

Improved control of sour rot on citrus

Exploring potential fungicide solutions for improved control of sour rot on citrus. By **Meagan van Dyk** and **Hanli Kellerman** (CRI)



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Valencia orange fruit expressing sour rot seven to eight days after inoculation. The fruit is very soft.

Sour rot is one of the most important postharvest diseases, and it is of particular importance during extensive cold storage (Rovetto et al., 2024). Mature turgid citrus fruit are most susceptible to the causal pathogen known as *Galactomyces citri-aurantii* (Baudoin and Eckert, 1982; 1985). The pathogen requires a wound in order to infect the fruit. The initial symptoms appear as water-soaked lesions that quickly develop over the whole fruit, leaving the fruit soft with a characteristic sour scent that attracts vinegar flies (Rovetto et al., 2024; **Figure 1**). Vinegar flies spread the pathogen from infected fruit to other injured fruit. In citrus orchards, the propagules of *G. citri-aurantii* can also be dispersed by wind and by water-splashing from contaminated soil onto the fruit surfaces, or by direct contact of the fruit with the contaminated soil (Rovetto

et al., 2024). The postharvest spread of this pathogen as stated by Rovetto et al., (2024) can also be through contaminated packhouse equipment and via contact with infected fruit in containers. Highly effective fungicide products are registered for use in SA for sour rot control. These products include active ingredients such as guazatine (210 SL) and propiconazole (250 EC), and can be applied in pre-packhouse fungicide drench systems and in packline fungicide baths. A wax formulated with guazatine can also be used for sour rot control. However, some of the most valued citrus export markets of SA do not tolerate residues of guazatine or propiconazole on the fruit (Kruger, 2021). Sour rot can be controlled to an extent by strict pre- and postharvest sanitation practices. However, these control



Tool used to inflict wounds for sour rot inoculations

measures do not offer long-term protection of the fruit against sour rot, so when sour rot conditions prevail, the pathogen may overcome these control measures. Generally recognised as safe (GRAS) chemicals have shown potential for use against postharvest decay, including sour rot. For example, a product containing clove oil has been registered in SA as a postharvest treatment of citrus fruit against waterborne sour rot fungal propagules and for suppression of *Penicillium* decay. Potassium sorbate is another GRAS chemical that has been well-studied and has also shown potential against sour rot (Smilanick et al., 2008). However, GRAS chemicals used in isolation do not offer the same level

of sour rot control as products containing guazatine and propiconazole when tested under extreme inoculation procedures. The aim of this study was to test GRAS products that are registered for use in SA, in various chemical packline combinations, to determine sour rot control measures that are as effective as the traditionally used packline systems containing guazatine. Registered GRAS products containing clove oil (Act 36 of 1947) and potassium sorbate (Act 5 of 2008) were tested in simulated packline systems for sour rot control. The ultimate goal of this on-going study is to be able to implement these control measures in South African packline systems in order to maintain a broad export market, while maintaining superior sour rot control.

Materials and methods

Valencia oranges that had not yet undergone any postharvest treatment were collected from a packhouse in Nelspruit during September 2024. The oranges were washed in calcium hypochlorite (150 ppm; pH 7.5) for one minute before storing them for no longer than two weeks. Then, the experimental treatments were applied. Spore suspensions of *G. citri-aurantii* were made in deionised water and diluted to a concentration of 1×10^5 spores/ml. Each fruit was wound inoculated by dipping a wounding tool (tapered screw; 6 mm long x 4 mm-in-diameter; **Figure 2**) into the spore suspension and then directly into each fruit. To simulate a long delay after harvesting before fungicide treatments, inoculated fruit were incubated for 18-20 hours at room temperature before submerging the fruit in the first experimental treatments. Chemical concentrations can be found in **Table 1**. The treatments involving simulated packline treatments with and without a simulated pre-packhouse drench can be found in **Tables 2 and 3**, respectively. The fruit that underwent a simulated pre-packhouse drench were incubated overnight after the pre-packhouse drench, before initiating the packline treatments.

The samples were air-dried after each of the packline steps (i.e., sanitation bath, fungicide bath and wax treatment). The treated fruit were packed manually onto pulp trays that were in plastic bags in cartons. One hundred and fifty millilitres of deionised water was added to the pulp trays to maintain a high relative humidity in the plastic bags, in order to facilitate sour rot development. Fruit was incubated for 14 days before rating sour rot infections. There were three experimental replicates, each consisting of a box of 12 fruit per experimental replicate and the experiment was repeated. The percentage disease was calculated for each treatment replicate as follows:

$$\text{disease (\%)} = \frac{\text{sum of infected fruit}}{\text{total number of inoculated fruit}} \times 100$$

