made on each fruit equidistantly around the stem end. Only the sensitive isolate was used for drench and wax application trials.

2.4. Dip treatment: PYR residue loading and effective residue values for curative and protective control of P. digitatum

The experiment was a 2 × 2 × 6 factorial design with 2 isolates (STEU6560 and 6590), 2 fungicide actions (curative and protective PYR treatment), and 6 PYR concentrations, with three repetitions of 12 fruit for each treatment combination. Six containers were prepared with 25 L solutions of different concentrations of PYR (Protector 400SC, ICA International, Stellenbosch, South Africa) in municipal water (pH 6.99): 0, 50, 100, 250, 500 and 1000 mg L^{-1}. The suspensions were continuously stirred during the trials. Fruit was immersed for 60 s in the respective concentrations at 18 °C, packed in in lock back table grape cartons (APL cartons, Worcester, South Africa) on count SFT13 nectarine trays (Huhtamaki South Africa (Pty.) Ltd Atlantis, South Africa). Each carton was enclosed in transparent polyethylene bags.

After treatment and inoculation, the fruit were incubated at 22 °C for 10 days. Infection was rated with a UV light (UV-A at 365 nm, Labino Mid-light; www.labino.com) after 4 days' incubation. The number of infected wounds was recorded using the UV light to indicate yellow fluorescing green mould lesions that could not yet be seen with the naked eye (Erasmus et al., 2011). Sporulation was rated after 10 days on a scale of 0–6 (0 = no sign of disease; 1 = visible lesion but no sporulation; 2 = sporulating area on lesion smaller than a quarter of the fruit; 3 = sporulating area larger than a quarter of the fruit, but smaller than half of the fruit; 4 = sporulating area larger than half of the fruit, but smaller than three quarters of the fruit; 5 = sporulating area larger than three quarters of the fruit, but smaller than the whole