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Orange oil postharvest dips for control of grey mould (*Botrytis cinerea*) of plums and strawberries, and green mould (*Penicillium digitatum*) of citrus

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Summary. Orange oil has antibacterial properties for uses in food and pharmaceutical industries. This study evaluated the efficacy of orange oil dip applications and treatment periods for protective and curative effects against *Botrytis cinerea*, (which causes grey mould on plums and strawberries), and *Penicillium digitatum*, responsible for green mould of citrus. Pure orange oil and two orange oil-based formulations (OR007B and OR79) were tested on inoculated fruit, at oil concentrations from 0.05% to 1.00%, and were compared to the fungicides fludioxonil at 300 mg. L^{-1} for *B. cinerea*, or imazalil at 500 µg mL^{-1} for *P. digitatum*, the respective South African registered doses for these fungicides. Orange oil treatments failed to control green mould on lemons and oranges, with disease incidence exceeding 90% even after 120 sec of exposure. Two plum cultivars had different susceptibilities to grey mould, and orange oil reduced the disease incidence (24 to 14%) as the oil concentration increased in the curative treatments of cv. African Delight, outperforming 1% fludioxonil (24%). The OR007B and OR79 formulations both gave low incidence of grey mould, with 0.5% of orange oil in these formulations resulting in up to 12% incidence (from OR007B) and 17% (from OR79). Cv. Laetitia, in contrast, had >60% incidence of grey mould across all treatments, including fludioxonil. Protective treatments showed similar trends in both cultivars. On strawberries stored at 4°C for 14 d, grey mould incidence was reduced from 82% (control treatment) to 55% (for orange oil), 21% (for OR007B), and 44% (for OR79), with the orange oil treatments having efficacy comparable to fludioxonil. These results demonstrate the potential of orange oil treatments as alternatives for grey mould management in plums and strawberries.

Keywords. Fungicide sensitivity, natural antimicrobial products, plant extracts, soft chemicals.

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INTRODUCTION

Occurrence of fruit losses due to decay, long distances to markets, and cold chain interruptions are major drawbacks for fruit industries (Nelson,

2010; Stander and Dyk, 2017; Cherono and Workneh, 2018), and use of synthetic fungicides is the most effective management strategy for control of postharvest decay-causing fungal pathogens. *Penicillium digitatum* (Pers.) Sacc., a causal agent of green mould, is responsible for citrus production losses of up to 90% (Macarisin *et al*., 2007; Costa *et al.,* 2019). This disease is managed using the fungicide imazalil (Smilanick *et al*., 1997, Smilanick *et al*., 2005; Erasmus *et al*., 2011). *Botrytis cinerea* Pers. (which causes grey mould) is responsible for postharvest fungal decay of up to 73% on stone fruit (Fourie and Holz, 1985). The fungicide fludioxonil is registered for postharvest application on stone fruit and is used to control grey mould infections (Förster *et al*., 2007).

Safety concerns over human health and environmental pollution threaten the use of synthetic chemicals for plant disease management. Synthetic fungicides, including some of those used postharvest (e.g. imazalil), are regarded as unsafe for human health and environmental toxic to the environment (e.g. fludioxonil), and consumers have become more aware of the impacts of these substances on their health (Sişman and Türkez, 2010; Tao *et al*., 2020; European chemical agency-ECHA, 2024). These increasing concerns have necessitated international and national regulatory authorities to formulate policies for phasing out and prohibiting the use of these products (Commission delegated regulation (EU) 2023/1656, 2023; Department of Agriculture, Forestry, and Fisheries DAFF, 2010). Recent consumer demands in important fruit export markets (including Europe) have caused some supermarkets to introduce lower fungicide residue limits than those set by government regulatory authorities. New systems such as the Globally Harmonized System (GHS) for safety classification of chemicals have identified highly hazardous substances at categories 1A and 1B, subsequently leading to their phasing out (Mudzunga, 2022). These pressures have resulted in a pesticide market shift from development of novel synthetic substances to investments in biopesticide development (Lucintel, 2024). Authorities have also instituted regulatory pathways specifically focused on biopesticides, ensuring fast-tracked processes with minimum delays (DALRRD, 2023).

