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The effect of temperature, exposure time and pH on imazalil residue loading and green mould control on citrus through dip application

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ABSTRACT

Imazalil (IMZ) is the most relied upon fungicide for use against citrus green mould, caused by *Penicillium digitatum*. In South Africa, the IMZ sulphate formulation is used as dip application in packhouses. Previous studies showed that IMZ efficacy and residue loading of this formulation is highly affected by pH in the application solution. This study investigated the effect of pH, exposure time and temperature on residue loading, green mould control and sporulation inhibition when citrus fruit were dipped in IMZ sulphate. Clementine mandarins, lemons and navel oranges were dipped in a 500 mg L^{-1} solution of IMZ sulphate for 15, 45 or 90 s, at a temperature of 23, 35 or 45 °C, and at pH levels of 3 or 6. At pH 3, similar residues were loaded on fruit regardless of fruit type, temperature or exposure time (1.18–1.51 mg kg^{-1}). At pH 6, both temperature and exposure time influenced residue loading: at 23 °C, higher residue levels were observed than at pH 3 (2.03–2.73 mg kg^{-1}, increasing with longer exposure time); at 35 °C and 45 °C, residue levels increased significantly with 15 s, 30 s and 45 s exposure time (3.34, 5.11, 7.24 mg kg^{-1} and 5.31, 9.65 and 13.10 mg kg^{-1}, respectively). Clementine mandarin fruit loaded higher residues at pH 6 (6.96 mg kg^{-1}) than lemons and navels (5.20 and 4.82 mg kg^{-1}, respectively). The MRL was frequently exceeded at longer exposure times. Green mould control was lower at pH 3 and was influenced by temperature and exposure time (92.9–97.4% and 42.8–45.1% for lemons and Clementine mandarins, respectively). Better control was observed at pH 6 regardless of temperature and exposure time (96.9–98.7% and 39.3–57.9% for lemons and Clementine mandarins, respectively). On navel oranges, increased exposure time led to increased green mould control (84.7–92.5%). Sporulation incidence was lower at pH 6 (12.5–28.8%, 19.1–39.2% and 4.6% for lemons, Clementine mandarins and navel oranges, respectively) than at pH 3 treatments (72.7–89.9%, 35.8–91.4% and 77.2% for lemons, Clementine mandarins and navel oranges, respectively). The results show that temperature, pH and exposure time are important parameters in dip application using IMZ sulphate formulation with significant effects on green mould control, more so than residue loading alone.

1. Introduction

South Africa exported 1,460,633 cartons (15 kg) of fresh fruit in the 2013/14 season (CGA, 2015), making this country the second largest exporter of citrus in the world, exceeded only by Spain (DAFF, 2010). Postharvest decay is frequently a problem, of which 90% is due to green mould, caused by *Penicillium digitatum* (Pers.:Fr) Sacc. (Kavanagh and Wood, 1967; Eckert and Eaks, 1989).

Penicillium digitatum is an ascomycete that survives on orchard debris and produce airborne spores (asexual conidia) that infect wounded and split fruit in the orchard (Brown et al., 1988). Being exclusively a wound pathogen *P. digitatum* enters through wounds on the fruit made by insects or during harvest (Smilanick et al., 2005). Moisture and nutrients in the wound facilitates conidial germination (Pelser and Eckert, 1977). Sporulating fruit may cause soilage of healthy neighboring fruit, which makes cleaning and repacking necessary, and leads to extra expenses (Brown et al., 1988).

Practices such as sanitation and careful handling of fruit during harvesting are essential to control this disease. In addition, fungicides are used for control, of which imazalil (IMZ) is currently

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the most reliable (Altieri and Renzo, 2005; Zhang, 2007; Liebenberg, 2011; Erasmus, 2014).

Imazalil is a sterol demethylation inhibitor (DMI) (Siegel et al., 1977), with anti-sporulant activity. The maximum residue limit (MRL) for IMZ in South Africa, Japan and most European countries are 5.0 mg kg^{-1} for citrus on whole fruit (Brown and Dezman, 1990). Residue levels of 2.0–3.5 mg kg^{-1} are required for green mould control and to inhibit sporulation (Kaplan and Dave, 1979; Brown and Dezman, 1990; Smilanick et al., 1997). It has been reported through recent studies that the sensitive strain of *P. digitatum* can be effectively controlled in the fungicide bath by IMZ residue of 0.97 mg kg^{-1}, while higher residue levels were required for sporulation inhibition (Erasmus et al., 2011; Njombolwana et al., 2013a). IMZ applications in packhouses in South Africa generally loaded lower residue levels (Erasmus et al., 2011).

For effective disease control and compliance with food safety standards, IMZ applications must be done as accurately and effectively as possible. Different application methods are commonly used: dip, drench or wax applications (Kaplan and Dave, 1979; Erasmus et al., 2011). The majority of South-African packhouses apply IMZ either as a single application in the fungicide bath, or as a dip application followed by IMZ incorporated in wax coating application. This additional application in wax improved IMZ residue loading, protective green mould control and sporulation inhibition (Njombolwana et al., 2013a).

