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Postharvest sour rot control in lemon fruit by natamycin and an Allium extract

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ABSTRACT

Citrus sour rot caused by *Geotrichum citri-aurantii* is one of the most important postharvest diseases in citrus fruit, causing huge economic losses. Traditionally, it has been controlled by the postharvest application of guazatine and propiconazole fungicides, but restrictions in their use make it urgent to find an alternative for sour rot management. Natamycin, a common food preservative, and the organosulfuric compounds extracted from *Allium* species are safe food additives that control different foodborne pathogens. In the present study, the curative activities of commercial formulations of natamycin (Fruitgard Nat 20) and an *Allium* extract (PTSO: propyl thiosulfinate oxide; Proallium FRD®), were evaluated for the control of *G. citri-aurantii* in artificially inoculated lemon fruit. Trials in laboratory and in commercial conditions were carried out to explore the feasibility of including both compounds as part of a safe postharvest sour rot disease control strategy. Under controlled laboratory conditions, sour rot was significatively reduced by 500 mg L^{-1} of natamycin, 580 mL L^{-1} of PTSO and 290 mL L^{-1} of PTSO + 4% of a food coat, applied by immersion. Nevertheless, the maximum dose of PTSO (580 mL L^{-1}) caused phytotoxicity on the fruit rind. In commercial drenching conditions, 290 mL L^{-1} of PTSO + 4% of a food coat reduced sour rot incidence similar to conventional treatment. In a packing line treatment, spray application of 500 mg L^{-1} of natamycin with a previous dip in sodium bicarbonate, resulted in nearly 70% reduction of disease incidence compared to conventional salt application. A second commercial experiment revealed that fruit drenching with 290 mL L^{-1} of PTSO + 4% food coat followed by an in-line cascade application of 500 mg L^{-1} of natamycin is completely effective for sour rot control after 20 days at 5 °C. Further exposure at room temperature for 7 d showed a 61% reduction in sour rot incidence compared to the control. Results revealed that natamycin and PTSO are promising tools for sour rot control used alone or combined as part of an integrated postharvest strategy.

1. Introduction

Citrus sour rot caused by *Geotrichum citri-aurantii* (Ferraris) E.E. Butler, (1988) (teleomorph: *Galactomyces citri-aurantii* E.E. Butler, (1988)) is one of the most important postharvest diseases in citrus fruit causing huge economic losses (McKay et al., 2012a; Talibi et al., 2014; Zacarias et al., 2020). The pathogen penetrates strictly through wounds (Brown, 1979) and the disease progress at temperatures between 23 °C and 29 °C, being favoured by the presence of free water on the fruit surface (Bussel and Shavit, 1975; Eckert, 1959). Further, the aggressiveness of the pathogen increases as the fruit ripens (Brown, 1979), being particularly destructive when the harvest is delayed and fruit overripe or during periods of high field relative humidity (Baudoin and Eckert, 1982; Duan et al., 2016; Eckert, 1959). All commercial citrus species are susceptible to this pathogen (Butler et al., 1965), but lemons and mandarins are more sensitive than oranges and grapefruit (Brown, 1979; Eckert, 1959). *G. citri-aurantii* is not controlled by conventional fungicides such as imazalil (IMZ), pyrimethanil (PYR) or thiabendazole (Mercier and Smilanick, 2005). Traditionally, sour rot has been controlled by the postharvest application of the fungicides guazatine and propiconazole (Brown, 1988; McKay et al., 2012b; Rippon and Morris, 1981; Wild, 1992). However, there are several reasons

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hazarding their continuity in the citrus industry worldwide. Different countries ban or limit their use such as United States of America (US), where guazatine is not registered for its use on citrus (McKay et al., 2012a). In the case of the European Union (EU), the Commission Implementing Regulation of the EU set the maximal residue limit for guazatine in 0.05 mg kg^{-1} ([EU] 2015/1910 of 21 October 2015) and for propiconazole in 0.01 mg kg^{-1} (EU 2021/155 of 2/9/2021), being these concentrations below the effective residue need for *G. citri-aurantii* control (Pérez et al., 2015). Although, the potential of *G. citri-aurantii* to develop resistance to propiconazole is low (McKay et al., 2012b), the continuous application of fungicides with a single action site could favour the selection of resistant isolates (Brent and Holloman, 2007; Deising et al., 2008; Hollomon, 2015). Finally, there is a rising trend toward the commercialization of fungicide residue-free fruit and produced applying good agricultural practices (Palou, 2011). Consequently, it seems urgent to find an effective strategy for sour rot control that fulfils regulations, market requirements and consumers' expectations.