Biopesticides are defined as products that are of natural origin, either from plants or microorganisms, that are applied to crops to control, repel or prevent occurrence of specific pests and diseases (Hezakiel *et al*., 2023). They are "generally regarded as safe" (GRAS). Interest in these substances is largely due to their natural origins, and that they have been described as GRAS by the Food and Drug Administration (FDA, 2006). These products should have known or minimal toxicological effects on the environment and mammals and not be regulated for chemical residues on fresh produce (Palou *et al*., 2016). Essential oils are GRAS, and are liquid products extracted from plants through several methods, including, for oils from citrus, hydro distillation, steam distillation, and cold expression (Tao *et al*., 2009; Pejin *et al*., 2011; Botrel *et al*., 2015).

The complex composition of essential oils is an advantage for their use as antifungal products, since synergism of compounds has been shown against specific targets and pathogens (Sharifi-Rad *et al*.; 2017; Zareiyan and Khajehsharifi, 2021). Although limonene has been found as the largest proportion (76%), of the essential oil compounds from *Citrus sinensis*, other compounds including monoterpenes (geranial, neral), that are lesser components, also have antimicrobial activity and effectively inhibit fungal pathogen growth (Tsao and Zhou, 2000; Hanafy *et al*., 2021; Hamdan *et al*., 2024). This synergism would be advantageous in postharvest disease management, reducing the likelihood of target pathogens developing resistance to combined components (Tripathi and Dubey, 2004). Rammanee and Hongpattarakere (2011) showed that the individual components of essential oils were less effective than the natural essential oils, demonstrating synergism between different compounds.

It would be advantageous to incorporate essential oils into postharvest disease management, due to their high biodegradability, and to their likely lack of residue issues (Talibi *et al*., 2014). Therefore, the Environmental Protection Agency (EPA) and European Union have eliminated the need to establish permissible residue levels for sweet orange oil, when used during pre- and postharvest at less than 10% (Federal Register, 2014).

Citrus peel is produced as waste in large amounts during processing, so presents an opportunity for essential oil extraction (Zareiyan and Khajehsharifi, 2021). Orange oil is obtained from fruit rind via cold pressing and steam distillation of mature sweet orange fruit (*Citrus sinensis* Linnaeus) (Botrel *et al*., 2015). As is typical for all essential oils, orange oil is analysed using gas chromatography or mass spectrometry, and approx. 27 compounds have been identified. Monoterpene D-limonene is the principal component (> 70%) and with other monoterpene hydrocarbons they make up 90% of the total essential oil content (Droby *et al*., 2008; Tao *et al*., 2009; Regnier *et al*., 2014). As the main component, D-limonene has been classified as an insect repellent and a natural pesticide by the EPA (John *et al*., 2017). Although orange oil use is well documented for pharmaceutical and food industries for its antibacte-

rial and food preservation benefits (Guo *et al*., 2018; Jantrawut *et al*., 2018; De Andrade *et al*., 2022), little information is available on its efficacy against postharvest fruit pathogens, although several studies have previously tested its efficacy on other products including potato slices and cucumber, and one study evaluated its use on fresh-cut oranges (Radi *et al*., 2018; Shi *et al*., 2018; Ziedan *et al*., 2022).

The objective of the present study was to investigate post-harvest applications of orange oil as an alternative for the management of *B. cinerea* on plums and strawberries and *P. digitatum* on citrus. These applications were evaluated for curative and protective activities, and incubation times, and treatment bath exposure periods were also assessed.

MATERIALS AND METHODS

Treatments

Two orange oil-based products were obtained from ORO AGRI SA (Pty) Ltd, Strand, South Africa. One formulation (OR007B) contained 5% orange oil, and the second (OR-79) contained 10% orange oil. These products were compared as fruit dip applications. Pure essential orange oil, containing 97% d-limonene, was also obtained from Puris Natural Aroma Chemicals, Paarl, South Africa. Prior to treatments, fruit treated with the orange oil extracts was sent to HORTEC (Pty) Ltd, Somerset West, South Africa, for imazalil residue analyses, to validate the purity of the oil. Thyme oil (from *Thymus vulgaris* L.) containing > 40% of thymol and obtained from Clive Teubes Africa (Pty) Ltd, Johannesburg, South Africa, was used in these trials to compare with orange oil. Dip treatments in tap water were included in experiments as negative control treatments. The fruit industry standards for postharvest fungicide applications, fludioxonil (Teacher 230 SC) for plums, or imazalil sulfate (Imazacure; 750 g kg^{-1} SP) for citrus, were used as positive control treatments. These fungicide products were obtained from ICA International Chemicals (Pty) Ltd, Plankenburg, Stellenbosch, South Africa. Fludioxonil was applied as a dip at the recommended concentration of 300 mg L^{-1} for *B. cinerea* in plums, and imazalil (IMZ) at 500 mg L^{-1} for *P. digitatum* on citrus. Since no postharvest treatments are used commercially for strawberries, no commercial product was included in the experiments. All fruit used in the experiments was collected from local packhouses (near Stellenbosch, South Africa), and was used within a week after harvest.

