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Improvement in imazalil treatments in commercial packinglines to control green mold on lemon fruit

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ABSTRACT

Long distance to markets limits global competitiveness in lemon fruit production due to fungal postharvest decays, such as green mold caused by *Penicillium digitatum*. Imazalil, the main fungicide used, is formulated as an emulsifiable concentrate (IEC) and as a water-soluble sulphate salt (IS), the later being less commonly applied in most of the producing countries. The aim of the present report was to improve the effectiveness of imazalil treatments to control green mold in commercial scale. In both laboratory and commercial applications, IS was more effective than IEC. IS formulation applied in water followed by IEC applied in wax is an improved option that provided suitable disease control and optimal residue loading >2.0 mg L^{-1} without exceeding the maximum residue levels. Additionally, IMZ residues remained at acceptable levels after 28 days at 7 °C, assuring the protection of lemon fruit for long periods of storage and transports.

1. Introduction

Control of fungal postharvest diseases is central to maintain fruit quality for long period of time. Argentina produces approximately 28% of the world lemon (Federcitrus, 2010), where Tucumán, located in between 26 and 28 °S latitude, contributes with 90% of this total fruit (Palacios, 2005). Because this production area is 10,000–17,000 km from the overseas markets, fresh fruit require 25–40 days to get to the final consumers; this transit time constitute a barrier that limits global competitiveness.

Green mold, caused by *Penicillium digitatum* Sacc., is the most common postharvest disease of citrus fruit, leading to significant economic losses to growers and packers (Eckert and Brown, 1986; Eckert and Eaks, 1989). The main fungicide used to control this and other fungal diseases is imazalil (IMZ) (Gutter et al., 1981; Kaplan and Dave, 1979). IMZ is available in two different formulations: IMZ sulphate (IS), water soluble granules or powders recommended for aqueous application, and IMZ emulsifiable concentrate (IEC), an oily emulsion often used for wax applications. IEC is the most employed formulation all over the world. A few reports on IS usage are available from South Africa, where this formulation is currently applied (Erasmus et al., 2011, 2015).

In lemon packinghouses of Argentina, at present, IMZ is only used as IEC, both in aqueous and wax applications. The aim of this work was to improve fruit conservation during the long time of storage employing IS, IEC or the combination of both formulations, keeping IMZ residue levels on fruit between 2 and 5 mg kg FW^{-1}. The imazalil residue levels on citrus fruit of 2 mg kg FW^{-1} are biologically effective and recommended (Smilanick et al., 1997) and 5 mg kg FW^{-1} is the maximum residue limit (MRL) accepted by the main European markets (Dezman et al., 1986).

2. Materials and methods

2.1. Fungicides, conidial suspension and fruit samples

Two formulations of IMZ were used: IEC (44.6% imazalil, Fungaflor 500 Emulsifiable Concentrate, Janssen PMP) and IS (75% imazalil, Fungaflor 75SP, Janssen PMP). Fungicide solutions of 1000 mg L^{-1} in tap water have pH 7 for IEC or pH 5.5 for IS. Conidial suspension of 1×10^6 conidia ml^{-1} was prepared using as described by Cerioni et al. (2012). *P. digitatum* isolate cultured for 7–14 days on potato dextrose agar (PDA, Difco Laboratories, Detroit) at 25 °C. For fruit inoculation, 'Eureka' lemons (*Citrus limon* (L.) Burm), collected

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from commercial orchards, were employed in lab and packingline trials within the 24 h after harvest.

2.2. Fruit inoculation

For fruit inoculation, the tip of a stainless steel rod (1 mm wide and 2 mm in length) was immersed in the conidial suspensions and then inserted in the fruit rind, once per fruit.