IMZ application in fungicide baths or dip tanks was the most effective application method for curative control (Erasmus et al., 2011; Njombolwana et al., 2013a). However, there was a lot of variation in specification of fungicide baths used by different packhouses, including the pH and temperature of the solution. Erasmus et al. (2011, 2013, 2015a) studied the effects of pH on IMZ residue loading and green mould control, specifically using the IMZ sulphate salt formulation, which appeared to be very sensitive to pH effects.

Several studies reported on the application of the IMZ emulsifiable concentrate (EC) formulation in dip tanks (Smilanick et al., 1997; Schirra et al., 1998; Cabras et al., 1999; Smilanick et al., 2005), but not much on the IMZ sulphate formulation, which is almost exclusively used in South Africa. Erasmus et al. (2011) showed the pH of a IMZ sulphate solution prepared at the registered 500 mg kg^{-1} in pH neutral water declined to ≈pH3. At this low pH, fruit exposure time and solution temperatures at 25 and 35 °C had no effect on IMZ residue loading when fruit was dip treated, but the MRL was exceeded after 45 s in a pH 8 solution. It was highlighted that IMZ sulphate has a pK_a level of 6.5 and dissociated at higher pH solutions (Siegel et al., 1977; Erasmus et al., 2011). Erasmus et al. (2013) showed that residue loading increased at longer exposure times when fruit was dipped in an IMZ sulphate solution at pH 6; however, exposure times did not affect residue loading at pH 3, although improved green mould control was observed. One of the factors that have not been investigated fully in these studies with the IMZ sulphate solution was solution temperature. As temperature also influenced residue loading and green mould control (Cabras et al., 1999; Erasmus et al., 2011), the influence of temperature and pH needed to be studied further. Therefore, the aims of this study were to determine combined effects of temperature, exposure time and pH of IMZ sulphate dip applications on curative control of green mould, sporulation inhibition and residue loading.

2. Material and methods

2.1. Penicillium digitatum *isolates*

One isolate of *P. digitatum* were used in all the trials: isolate STE-U 6560 that is sensitive to TBZ and IMZ (Erasmus et al., 2011; Kellerman et al., 2014). The isolate was plated out from −80 °C storage culture onto potato dextrose agar (PDA; DIFCO, Becton, Dickinson and Company, USA) and grown for 7–14 days at 25 °C before each trial. Spore suspensions were freshly prepared each day of inoculation by filtering the culture grown on PDA through two layers of cheesecloth with deionised 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 final spore concentration adjusted to 10^6 spores mL^{-1}. Viability of spores was verified after each trial by plating out the used spore suspension on PDA.

2.2. Fruit

Untreated export quality citrus fruit were collected from various citrus packhouses in the Western Cape province of South Africa. This was done during the 2013 harvest season as the specific citrus type, lemons (cv. Eureka), mandarin (cv. Nules Clementine) and early navels (cv. Washington), became available for trials. Before the trials commenced, fruit was washed with chlorine (70 mg L^{-1}), air dried and stored at 3.5–7 °C for ± 3 days. Fruit was transferred from cold storage to ambient (± 23 °C) a day before each trial.

2.3. Inoculation

Inoculations for curative treatments were done 24 h before IMZ dip treatment. Wounding and inoculation were conducted simultaneously by dipping a wounding tool into a spore suspension of *P. digitatum* (10^6 spores mL^{-1}) immediately prior to wounding (Erasmus et al., 2011). Wounding tools consisted of a 7 mm diameter cylindrical stainless steel rod with a protruding tip 2 mm long and 1 mm in diameter. With equal distances apart, four wounds were induced on each fruit around the calyx. Three replications were done for every treatment, with 12 fruit being inoculated per treatment combination. Untreated fruit served as controls.

2.4. Temperature and pH trial

A 3 × 3 × 2 factorial experiment was done for each fruit type with three different temperatures (23 °C, 35 °C and 45 °C), three exposure times (15, 45 and 90 s) and two pH levels (3 and 6). For every citrus type the experiment was done twice. Temperature-controlled stainless steel warm water baths (25 L capacity; Unitemp, Baird and Tatlock Ltd., Essex, UK) were used as IMZ dip tanks at 23 °C, 35 °C and 45 °C. Fruit from the various treatment combinations were dipped in a 500 mg L^{-1} solution of IMZ sulphate (Imazacure, 750 g kg^{-1} SG, ICA International Chemicals, Stellenbosch, South Africa). The pH of the IMZ sulphate solutions was adjusted to a pH of 6 by using sodium bicarbonate ($NaHCO_3$; Alkalinity Plus, Pool Perfect, Bellville, South Africa) and pH was measured using a pH meter with temperature probe (HI 991002; Hanna Instruments, Woonsocket, Rhode Island). Fruit were left to dry at ambient temperature (23 °C) after treatment.

On count 13 soft fibreboard trays (SFT) nectarine trays (Huhtamaki South Africa (Pty) Ltd, Atlantis, South Africa), 12 fruit from each treatment combination were packed in lock back table grape cartons (APL Cartons, Worcester, South Africa). Each carton was covered with a transparent polyethylene bag. This created a humid incubation chamber, and also excluded cross-contamination. The cartons were incubated at ambient temperature (22–25 °C).

Infection was rated with a UV light (UV-A at 365 nm, Labino Mid-light; www.labino.com) after 4 – 5 days' incubation. The number of infected wounds was recorded using the UV light to