Inoculation and treatment application

Citrus experiments

During the 2020 and 2021 fruit packing seasons, high quality export citrus fruit of 'Eureka' lemon [*Citrus* x *limon* (L.) Osbeck], and 'Midnight Valencia' orange [*Citrus* × *sinensis* (L.) Osbeck], were obtained from packhouses in the Citrusdal and Stellenbosch regions, Western Cape, South Africa. Fruit was washed upon arrival from packhouses, using a custom-built packline designed to mimic a commercial system to apply wash solution containing 200 mg L^{-1} of calcium hypochlorite. After washing, the fruit was dried in a drying tunnel.

To commence the experiments, inoculations were conducted as previously described in Njombolwana *et al.* (2022), using *P. digitatum* isolates previously classified as sensitive (isolate STE-U 6560) or resistant (isolate STE-U 6590) to IMZ (Erasmus *et al,.* 2011). The IMZ-resistant isolate was obtained from Citrus Research International (CRI), Nelspruit, South Africa, and the IMZ-sensitive isolate was obtained from a satsuma orchard at the Stellenbosch University experimental farm, Welgevallen, Stellenbosch, South Africa. These isolates were used to prepare conidium suspensions, following 2-week incubation on potato dextrose agar (Merck) containing streptomycin sulfate at 0.04 mg L^{-1} (PDA+). The conidium suspension was prepared in autoclaved deionized water amended with 0.01 mL L^{-1} Tween 20 (Sigma-Aldrich) and was filtered through two layers of sterile cheesecloth. A Neubauer improved haemocytometer was used to adjust the conidium suspension to 1×10^6 conidia mL^{-1}, according to Plaza *et al*. (2004). Ten unblemished fruits of similar size and colour per replicate were inoculated using a metallic rod wounding tool to create four wounds (0.5 mm deep and 2 mm wide) around each fruit stem end. Treatments were applied either 1 h before (protective) or 12 h after (curative).

The water bath solutions containing different treatments were prepared along with a negative control treatment (a bath containing water). In all the experiments, IMZ at 500 µg mL^{-1} was included as a standard disease control treatment (as conventionally used by industry). For orange oil only and thyme oil only treatments, the required concentrations of essential orange oil were premixed with 0.50% v/v Tween 80 to obtain suspensions of the oils in water. The essential oils were applied at 0.05, 0.1 0.5 or 1% concentrations. The OR-007B and OR-79 formulated products were adjusted to these concentrations. Treated fruit was submerged for 30 s in the aqueous solutions and allowed to dry for packing or inoculations depending on the treatment schedules.

In addition, 'Eureka' lemons and 'Midnight Valencia' oranges were used to evaluate effects of exposure times in the treatment bath. The fruit was submerged in the bath for 60, 90, or 120 s following a 12 h incubation period at 25°C. The preparation of the treatments and the procedures were as described above.

In all the experiments, the laboratory temperature was maintained. Fruit was stored in plastic crates on count SFT15 nectarine trays (AgriMark, Paarl, South Africa). Each crate was covered with a transparent polyethylene bag, which was sealed, and then incubated at 25°C for 4 d, after which the infected fruit wounds were evaluated. This was done using a light source (UV-A at 365 nm, Labino Mid-light; www.labino.com), with each wound visible as yellow fluorescence on the fruit surface. Six to 8 d later, incidence of conidium production was assessed, using the sporulation index described by Erasmus *et al.* (2011), which includes scores of 0 = absence of conidia, to 6 = conidia covering the whole fruit.

Plum experiments

Commercially harvested plums [*Prunus salicina* Lindley ('Angelino,' 'African Delight', 'Laetitia') were collected from packhouses in Wellington region, Western Cape, South Africa. The fruit was brought to the laboratory without receiving prior treatments at the packhouses. The plums were surface sterilized before the experiment, by submerging the fruit for 1 min in a waterbath containing 0.48 mg L^{-1} of sodium hypochlorite (Chlorguard sanitiser bleach), combined with 0.005% Tween 20 as a surfactant. The fruit was then allowed to dry on net stainless steel trays.