The statistical analyses (ANOVA and Tukey's tests) were performed on the normalised percentage control:

$$\text{control (\%)} = \frac{\text{disease (\%)} \text{ of negative control} - \text{disease (\%)} \text{ of treatment}}{\text{disease (\%)} \text{ of negative control}} \times 100$$

Results and discussion

Fruit of the negative controls were sufficiently infected with sour rot (>70% infection) for an informative comparison of the treatments tested in this study. The positive controls (i.e., treatments containing GZT) provided 100% sour rot control for experiments that were treated with and without pre-packhouse drenches. The pre-packhouse drenches containing TBZ+FLU-PYR+CLO and TBZ+OPP+PS+2,4-D provided significantly better sour rot control (>90%) than the current industry standard ($17.25 \pm 29.05\%$ control; **Table 2**). Where no pre-packhouse drench was applied, several treatments provided significantly better sour rot control than the industry standard ($70.69 \pm 13.31\%$ control), when evaluated 14 days after inoculation (**Table 3**). Besides the positive control treatment, treatments that provided significantly better control than the industry

Table 1: Concentrations of active ingredients and GRAS chemicals used in this study

Active	Concentration (ppm)
Pre-packhouse drench	
2,4-dichlorophenoxyacetic acid (2,4-D)	250
Clove oil	2000
Didecyltrimethylammonium chloride (DDAC)	1500
Fludioxonil (FLU) + Pyrimethanil (PYR)	200 + 800
Guazatine (GZT)	1000
Ortho-phenyl phenol (OPP)	2000
Peracetic acid + hydrogen peroxide (PAA)	150 + 230
Potassium sorbate	3000-7500*
PYR	1000
Thiabendazole (TBZ)	1000
Sanitation bath	
Calcium hypochlorite	150
Fungicide bath	
Clove oil	2000
GZT	1000
IMZ-S (imazalil-sulphate)	500
OPP	2000
PAA	150 + 230
Potassium sorbate	3000-7500*
TBZ	100
Wax treatment	
2,4-D	250
IMZ-EC (imazalil)	2000
Sodium ortho-phenyl phenate (SOPP)	2000
TBZ	4000
* Concentration of the active ingredient of the product is given as a range	

Table 2: Treatments tested for sour rot control where a pre-packhouse fungicide drench was applied

Reference	Pre-packhouse drench ^a	Sanitation bath ^b	Fungicide bath ^c	Wax treatment ^d	Sour rot control (14 days)
Positive control	TBZ+GZT+2,4-D+DDAC	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ	100.00±0.00% a
	TBZ+FLU-PYR+CLO	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ+IMZ-EC	91.73±7.23% a
	TBZ+OPP+PS+2,4-D	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ+IMZ-EC	90.08±14.18% a
	TBZ+OPP+PS+2,4-D+PAA	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ+IMZ-EC	87.31±12.16% a
	TBZ+PYR+PS+2,4-D+PAA	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ+IMZ-EC	84.28±10.52% a
	TBZ+PYR+CLO+2,4-D	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ+IMZ-EC	80.56±13.37% a
	TBZ+OPP+CLO+2,4-D	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ+IMZ-EC	80.36±5.96% a
	TBZ+PYR+PS+2,4-D	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ+IMZ-EC	77.66±18.30% a
Industry practice	TBZ+PYR+2,4-D+PAA	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ+IMZ-EC	17.25±29.05% b
Negative control	n/a	Ca(ClO) ₂	n/a	Wax only	0.00±12.59% b

^a pH not adjusted (pH 7.10±1.28); Ambient temperature: 20.14±0.13°C; Exposure: 2 min
^b pH adjusted to 7.50±0.01; Ambient temperature: 20.8±0.07°C; Exposure: 1 min
^c pH adjusted to 6.00±0.02; Ambient temperature: 20.28±0.47°C; Exposure: 1 min
^d Actives added to a 20% polyethylene-based wax
* 2,4-D = 2,4-dichlorophenoxyacetic acid; Ca(ClO)₂ = calcium hypochlorite; CLO = clove oil; DDAC = didecyldimethylammonium chloride; FLU = fludioxonil; GZT = guazatine; IMZ = imazalil; OPP = Ortho-phenyl phenol; PAA = peracetic acid + hydrogen peroxide; PYR = pyrimethanil; TBZ = thiabendazole; Mixing order: water soluble granules (SG) – suspension concentrate (SC) – emulsifiable concentrate (EC) – soluble liquid concentrate (SL)

Sour rot is of particular importance during extensive cold storage.

