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Effectiveness Of Fungicide Application Systems In The Control Of Penicillium Digitatum In Oranges In Tucuman Argentina

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Abstract

The citrus green mold caused by *Penicillium digitatum* produced the main postharvest decay and its control depends on fungicides application. At the present a rotating spray system is used in the packing for fungicide application. The aim of this work was to compare the effectiveness of the rotating spray (rss) and immersion systems (is) for fungi control, measuring the residual active ingredients (rai) in fruits. Orange fruits were inoculated using a punch previously dipped in a spore suspension and stored for 24h at 20 ℃ and 90% RH. Then fruits received the following treatments: T1 water (control), T2 0.16% IMZ+PRM applied with rss at the packing line, T3 wax+0.2% IMZ with rss at the packing line, T4 dipping in 0.16% IMZ+PRM for 30 seconds, T5 dipping in wax+0.2% IMZl for 30 seconds. Fruits were stored at 4 ℃ and 90% RH during 15d and then at 20 ℃ and 90% HR during 7d. Fruit damages were evaluated as % of control (incidence). The residual active ingredients (rai) residue levels were measured in fruit, flesh and essential oil. Results indicated that the best treatment was T4 with 100% control capacity. The rai in fruit were: 2 mg/kg of PRM and 3.8 mg/kg of IMZ, and in essential oil: 104mg/kg of PRMl and 182mg/kg of IMZ. T2 treatment yielded 30 % of control and the rai in fruit were: 1.2mg/kg of PRM and 0.69mg/kg of IMZ and in essential oil: 89mg/kg of PRM and 37mg/kg of IMZ. No residue was detected in the flesh. The effectiveness of application system was directly related to the rai level found in fruits and essential oil.

Keywords

fungicide, residue, green mold

1. Introduction

The Province of Tucuman produces 95 % of the lemon of Argentina and is the main lemon producer and exporter in the world. In the 2012 season Tucuman produced 1,264,000tn and exported 264,000tn as fresh fruit.
Because an important part of this production is shipped to oversea markets, i.e. the European Union, extensive efforts are devoted to ensure the high standards of quality demanded by these markets. Postharvest decay that causes losses estimated between 5% and 10% (Viñas, 1997) are among the problems that affect the quality of fresh fruit.

Green mold caused by *Penicillium digitatum* Sacc. is regarded as one of the most important postharvest diseases due to its frequency and severity. The pathogen is present in Tucuman throughout the export season (May to September), but causes more damage early in the season when the disease development is favored by environmental conditions mainly temperature and rainfall (Stein et al 1983).
In Tucumán lemon export started in 1971. At that time postharvest decay was controlled with 1000ppm of benomyl or thiabendazole in wax or 2000 ppm of benomyl in water. During the 1980s strains of *Penicillium* sp. with resistance to benomyl were detected, hence imazalil started being used (Eckert et al 1981). In 1997, 1998 and 1999 imazalil resistant strains of *P. digitatum* were detected in various citrus packinghouses in Tucumán, Argentina (Fogliata et al 2000; Torres Leal et al 2000).
Commercial postharvest citrus fungicides were applied frequently as non recovery sprays. At the present a rotating spray system is used in the packing for fungicide application.
The problematic produced for inadequate and low fungicide application effectiveness generated pathogen resistance, residue presence and appearance of secondary pathogens (Bogliani et al 2005).
The aim of this work was to compare the effectiveness of the rotating spray (rss), and immersion systems (is) for fungi control, measuring the residual active ingredients (rai) in fruits.