Inoculations for the experiments were carried out using a *B. cinerea* isolate STE-U 9254, obtained from the Stellenbosch University Plant Pathology culture collection. 1-week-old mycelium PDA+ cultures were grown for 4 d at 25ºC then for 3 d exposed to ultraviolet (UV) light to induce conidium production. Conidium suspensions were prepared as described above but were adjusted to concentration of 1×10^5 conidia mL^{-1} (Lopez-Reyes *et al*., 2013). Ten individual fruit per replication were wound inoculated using a metallic rod wounding tool (2 mm depth, and 2 mm wide). The wounded sections were carefully marked using an Artline 70 black permanent marker.

To evaluate the curative and protective effects of the treatments against *B. cinerea* on fruit of 'African Delight' and 'Laetitia', fludioxonil bath solution at the recommended commercial concentration for plums of 300 mg $^{-1}$ was used. All other treatments and applied concentrations were kept the as described above for the citrus experiments. Applications of thyme oil at all concentrations and 1% adjusted concentration of orange oil in the OR007B and OR79 treatments was discontinued due to the observed phytotoxicity in a pilot trial (data not shown). The fruit was submerged for 30 s in the aqueous solutions and allowed to dry for packing.

To evaluate the effect of incubation time on 'Angelino', a similar treatment procedure as described above was followed, but the fruit was treated after 8, 16, or 24 h. The fruit were allowed to dry, and were then packed on to SFT13 nectarine trays. For the duration of the trials, plums were kept in moisture chambers with temperature maintained at 25°C, and evaluations were carried out on day 7 and day 12 after the treatments were applied.

Form both application methods (preventative or curative), evaluations were made for disease incidence (total number of infected fruits per treatment replicate) and severity (lesion diameters, length and width at the inoculation site). Lesion size was measured using a digital calliper (Miyumoto, MTMA). A repeat of two trials per cultivar was conducted using 'African Delight' and 'Laetitia' cultivars.

Strawberry experiments

During the 2021 fruit packing season, strawberries *Fragaria* x *ananassa* (Duchesne ex Weston) Duchesne ex Rozier; 'Albion'), were collected from packhouses in Stellenbosch, Western Cape, South Africa. The fruit were surface sterilized using a 25 mg L^{-1} sodium hypochlorite solution for 3 mins, followed by a 5 min dip in sterile distilled water, and allowed to dry for 1 h in a laminar flow hood.

Fruit inoculations were carried out to evaluate curative effects of the treatments, after 3 h incubation. The fruit was treated by submerging in a bath containing orange oil, orange oil-based formulated products (using concentrations at 0.05, 0.1, 0.5 and 1% as described above for plums and citrus), and an untreated experimental control, with an exposure period of 30 s in the bath. No standard fungicide treatment was included, since this is not currently practiced by industry. Following treatments, the fruit was allowed to dry, and was then packed into plastic crates lined with black polystyrene pear trays (AgriMark). The fruit was stored at 4°C for 14 d, with monitoring for disease development in the untreated controls. On day 14, evaluations were carried out for appearance of superficial mycelia resembling grey mould, in the untreated fruit. The fruit was then transferred to 25°C and further development of the disease was evaluated on day 17 (i.e. after 3 d storage).

Statistical analyses of data

For disease incidence, severity, fungus sporulation, and control of *P. digitatum* or *B. cinerea* decay, data were analysed using appropriate analyses of variance (ANOVA) and generalized estimated models, using Statistica (v.13.5). These analyses were carried out by the Department for Statistical Analysis, Stellenbosch University. Dependant variables within the analyses were continuous, so Fisher's LSD was an appropriate method for detecting statistically significant differences (95% confidence interval).

RESULTS

Curative and protective efficacy of orange oil and orange oil-based dip applications

The curative and protective effects of the orange oil and orange oil-based treatments OR-007B and OR-79 were evaluated on plums and strawberries. In the preliminary investigations, thyme oil was incorporated and completely inhibited *B. cinerea* at 0.5 and 1%. However, heavy phytotoxicity symptoms were caused by these treatments, as fruit hardening and white patches, observed 24 h after application (Figure 1, B). These treatments were excluded from the main experiments.