Postharvest application of biocontrol agents, plant extracts, essential oils, antimicrobial peptides and biopreservatives reduces *G citri-aurantii* incidence in citrus fruit (Duan et al., 2016; Karim et al., 2017; Mercier and Smilanick, 2005; Regnier et al., 2014; Soto-Muñoz et al., 2020; Wang et al., 2020). However, limitations related to their actual efficacy under commercial conditions (scaling up or formulation development) and some negative effects on fruit quality have limited their adoption by the citrus industry (Feng et al., 2011). In this sense, efforts are necessary to overcome these limitations and to underpin the adoption of new safe alternatives.

Natamycin (Nat), also known as pimaricin, is a polyene antifungal produced by *Streptomyces natalensis* and related species during fermentation (Roberts et al., 2011). It has been classified as a biofungicide by the U.S. Environmental Protection Agency (U.S.EPA, 2012). The elucidated mechanism of action of natamycin involve the damage of the plasma membrane (He et al., 2019; te Welscher et al., 2008), disturbance of the nutrient transportation (te Welscher et al., 2012) and mitochondrial dysfunction (Awasthi and Mitra, 2018). It is employed as a food additive in more than 40 countries and due to its very low toxicity to human and mammalian cells, it has been considered as a GRAS product by the FDA (Koontz et al., 2003). The European Food Safety Authority (EFSA), Panel on Food Additives and Nutrient Sources of Foods, has considered the natamycin (E235) as a safe food additive (adequate margin of safety), being poorly absorbed by humans and without concerns for the induction of antimicrobial resistance (Aguilar et al., 2009). In 2017, natamycin was registered in United States as a postharvest fungicide exempt from residue tolerance (Adaskaveg et al., 2019). Several studies have demonstrated its efficacy to control different postharvest pathogens as *Alternaria alternata*, *A. arborescens*, *Botrytis cinerea*, *G. citri-aurantii*, *Monilia fructicola*, *Mucor piriformis*, *Penicillium digitatum*, *P. expansum* and *Rhizopus stolonifer* (Chen et al., 2016; Wang et al., 2021; Zhang et al., 2020). In citrus fruit, natamycin was effective for gray mold control on mandarin (Saito et al., 2020) and blue and green mold control on orange and lemon, respectively (Yiğiter et al., 2014). *In vitro* experiments revealed that 3.20 mg L^{-1} of natamycin inhibited mycelial growth of *G. citri-aurantii* (Chen et al., 2016) while could provide an 89% of sour rot control on lemon fruit (Adaskaveg et al., 2018). However, little information is available about natamycin dose-related efficacy or its real effect during commercial citrus postharvest management.

The genus *Allium* is rich in organosulfur compounds that have been characterized as the main bioactive molecules present in this gender (Ramirez et al., 2017). One of these extracts is propyl thiosulfinate oxide (PTSO) (Llana-Ruiz-Cabello et al., 2015b; Putnik et al., 2019) which has been evaluated in active food films and packaging for its fungicidal and bactericidal effects (Cosmai et al., 2017; Llana-Ruiz-Cabello et al., 2015a, 2018). Its use was proposed to guarantee the food safety and extend the shelf life of organic food (Cosmai et al., 2017; Llana-Ruiz-Cabello et al., 2018; Mylona et al., 2019) but its fungicidal effect and efficacy to control citrus postharvest diseases has not been yet evaluated.

In the present study, the curative activity of the food additives natamycin and PTSO was evaluated with the objective of reducing *G. citri-aurantii* postharvest incidence in artificially inoculated lemon fruit. Experiments under laboratory and commercial conditions were carried out to explore the feasibility of including both compounds as part of a safe postharvest sour rot disease control strategy.