2.3. Evaluation of IMZ formulations in laboratory assays

To determine preventative (protective) activity of IMZ formulations, fruit were dipped for 1 min in 10 L of fungicide solutions and inoculated at 0, 7, 14 or 21 days after storage at 7 °C. To determine curative (eradicant) activity of IMZ formulations, fruit were inoculated and 0, 2, 4, 6, 8, 10, 12, 18, 36 and 48 h later were dipped for 1 min in 10 L of fungicide solutions. In addition, injury protection was determined in wounded fruit dipped in 1000 mg L^{-1} IS followed by inoculation of the fruit by immersion for 1 min in a conidial suspension 24 h later. In all cases, lemons were incubated at 24 °C and 95% RH and green mold incidence was evaluated 7 days after inoculation. Positive controls were included in each trial.

To examine the effect of pH on IMZ solution effectiveness, lemons inoculated 24 h previously were immersed for 1 min in 10 L of 1000 mg L^{-1} IEC or IS at pH 3, 5, 7, or 10. Fungicide solutions had been adjusted to each pH with 1N NaOH or 65% HNO_3 stock solutions. To examine the effect of dip exposure time, inoculated lemons were immersed for 15, 30, 60, 90, 180, or 540 s in 10 L of 1000 mg L^{-1} IEC or IS.

2.4. Evaluation of IMZ formulations in commercial packingline assays

The effectiveness of different fungicide treatments applied to lemons inoculated 24 h previously were done on a commercial packingline. In all cases, fungicides were sprayed over natural bristle brushes without recovery. In a complete treatment, lemons were dipped in a 3% (v/v) $NaHCO_3$ tank followed by an aqueous fungicide application (2000 mg L^{-1} IEC or 1000 mg L^{-1} IS), pre-dried for 15 to 20 s at 40 °C and waxed with 4000 mg L-1 IEC. Other treatments evaluated were the following: (1) 2000 mg L^{-1} IEC in water; (2) 1000 mg L-1 IS in water; or (3) 4000 mg L^{-1} IEC in wax. Assayed fruit were recovered from the packingline and disease incidence was determined 7 days after incubation at 24 °C and 95% RH.

2.5. Determination of IMZ residue levels

Non-inoculated fruit were included in each test for IMZ residue determinations. The methodology was described by Lehotay (2007). Briefly, whole fruit were weighed, macerated in a blender (Metvisa, Metalurgica Visa, SP Brazil) and samples were frozen (−20 °C). A sub-sample of macerated fruit was extracted with solvents and the extract was analyzed with a GC-NPD chromatograph (Agilent model 7890, Agilent Technologies, CA, USA). Fungicides residues were reported as mg kg FW^{-1}.

2.6. Statistical analysis

For decay incidence determination, treatments were applied to 4 replicates of 25 fruit each and the tests were performed in triplicate. For residue analysis, assays were carried out at least four times using 20 fruit for each condition. Data were subjected to analysis of variance (ANOVA) followed by Tukey's test (Statitix 9.0 Analytical Software 2008 for Windows, USA). Differences at $p < 0.05$ were considered significant.

Table 1
Preventive activity and residue levels of imazalil sulfate (IS) and imazalil emulsifiable concentrate (IEC). Lemons were dipped for 1 min in 1000 mg L^{-1} solutions and inoculated at indicated storage periods.

Days of storage at 7 °C	Incidence[a] (%)		Residues[b] (mg Kg FW^{-1})	
	IS	IEC	IS	IEC
0	0.0c	0.0c	3.3 ± 0.03	4.9 ± 0.05
7	3.7c	2.5c	3.0 ± 0.03	4.7 ± 0.05
14	7.5c	12.5c	2.8 ± 0.02	nd
21	67.5b	83.8a	2.4 ± 0.03	nd

[a] For each formulation, different letters indicate significant differences according to Tukey's test with a p-value of 0.05.
[b] Residues of imazalil determined immediately after treatments. nd: not determined.