2. Materials and methods

Fruit. Oranges (Citrus sinensis (L) Osbeck) Delta seedless variety. Delta Valencia orange, this South African variety produces a seedless fruit of high quality that resembles the Valencia but matures somewhat earlier. Reuthers 1967
Inoculation. An imazalil resistant isolate of P. digitatum was used in experiments. Spore suspension was prepared adjusting their concentration to 10^6 spores/mL, and used in the following experiments.
Mature and sound fruit of Delta seedless orange were surface sterilized with 70 % ethyl alcohol and then inoculated on two opposite sites along the equatorial line using a punch previously dipped in the spore suspension. A total of 150 fruit were inoculated with strain, and stored for 24h at 20 ℃ and 90% RH.
Fungicide treatments Then fruits received the following treatments: T1 water (control), T2 0.16% IMZ+PRM applied with rss at the packing line, T3 wax+0.2% IMZ with rss at the packing line, T4 dipping in 0.16% IMZ+PRM for 30 seconds, T5 dipping in wax+0.2% IMZl for 30 seconds. Fruits were stored at 4 ℃ and 90% RH during 15 days and then at 20 ℃ and 90% HR during 7d.
Evaluation of decay Fruit damages were evaluated as % of control (incidence).
Residue analyses The active ingredient (ai) residue level was measured in fruit, flesh and essential oil. The fungicides present in homogeneous and representative aliquots were extracted with ethyl acetate. Magnesium sulfate was added to eliminate water residues, and sodium acetate as pH regulator. To improve fungicide extraction, extracts were vigorously hand shacked for 1 minute and then centrifuged at 5000 x g to separate the liquid and solid phase (Payá et al 2007). Organic extracts cleanup were carried out by solid phase technique (SPE) with PSA (i.e. Primary Secondary Amine). The fungicide residues were analyzed and quantified directly from extracts by gas chromatography using a nitrogen-phosphorus detector (NPD). The confirmation of residue identity was carried out by gas chromatography with Mass Spectrophotometer detector (MSD) and by the Pesticides EU – MRL Regulation EC N 396. 2005, MRL Data base USDA.

3. Results and discussion

The results obtained in this work are shown in the following figures and tables.
Fruit inoculated with the imazalil resistant isolate of *Penicillium digitatum* and treated with 0.16% IMZ+PRM dipping for 30 seconds had 0 % of decayed fruits (100 % control) (Figure 1) the residual active ingredients (rai), in fruit (Figure 2) were: 2 mg/kg of PRM and 3.8 mg/kg IMZ, and in essential oil (Figure 3): 104mg/kg of PRMl and 182mg/kg of IMZ. But 70 % of decayed (30 % control) fruit treated with 0.16% IMZ+PRM applied with rotating spray systems; the rai in fruit were: 1.2mg/kg of PRM and 0.69mg/kg of IMZ and in essential oil: 89mg/kg of PRM and 37mg/kg of IMZ.
Dipping in wax+0.2% IMZl for 30 seconds showed 60% of decayed fruit (40% control). The remaining treatment presented low percentage of control. No residue detected in flesh.

Table 1. Effectiveness of Fungicide Application Systems

N º	Treatment	% Control
1	Control	1
2	Imazalil + Pyrimethanil	30
3	Imazalil packing line	20
4	Imazalil + Pyrimethanil bath	100
5	Imazalil bath	40

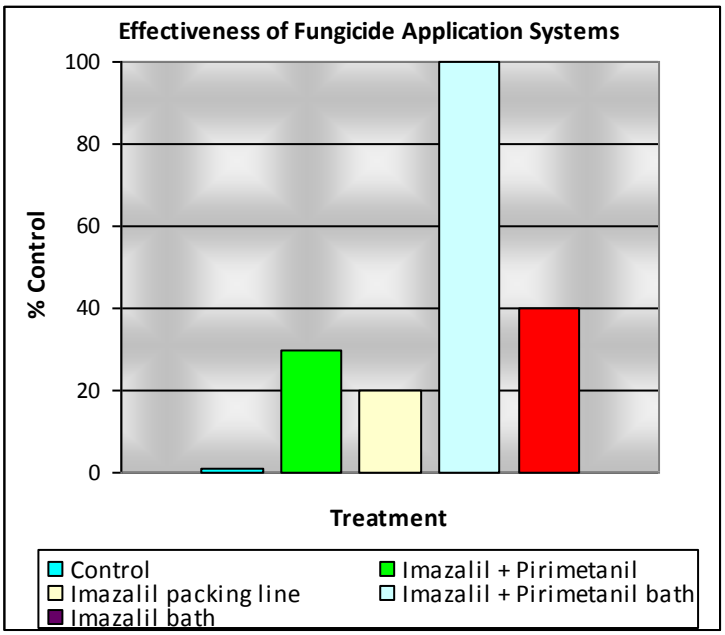

Figure 1

Table 2. Residues of Imazalil and Pyrimethanil recovered from fruit of delta seedless orange stored at 4 ℃ for 15 days and then at 20 ℃ and 90% HR during 7 days

N º	Treatments	Pyrimethanil residue (mg/Kg)	Imazalil residue (mg/Kg)
1	Control	0.02	0.02
2	Imazalil + Pyrimethanil packing line	1.20	0.69
3	Imazalil packing line	1.50	0.87
4	Imazalil + Pyrimethanil bath	2.10	3.80
5	Imazalil bath	0.02	1.50