2. Material and methods

2.1. Fungal isolate and inoculum preparation

The isolate of *G. citri-aurantii* (G0) used in all experiments belongs to the fungal collection of the Phytopathology laboratory of INIA Salto Grande, Uruguay. The isolate was obtained from a rotten lemon fruit collected in a citrus packinghouse in Salto, Uruguay. After being purified, the pathogenicity of a monosporic culture was tested in orange, mandarin and lemon. The isolate has been characterized as sensitive to propiconazole (Pérez-Faggiani, 2017). For the present work, a loop of the fungi suspension cryopreserved at −80 °C was recovered in potato dextrose agar (PDA) medium (Oxoid Ltd., Basingstoke, Hampshire, UK) and conserved at 4 °C. *In vitro* experiments, the effect of natamycin on the mycelial growth of *G. citri-aurantii* isolate G0 used in the experiments was determined by the absence of fungal growth in plates of 24 well (6 rows by 4 columns). A stock solution (100 mg L^{-1}) was dissolved in sterile distilled water. Wells of the same column were filled with 1.5 mL of PDA medium amended with natamycin at 1.0, 2.0, 3.0, 4.0 or 5.0 mg L^{-1}. One column without natamycin was used as a control. Aliquots of 5 μL each of arthrosporic suspension droplet (1×10^7 conidia L^{-1}) were inoculated on the centre of each well without agitation. The inoculated plates were kept at 25 °C in the dark for 7 days and presence/absence of visible fungi growth was evaluated. Four replicates per treatment were used and the entire assay was performed twice. Technical grade natamycin (95.5%) provided by Enzur S.A. (Montevideo, Uruguay) was used. The natamycin concentration was checked by HPLC method, with Restek Ultra C18 column (150 × 4.5 mm, 5 μm particle size) and acetonitrile/water 40:60, flow rate of 0.5 mL min^{-1}.

2.2. Fungal and fruit material

For all inoculations (fruit and plates), arthrosporic suspensions were prepared adding 10 mL of 0.05 M KH_2PO_4 with 0.01% (p/v) Triton X-100 over the surface of a 7-day-old fungi culture grown on petri dishes with PDA medium. The arthrospore suspension was rubbed from the agar surface with a rod and filtered through two layers of sterile cheesecloth. Immediately, the suspension was adjusted using a Neubauer counting chamber. The arthrospore concentration used in fruit was adjusted to 1×10^9 spores L^{-1} and 10% of a 10 mg L^{-1} cycloheximide solution was added. Cycloheximide is recommended because inhibits wound healing but has no significant effect upon the growth of. *G. citri-aurantii* (Eckert and Brown, 1986).

Mature lemon fruit var. Lisbon was harvested from commercial orchards the day before inoculation, selected for uniform size, colour and the absence of apparent lesions. In laboratory, fruits were randomized, washed and disinfected superficially by immersion for 1 min in a solution with 0.2 mL L^{-1} of active chlorine. Then, the fruit were treated by immersion for 1 min in a 100 mL L^{-1} IMZ solution to minimize interference by infection from *Penicillium* spp., and remained at room temperature for 6 h until inoculation. The fruit inoculation was made at the equatorial zone with a 2 mm diameter and 1 mm depth needle, previously dipped with the inoculum suspension (Eckert and Brown, 1986). Subsequently, the fruit were kept at room temperature for 18–20 h until treatments were applied.

2.3. Food additives

A commercial product with 14,5% of PTSO (Proallium FRD®) and a food coat of vegetable fatty acids (Food Coat®) were provided by Domca S.A. (Entre Ríos, Argentina). A commercial product with 20% of natamycin (Fruitgard Nat 20), was provided by Enzur S.A. (Montevideo, Uruguay). Based on preliminary research (data not shown) a range between 145 and 580 mL L^{-1} of PTSO was evaluated. Doses of natamycin (500 to 1500 mg L^{-1}) were determined according to the available literature (Adaskaveg et al., 2019).

2.4. In vivo experiments on artificially inoculated lemons

The evaluation in artificially inoculated lemons was carried out under laboratory conditions and repeated twice for each additive. Different concentrations of each food additive were applied by immersion for 1 min (Table 1). The incidence of rotten fruit (rotten fruit in the inoculation zone/total fruit per replicate * 100) and severity (diameter of fungus growth) were evaluated in a maximum period of 11 days at room temperature (25 °C ± 1). Based on results, an additional experiment was performed, comparing this effective treatment to 290 mL L^{-1} of PTSO plus 4% of food coat (PTSO ×2 + FC), following manufacturer recommendations. Control treatment consisted in fruit inoculated and treated with tap water. After treatments, each replicate was placed in individual boxes and covered with plastic bags. Six replicates of 20 or 23 fruit were used. High humidity conditions inside the boxes were maintained by sprinkling sterile distilled water on the day of the inoculation and every 48 h until the end of the experiment.