3. Results and discussion

3.1. IMZ effectiveness and residue levels in laboratory assays

Curative activity of IS or IEC formulations was evaluated at different time after fruit inoculation. In laboratory treatments, IS was effective up to 36 h, while IEC presented green mold incidence of 20% (data not shown). When preventive activity was evaluated (Table 1), both formulations were effective up to 7 days after fungicide application and only moderately effective up to the 14 days. When fruit was inoculated 21 days after treatment, green mold incidence was 67.5 and 83.8% for IS and IEC, respectively. Residue levels were between 2 and 5 mg Kg FW^{-1} in all treatments evaluated. For curative and preventive activities, IS was more successful than IEC. Even, IS was nearly 80% effective when injury protection was evaluated. Data indicate that IS in aqueous solutions is more available to exert fungicide action than the less water-soluble IEC, in agreement with results reported by Erasmus et al. (2011).

Green mold was completely controlled by all laboratory treatments in which pH and time of exposure were evaluated. Residues increased with pH of solution and dip exposure time using both IMZ formulations (Fig. 1), in agreement with previous reports performed with IEC (Cabras et al., 1999; Dore et al., 2009; Holmes et al., 1994; Smilanick et al., 1997, 2005). The effect of pH on residues was higher with IS than with IEC, reaching at pH 10 residues up to 35 mg Kg FW^{-1} and 10 mg Kg FW^{-1}, respectively. When pH was increased above the pKa of IMZ (6.5), the proportion of anhydrate base molecules increases, IMZ molecules become less water-soluble and they would precipitate (Erasmus et al., 2011). Exposition to IMZ for 60 s or more time deposited excessive residues, mainly for IEC. Short exposure time of 15 s loaded acceptable residue levels for both formulations. Erasmus et al. (2011) reported that the fruit residence time in IS solution could be critical, except at pH 3, pointing the need to remove fruit from tanks promptly to avoid the deposition of illegal residues during pauses in the processing. Taken together, a good control of the application time is needed to minimize the risk of excessive residues occurring when IEC is employed.

3.2. IMZ effectiveness and residue levels in commercial assays

IMZ treatments in a commercial packingline included spray applications of IMZ in aqueous solutions and/or in wax (Table 2). In aqueous applications, IS residues were below the recommended minimum of 2 mg Kg FW^{-1} and lower than those of IEC. Green mold incidence was 8.2 and 16.3% for IS and IEC, respectively, consistently with better IS laboratory performance in respect to IEC. It should be considered that increased fungicide residues not always imply increased effectiveness. Applications of only 4000 mg L^{-1} IEC in wax, which also left fungicide residues below the recommended