standard practise and had no more than three active ingredients in the fungicide bath, were those that contained: IMZ-S+PS+PAA (96.05±6.12%), IMZ-S+CLO (93.99±6.60%) and IMZ-S+OPP+PS (93.99±6.60%; **Table 3**). The first two of these treatments were applied on the packline with wax containing the standard industry active ingredients, while the third treatment contained the standard active ingredients with SOPP added to the wax (**Table 3**). However, with extended storage at room temperature for 26 days after

inoculation, infections continued to develop in these treatments, unlike for the treatment containing GZT (**Table 3**). This finding was not expected and extended storage was not planned for sour rot evaluation. Thus, only four treatments of one trial were available for evaluation, and the percentage sour rot control was calculated for these treatments using the percentage sour rot control of the negative controls at 14 days after inoculation, without considering any additional infections thereafter. Considering the percentage sour rot control at 26 days after inoculations, the fungicide bath containing IMZ-S+CLO (88.72±11.28%) provided the highest level of control after the treatment applied with GZT (**Table 3**). In future extended storage will always be considered for sour rot control evaluations.

The results from this study demonstrate the potential of integrating CLO, OPP and PS into packline systems for increased sour rot control. These experiments were conducted with fungicide baths applied at ambient temperature. The most promising combinations recorded during this study will be selected for an extensive evaluation at typical packline fungicide bath temperatures that are used for optimal control of postharvest decay. During this study, residue levels and rind disorders will be considered under extended storage, including cold storage.

References

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Table 3: Treatments tested for sour rot control where no pre-packhouse fungicide drench was applied

Reference	Sanitation bath ^a	Fungicide bath ^b	Wax treatment ^c	Sour rot control (14 days)	Sour rot control (26 days)
Positive control	Ca(ClO) ₂	IMZ-S+GZT	2,4-D+TBZ	100.00±0.00% a	100±0.00% a
	Ca(ClO) ₂	IMZ-S+PS+PAA	2,4-D+TBZ+IMZ-EC	96.05±6.12% a	66.17±19.53% a
	Ca(ClO) ₂	IMZ-S+CLO	2,4-D+TBZ+IMZ-EC	93.99±6.60% ab	88.72±11.28% a
	Ca(ClO) ₂	IMZ-S+OPP+PS	2,4-D+TBZ+IMZ-EC+SOPP	93.99±6.60% ab	
	Ca(ClO) ₂	IMZ-S+TBZ+OPP+CLO	2,4-D+TBZ+IMZ-EC	93.99±10.25% ab	69.92±28.38% a
	Ca(ClO) ₂	IMZ-S+OPP+PS+PAA	2,4-D+TBZ+IMZ-EC+SOPP	92.11±9.95% ab	
	Ca(ClO) ₂	IMZ-S+TBZ+PS+PAA	2,4-D+TBZ+IMZ-EC	91.92±10.04% ab	
	Ca(ClO) ₂	IMZ-S+TBZ+OPP+PS+PAA	2,4-D+TBZ+IMZ-EC+SOPP	90.60±11.09% ab	
	Ca(ClO) ₂	IMZ-S+PS	2,4-D+TBZ+IMZ-EC	90.41±8.55% abc	
	Ca(ClO) ₂	IMZ-S+OPP+CLO	2,4-D+TBZ+IMZ-EC	90.23±8.61% abc	
	Ca(ClO) ₂	IMZ-S+TBZ+CLO	2,4-D+TBZ+IMZ-EC	90.04±11.68% abc	
	Ca(ClO) ₂	IMZ-S+TBZ+PS	2,4-D+TBZ+IMZ-EC	86.28±8.39% abc	
	Ca(ClO) ₂	IMZ-S+TBZ+OPP+PS	2,4-D+TBZ+IMZ-EC+SOPP	74.45±13.45% bc	
Industry practice	Ca(ClO) ₂	IMZ-S	2,4-D+TBZ+IMZ-EC	70.69±13.31% c	
Negative control	Ca(ClO) ₂	n/a	Wax only	0.00±13.60% d	n/a

^a pH adjusted to 7.46±0.02; Ambient temperature: 22.43±0.37°C; Exposure: 1 min
^b pH adjusted to 6.03±0.04; Ambient temperature: 21.72±0.39°C; Exposure: 1 min
^c Actives added to a 20% polyethylene-based wax
* 2,4-D = 2,4-dichlorophenoxyacetic acid; Ca(ClO)₂ = calcium hypochlorite; CLO = clove oil; DDAC = didecyldimethylammonium chloride; FLU = fludioxonil; GZT = guazatine; IMZ = imazalil; OPP = Ortho-phenyl phenol; PAA = peracetic acid + hydrogen peroxide; PYR = pyrimethanil; SOPP = sodium ortho-phenyl phenate; TBZ = thiabendazole; Mixing order: water soluble granules (SG) – suspension concentrate (SC) – emulsifiable concentrate (EC) – soluble liquid concentrate (SL)

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