2.5. Experiments under commercial conditions

Sour rot control in yellow lemon fruit was evaluated under commercial conditions: in drencher and in packing line (separately or as part of a combined strategy). For all commercial experiments, no fruit disinfection was done before inoculation and experiments were repeated, presenting average results. The incidence of rotten fruit was evaluated after 7 d of storage at 25 ± 2 °C in first two trials, or after storage for 20 d at 5 ± 2 °C plus 7 days at room temperature (23 ± 3 °C) in the third trial.

In a first trial, PTSO + FC was applied in a commercial drencher and was compared with the conventional treatment usually employed at the packinghouse (Table 2, Trial A).

In the second trial, carried out in a commercial packing line, application of natamycin-based product was done by spraying with a fixed flat fan nozzle (using a flow rate of 4.6 L ton^{-1}) over cylindrical nylon rotating brushes. Fruit have previously received a conventional treatment (120 s dip treatment with sodium bicarbonate 3%). The conventional dip treatment, ineffective against sour rot (data not shown) served as a control (Table 2, Trial B).

A third experiment simulating the commercial process was conducted combining the drencher with the in-line strategy and compared with a conventional treatment only with imazalil. Fruit was treated in drencher for 30 s with 100 mL L^{-1} of imazalil, 290 mL L^{-1} of PTSO and 4% food coat following by an in-line cascade of 500 mg L^{-1} of natamycin (Table 2, Trial C).

Table 1
Treatments evaluated for the control of sour rot in lemon fruit var. Lisbon, applied by immersion for 1 min.

Treatment	Active ingredient	Dose
Control	Water	–
PTSO ×1	Propyl thiosulfinate oxide (PTSO)	145 mL L^{-1}
PTSO ×2		290 mL L^{-1}
PTSO ×3		435 mL L^{-1}
PTSO ×4		580 mL L^{-1}
PTSO ×2 + FC	PTSO + food coat of vegetable fatty acids	290 mL L^{-1} + 4%
Nat 500	Natamycin	500 mg L^{-1}
Nat 1000		1000 mg L^{-1}
Nat 1500		1500 mg L^{-1}

Table 2
Treatments evaluated for sour rot control in lemon fruit var. Lisbon, in commercial conditions.

Trial	Treatment	Application method	Active ingredient	Dose
A	Conventional 1	Drencher	Imazalil (Fruitgard IS 7.5[a])	0.975 mL L^{-1}
			Propiconazole (Fruitgard PZ 100[b])	0.700 mL L^{-1}
			Heptamethyltriloxane (Adjuvant - Silwet Lag[b])	0.837 mL L^{-1}
			Dimethyl polysiloxane (Antifoam – Antiespuma PC[a])	250 mL L^{-1}
	PTSO ×2 + FC	Drencher	PTSO (propyl thiosulfinate oxide)	290 mL L^{-1}
			Food coat of vegetable fatty acids	4%
B	Conventional 2	Immersion	Sodium bicarbonate[c]	3%
	Conventional 2/ Nat 500	Immersion	Sodium bicarbonate	3%
		Spray	Natamycin	500 mg L^{-1}
C	Conventional 3	Drencher	Imazalil	100 mL L^{-1}
	Conventional 3 + PTSO ×2 + FC/Nat 500	Drencher	PTSO	290 mL L^{-1}
			Food coat of vegetable fatty acids	4%
		Cascade	Natamycin	500 mg L^{-1}

[a] Enzur S.A. Uruguay.
[b] Rizobacter S.A. Argentina.
[c] Nortesur S.A. Uruguay.

2.6. Experimental design and statistical analysis

In all trials, a completely randomized design with 3 or 6 replicates per treatment was followed and the experimental unit consisted of 20 fruits. The pathogen incidence was analysed using mixed generalized linear models and the comparison of means was performed by the DGC test procedure ($p \leq 0.05$) using Infostat (Di Rienzo et al., 2018) or R software v.3.6.1 (R Core Team, 2019).