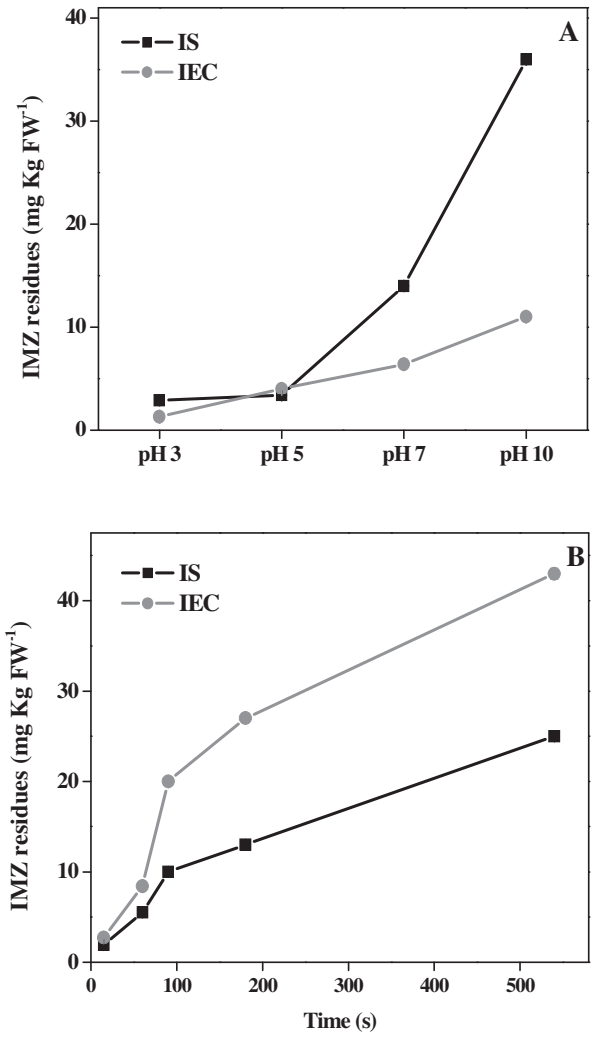

Fig. 1. Imazalil residues on lemons after application of either imazalil sulphate (IS) or imazalil emulsifiable concentrate (IEC). (A) Residues were determined after dipping the fruit for 1 min in 1000 mg L^{-1} solutions at the indicated pHs. (B) Residues were determined after dipping the fruit in 1000 mg L^{-1} solutions during the indicated exposure times.

Table 2
Comparison of imazalil effectiveness and residue levels in commercial packingline assays. Lemons were treated with overhead spray of aqueous, wax or aqueous/wax fungicide applications using imazalil sulfate (IS) and imazalil emulsifiable concentrate (IEC) at the indicated rates.

Spray treatments	Residues (mg Kg FW^{-1}) 0 d[b]	28 d[b]	Incidence[a] (%)
Aqueous (1000 IS)[c]	1.40 ± 0.02	nd	8.2 c
Aqueous (2000 IEC)[c]	2.20 ± 0.03	nd	16.3 b
Wax (4000 IEC)[c]	1.68 ± 0.02	0.90 ± 0.01	34.0 a
Aqueous (1000 IS)[c]/wax (4000 IEC)[c]	2.75 ± 0.04	1.99 ± 0.03	0.0 d
Aqueous (2000 IEC)[c]/wax (4000 IEC)[c]	3.21 ± 0.04	2.03 ± 0.03	1.2 d

[a] For each formulation, different letters indicate significant differences according to Tukey's test with a *p*-value of 0.05.
[b] Days of storage at 7 °C.
[c] IMZ concentrations in mg L^{-1}. nd: not determined.

minimum, were ineffective to control green mold (Table 2). Applications of IMZ in water followed by wax deposited residues within acceptable levels and effectively controlled green mold. As previously reported (Schirra et al., 1997), IMZ residues in fruit declined during storage. In our trials fungicide residues decreased ~30%, to 2 mg kg FW^{-1}, after fruit storage during 28 days at 7 °C (Table 2), but within the recommended range to control green mold. The combination of two IMZ formulations, IS in water followed by IEC in wax, was the optimum regime for the use of this fungicide, with residue levels below the MRL required by fresh fruit markets but retaining the biologically effective residue of 2 mg kg FW^{-1}. Our work corroborates earlier reports that formulation and mode of application greatly influence IMZ residues and efficacy (Erasmus et al., 2011). IEC controlled green mold significantly better when applied in water than in wax (Brown et al., 1983; Brown, 1984; Eckert et al., 1994). IMZ loses effectiveness in wax because a substantial portion of the oil-soluble residue remains immobilized and entrapped in the wax (Brown, 1984). However, the application of IMZ in wax has continued due to its anti-sporulant activity. This minimizes the cosmetic defect of the unsightly contamination of adjacent fruit by conidia, termed spoilage (Eckert and Brown, 1986), reduces the production of airborne inoculum within citrus packinghouses (Eckert et al., 1994), and protects the fruit from later infection (Njombolwana et al., 2013).

In conclusion, laboratory assays are useful to conduct precise comparisons among treatments, but they provide only indicative data to predict treatment effectiveness under commercial conditions. In commercial packing, the use of IS in water followed by IEC application in wax is recommended to improve the postharvest treatments. Optimal residue loading >2.0 mg L^{-1} without exceeding the MRL was achieved in our commercial scale applications, and they remained at acceptable levels after 28 days at 7 °C, assuring the protection of lemon fruit for long periods of storage and transports.

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