On plums, for disease incidence and severity, the ANOVA analyses showed a statistically significant factor interaction of cultivar × action × treatment × day ($P < 0.001$). The interaction was attributed to differing levels of disease susceptibility between the cultivars (Table 1). In 'African Delight' plums, orange oil at all the tested concentrations reduced *B. cinerea* incidence, both curatively and as a protectant. There were statistically significant interactions for the incidence data ($P = 0.06$), and the severity data ($P < 0.001$), between cultivar, action (curative or preventative), and treatment.

Data for each plum cultivar are presented separately. Disease incidence for 'African Delight' was low in the untreated controls (mean = 50%; Table 1) in the curative experiments. The orange oil treatment decreased incidence with increasing concentration. The 0.5% concentration of orange oil (least mean incidence = 14%)

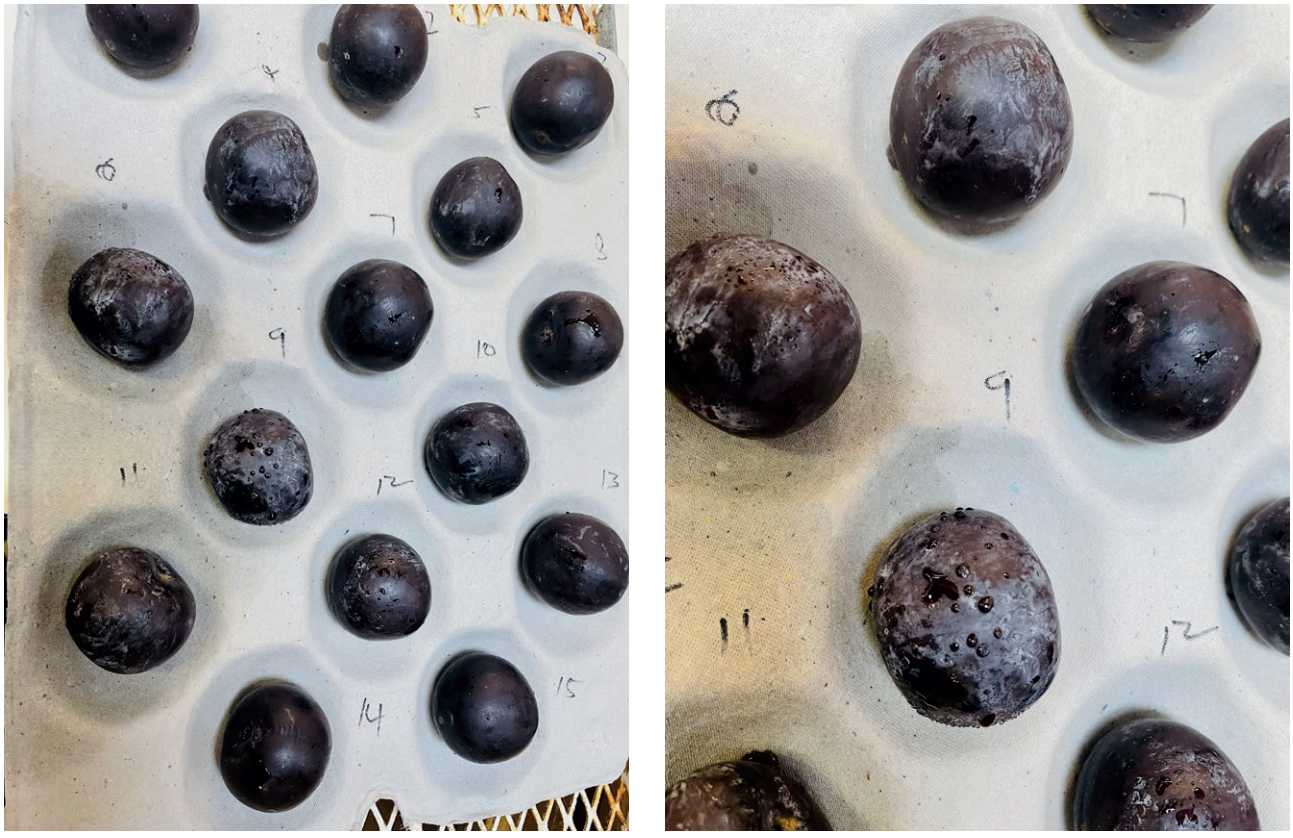

Figure 1. 'Angelino' plums treated with dip applications of thyme oil (0.05-0.5%) for control of *Botrytis cinerea*. White patches on the fruit surfaces indicate damage and hardened texture 24 h after oil application. **A,** thyme oil at 0.05%. **B,** thyme oil at 0.5%.