3. Results

3.1. Effect of natamycin on the mycelial growth of G. citri-aurantii

Mycelial growth of the G0 isolate of *G. citri-aurantii* was completely inhibited in presence of 2 mg L^{-1} of natamycin incorporated into PDA culture medium.

3.2. In vivo experiments on artificially inoculated lemons

3.2.1. Propyl thiosulfinate oxide (PTSO)

The application of 580 mL L^{-1} of PTSO (PTSO ×4) reduced the incidence of sour rot in artificially inoculated lemons on all days evaluated, while lower doses were ineffective in controlling decay incidence (Fig. 1A). However, this effective dose produced severe spots (phytotoxicity) on the fruit rind (Supplementary Fig. 1).

The disease severity was reduced after treating lemons with 435 and 580 mL L^{-1} of PTSO (PTSO ×3 and PTSO ×4), while the other treatments remained similar to the control. At the end of the experiment (day 7) only PTSO ×4 reduced pathogen growth compared to the control (Fig. 1B).

In order to avoid phytotoxicity without losing control effectivity, the

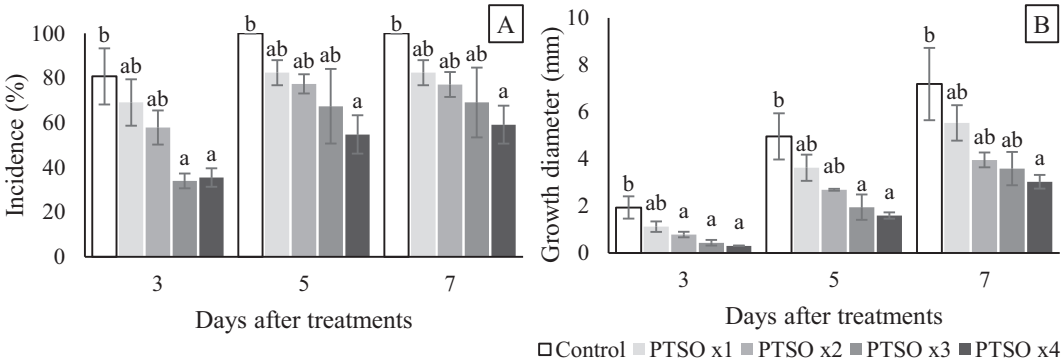

Fig. 1. Incidence (A) and severity (B) of sour rot in lemon fruit var. Lisbon artificially inoculated with *Geotrichum citri-aurantii* and treated by immersion with 145, 290, 435 or 580 mL L^{-1} of PTSO for 1 min. Evaluation at 3, 5 and 7 days of storage at 25 ± 2 °C and RH > 85%.
Means (±standard error) with different letter between treatments differ significantly (incidence by DGC $p \leq 0.05$; Severity by Tukey $p \leq 0.05$). References: Control: Treatment with water; PTSO ×1, ×2, ×3, ×4: PTSO at 145, 290, 435 and 580 mL L^{-1} respectively.

combination of PTSO with a food coating was explored. The treatment with PTSO plus the food coat (PTSO ×2 + FC) applied by immersion effectively controlled sour rot after 11 days (similar to PTSO ×4) without causing phytotoxicity (Fig. 2).

3.2.2. Natamycin

Natamycin application reduced sour rot incidence by nearly 30% after 7 days at 25 °C (Fig. 3A) while lesion diameter was barely affected (Fig. 3B). Early evaluations (3 and 5 d at 25 °C) registered similar decay incidence and growth diameters among treatments. At the end of the experiment (7 d), natamycin reduced pathogen incidence for all tested doses, impairing lesion diameter consistently only with the lowest dose of 500 mg L^{-1} (Fig. 3B).