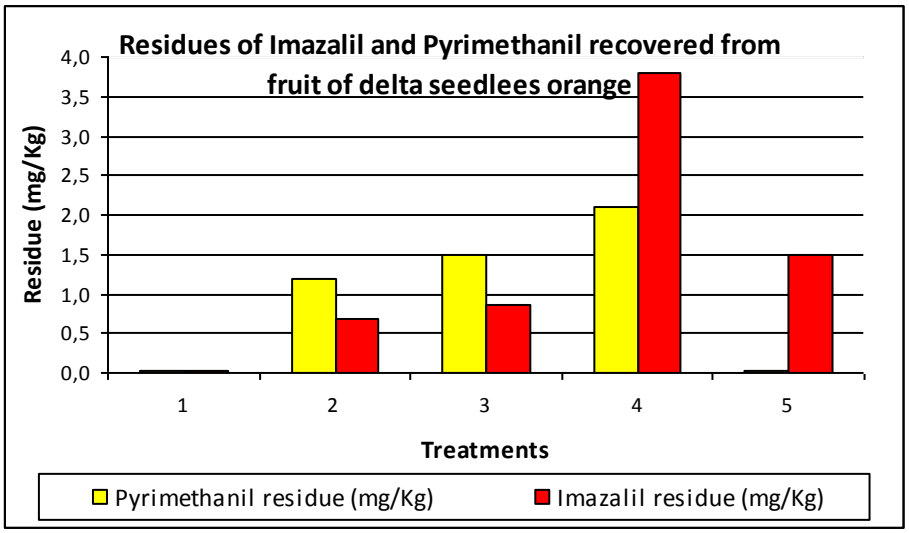

Figure 2

Table 3. Residues of Imazalil and Pyrimethanil recovered from esential oil of delta seedless orange stored at 4 ℃ for 15 days and then at 20 ℃ and 90% HR during 7 days

N º	Treatments	Pyrimethanil residue (mg/Kg)	Imazalil residue (mg/Kg)
1	Control	0.80	0.80
2	Imazalil + Pyrimethanil packing line	89.00	37.00
3	Imazalil packing line	4.50	4.80
4	Imazalil + Pyrimethanil bath	104.00	182.00
5	Imazalil bath	9.60	5.20

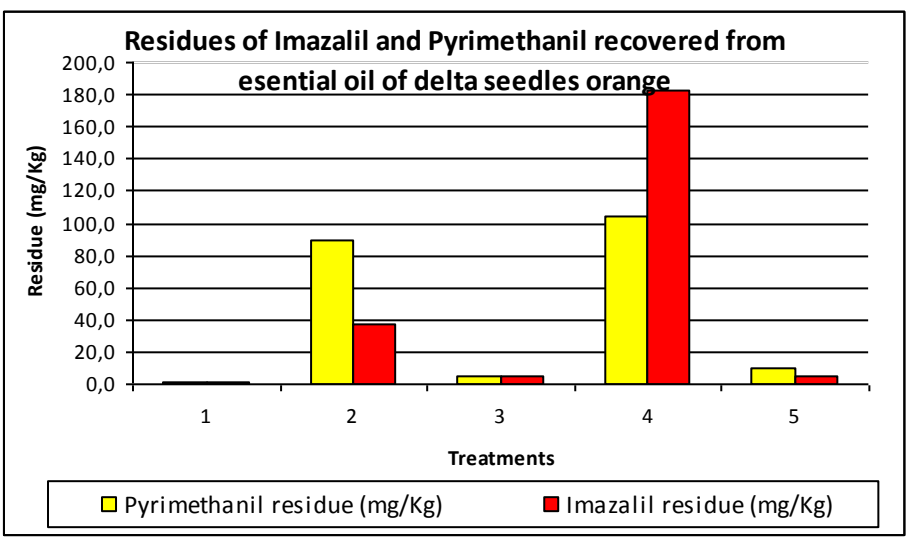

Figure 3

Discussion

Applications of bath containing pyrimethanil and imazalil resulted in better control of *Penicillium digitatum* than when these fungicides were applied with rotating spray system in the packing line, as well as the level of fungicide residue of active ingredients. Application of bath containing imazalil in water based wax yield a better control and higher residue than when they were applied in packing line.

The effectiveness of application system was directly related to the residue active ingredient (rai) level found in fruits and essential oil. The level of fungicide residue found in fruit and essential oil was correlated with *Penicillium digitatum* incidence.

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