3.3. Evaluation of sour rot control in commercial drencher and packing line

The application of 290 mL L^{-1} of PTSO plus 4% of food coat (PTSO ×2 + FC) in a commercial drencher had similar efficacy than conventional treatment containing propiconazole (Fig. 4, Trial A). At the commercial packing line, the addition of 500 mg L^{-1} of natamycin to the conventional treatment with 3% sodium bicarbonate, significantly improved disease control, showing nearly a 70% reduction in sour rot incidence after 7 d at 25 °C (Fig. 4, Trial B). The combination of both treatments under commercial conditions where PTSO + food coat was applied in drencher and natamycin in cascade, completely controlled disease incidence after 20 d of cold storage. After 7 d at room temperature significant differences were observed respect to the conventional treatment of imazalil alone (Fig. 4 Trial C).

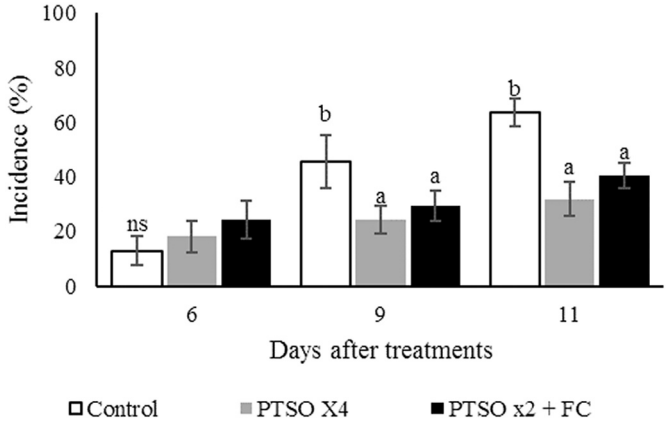

Fig. 2. Incidence of sour rot in lemon fruit var. Lisbon artificially inoculated with *Geotrichum citri-aurantii* and treated by immersion for 1 min with 580 mL L^{-1} of PTSO or 290 mL L^{-1} of PTSO plus 4% of food coat (B). Evaluation in different days after treatments in storage conditions at 25 ± 2 °C and RH > 85%.
Means (±standard error) with different letter between treatments differ significantly (DGC $p \leq 0.05$). References: Control: Treatment with water; PTSO ×2, ×4: PTSO at 290 or 580 mL L^{-1} respectively; FC: food coat at 4%.

4. Discussion

In the citrus industry, there is an urgent need for new residue-free and safer strategies to replace conventional fungicides for the control of postharvest pathogens. Sour rot is one of the main challenges in citrus postharvest pathology since conventional fungicides used for its control are deeply questioned or limited in different markets (Soto-Muñoz et al., 2020). This study was focused on evaluating new alternatives (propyl thiosulfinate oxide and natamycin) for sour rot control in yellow lemon fruit addressing both laboratory and commercial conditions. Mature Lisbon lemon showed a high susceptibility to sour rot, registering the control fruit more than 60% spoilage under laboratory conditions, revealing to be a good fruit model for the evaluation of sour rot control strategies. *In vivo* laboratory trials revealed that PTSO at 580 mL L^{-1} and natamycin at 500, 1000 and 1500 mg L^{-1} reduced sour rot incidence and lesion development in lemon fruit. Further commercial experiments demonstrated that PTSO could be combined with an additive (Food coat) in order to reduce the effective dose and prevent phytotoxicity and that their combination with the food preservative natamycin in the packing-line, reduce the incidence of sour rot similarly or even better than conventional commercial treatments.

PTSO (Proallium FRD®) inhibits the growth of microorganisms responsible for food spoilage and food-borne pathologies and is used in organic food (Cosmai et al., 2017; Llana-Ruiz-Cabello et al., 2015a, 2018; Mylona et al., 2019). This work provides information supporting its use in citrus postharvest management and suggest an adjustment of the label recommendations for sour rot control, proposing a 2% commercial product (a.i. = 290 mL L^{-1}) instead of 0.5% (a.i = 0.725 mL L^{-1}) for controlling filamentous fungi. In the same line, the best antimicrobial effect of polypropylene (PP) and polylactic acid (PLA) films containing PTSO against *Penicillium* spp. isolates have been obtained with doses higher than 580 mL L^{-1} of PTSO (Llana-Ruiz-Cabello et al., 2015a, 2018). However, it is important to highlight that under a commercial drenching system, there was a reduction in disease expression on lemon inoculated fruit using half the recommended dose of PTSO though when combined with Food coat® (4%) without affecting fruit rind integrity. This synergy could be explained by the fact that the edible coat creates a semi-permeable barrier to O_2 and CO_2 minimizing moisture loss and fruit respiration rate, which could contribute to delay fruit aging

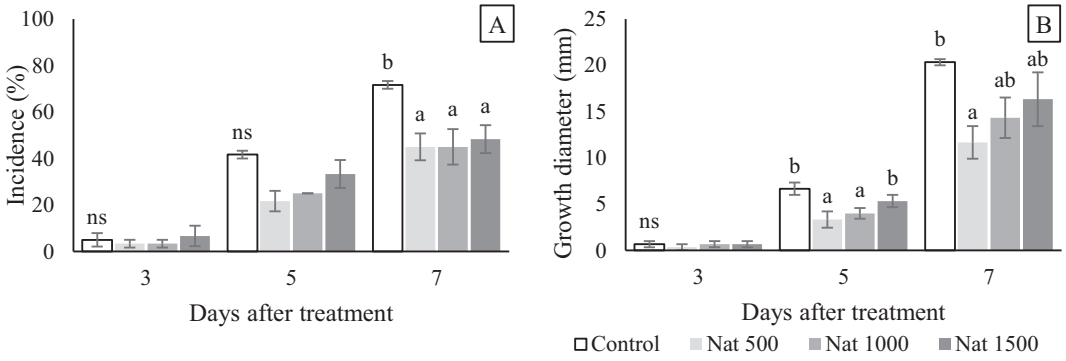

Fig. 3. Incidence (A) and severity (B) of sour rot in lemon fruit var. Lisbon artificially inoculated with *Geotrichum citri-aurantii* and treated by immersion for 1 min with different concentrations of natamycin. Evaluation at 3, 5 and 7 days of storage at 25 ± 2 °C.
Means (±standard error) with different letter between treatments differ significantly (incidence by DGC $p \leq 0.05$; severity by Tukey $p \leq 0.05$). References: Control: Treatment with water; Nat 500, 1000, 1500: Natamycin at 500, 1000 and 1500 mg L^{-1} respectively. One of two evaluation carried out is presented, since similar results were obtained.

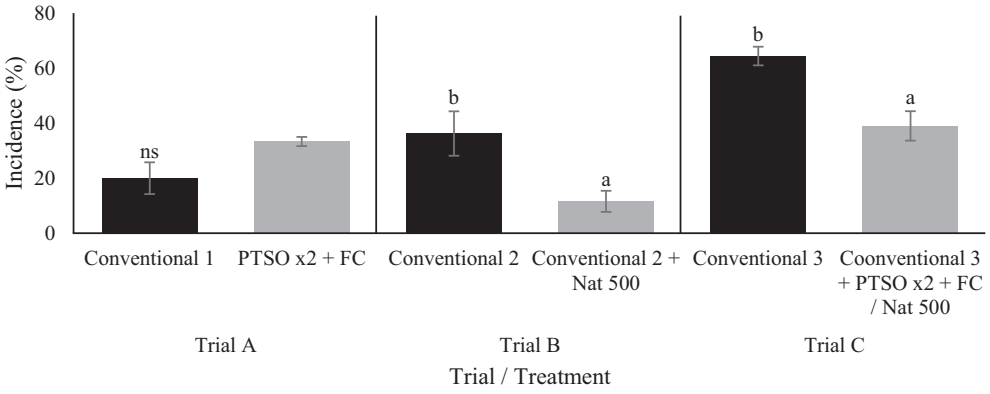

Fig. 4. Incidence of sour rot in fruit of lemon var. Lisbon, artificially inoculated with *Geotrichum citri-aurantii* and treated in a commercial drencher (Trial A), in a packing line (Trial B), or in drencher + packing line (Trial C). Evaluation after 7 days at 25 ± 2 °C (Trial A & B) or after 21 days at 5 ± 2 °C plus 7 days at room temperature (Trial C).
Means (±standard error) with different letter between treatments into each trial, differ significantly (DGC $p \leq 0.05$). References: Conventional1: 0.975 mL L^{-1} IMZ + 0.700 mL L^{-1} PZ + 0.100 mL L^{-1} adjuvant + 0.250 mL L^{-1} Antifoam; PTSO ×2 + FC: 290 mL L^{-1} PTSO + 4% food coat; Conventional 2: 3% sodium bicarbonate; Conventional 2 + Nat 500: 3% sodium bicarbonate + 500 mg L^{-1} natamycin. Conventional 3: 100 mL L^{-1} IMZ; Conventional 3 + PTSO ×2 + FC/Nat: drencher with 100,290 mL L^{-1}, 290 mL L^{-1} PTSO and 4% food coat, and cascade with 500 mg L^{-1} natamycin.

(Baldwin et al., 1995; Nisperos-Carriedo, 1994). This coating should then be generating unfavourable conditions for sour rot development, considering that the fruit susceptibility increases with maturity and senescence (Brown, 1979). Therefore, the PTSO (Proallium FRD®) most effective dose under commercial conditions should depend not only on the citrus species sensitivity, fruit condition and application technology, but also on other possible components present in the mixture.

The native *G. citri-aurantii* isolate used in this study was sensitive to natamycin, being inhibited in artificially inoculated fruit by lower doses than the EC_{50} value (3.20 mg L^{-1}) described in literature (Chen et al., 2016). In fact, in food-mold control trials, the MIC of natamycin was described to be lower than 10 mg L^{-1} (Brik, 1981), coinciding with information available for other plant pathogens such as *Alternaria alternata*, *A. arborescens*, *Botrytis cinerea*, *Colletotrichum acutatum* and *Penicillium expansum* (Haack et al., 2018; He et al., 2019; Saito et al., 2020; Wang et al., 2021; Zhang et al., 2020). The effective dose of natamycin for the control of sour rot in inoculated fruit was higher than those derived from *in vitro* assays. In fact, the fruit provides a better environment for pathogen development and represents a hurdle for the product (He et al., 2019). Moreover, the *in vivo* evaluations on lemon fruit showed that the effectiveness of natamycin was dose-dependent, as described in other pathosystems (He et al., 2019; Saito et al., 2020). The most effective dose (500 mg L^{-1}) for sour rot control in lemon inoculated fruit was slightly lower than the one suggested for sour rot control by Adaskaveg et al. (2018). Probably, the differences could rely on the different experimental conditions in both studies.

The incidence of the sour rot disease in drencher and in-line commercial experiments was reduced by the application of PTSO and natamycin both, separately and combined. Therefore, they represent promising alternatives to be used and tested under other commercial systems. The decision to propose PTSO for the drencher and not on the packing line was based on its strong smell since its use in a confined environment would generate concerns in the packinghouse operators. In addition, sensory analysis of food products has shown that the use of Proallium for food preservation, either as an additive or in packaging, alters the natural flavour (Cosmai et al., 2017; Llana-Ruiz-Cabello et al., 2018). However, under the conditions of our experiment (use of the product in a ventilated environment), the odour of the Allium extract dissipated in about 1 h, although changes in taste were not evaluated. In the case of natamycin, a fast application after fruit harvest is recommended (Saito et al., 2020; Wang et al., 2021) since it inhibits the spore germination but does not affect the infection process once initiated (He et al., 2019). Then, when the time lapse between harvest and packing line treatments is long, the effectiveness of the product may drop. In this sense, the efficacy of natamycin was significantly reduced in mandarin fruit inoculated with *B. cinerea*, when the natamycin was applied 12 h post inoculation compared to 1 or 6 h after inoculation (Saito et al., 2020). Results from this work revealed that infections that may occur 18–20 h before treatment application in lemon fruit should be controlled by 290 mL L^{-1} of PTSO plus 4% of food coat. Based on the available information, we suggest using both products as early as possible during the commercial citrus postharvest process. PTSO could be used in a field

drencher immediately after the fruit is harvested and could be combined with a later natamicyn spray application during in-line fruit processing, preferably during the first 24 h after drenching.

Results from this work support the feasibility of including 'green and alternative' Fruitgard Nat 20 and Proallium FRD® additives as promissory options for the control of citrus sour rot, applied alone or combined with salts. It is worth noting that to enhance the performance or improve the efficacy of both products, their postharvest application should be part of an integrated management program that a) reduces the arrival at packing houses of contaminated fruit and/or with a greater predisposition to fungal infections and b) emphasize the packinghouse cleaning and disinfection programs to reduce the presence of spores in all industrial plant environments and equipment.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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