Table 1. Mean percent incidence of grey mould after 12 d incubation at 25°C, on plums of 'African Delight' and 'Laetitia' following curative or protective treatments, including: fludioxonil (300 mg L^{-1}, orange oil (0.05, 0.1, 0.5 or 1%), OR007B (5% orange oil) or OR79 (10% orange oil). The orange oil treatment rates were adjusted to give orange oil concentrations of 0.05, 0.1 or 0.5%.

Treatment	Mean grey mould incidence (%)			
	'African Delight'		'Laetitia'	
	Curative	Protective	Curative	Protective
Control	50 i-k	74 d-f	88 a-f	84 a-c
Fludioxonil 300 mg L^{-1}	24 I-p	33 l-n	68 e-h	72 b-g
Orange oil 0.05%	24 no	24 m-p	59 g-i	74 g-i
Orange oil 0.1%	23 no	49 i-k	75 g-i	80 b-g
Orange oil 0.5%	14 o-q	49 l-n	65 f-i	82 f-i
Orange oil 1%	22 m-o	25 m-p	55 h-j	79 b-g
OR007B 0.05%	7 q	32 k-m	78 c-f	96 a
OR007B 0.1%	26 m-o	24 m-o	80 d-f	83 b-f
OR007B 0.5%	12 pq	37 k-m	75 b-g	82 b-f
OR79 0.05%	35 j-o	26 m-o	77 d-g	91 ab
OR79 0.1%	24 op	19 n-p	78 c-f	83 b-f
OR79 0.5%	17 n-q	41 j-l	61 g-i	84 b-d

Means accompanied by the same letters are not significantly different ($P > 0.05$).

Table 2. Mean percent grey mould severity after 12 d incubation at 25°C on plums of cvs. 'African Delight' and 'Laetitia' following curative or protective treatments, including: fludioxonil (300 mg L^{-1}, orange oil (0.05, 0.1, 0.5, 1.0%), OR007B (5% orange oil) and OR79 (10% orange oil) adjusted to orange oil concentrations of 0.05, 0.1, 0.5%.

Treatment	Mean grey mould severity (%)			
	'African Delight'		'Laetitia'	
	Curative	Protective	Curative	Protective
Control	100 j-n	100 b-d	100 ab	100 a-c
Fludioxonil 300 mg L^{-1}	61 n-q	47 k-o	79 c-f	93 b-d
Orange oil 0.05%	64 m-p	81 l-o	68 e-i	91 b-d
Orange oil 0.1%	63 m-p	36 m-q	66 e-i	87 b-e
Orange oil 0.5%	36 q-r	69 f-j	73 d-g	97 a-c
Orange oil 1%	58 o- q	81 l-o	60 g-k	92 b-d
OR007B 0.05%	12 r	46 m-q	88 b-d	100 a
OR007B 0.1%	68 m- q	34 i-m	91 a-d	95 a-d
OR007B 0.5%	25 qr	55 m-q	83 b-e	94 a-d
OR79 0.05%	57 o- q	35 i-m	88 b-d	100 ab
OR79 0.1%	66 o-r	27 l-q	87 b-d	93 b-d
OR79 0.5%	29 p-r	60 h-l	67 e-h	95 a-d

Means accompanied by the same letters are not significantly different ($P > 0.05$).

provided control similar to fludioxonil (mean incidence = 24%). The OR007B and OR79 treatments also reduced disease incidence, in some cases to significantly less than the fludioxonil treatment (i.e. 7% from 0.05% OR007B). For the protective treatments in 'African Delight' plums, the untreated controls had mean grey mould incidence of 74%, while the orange oil treatments reduced incidence to between 24 and 49% (similar to the fludioxonil treatment). Fludioxonil resulted in 33% disease incidence, which was also similar to the OR007B and OR79 treatments, where mean incidence was the least (19%) from the 0.1% OR79 treatment.

Disease incidence for 'Laetitia' (untreated control mean = 88%) was greater compared to 'African Delight' (Table 1). Disease incidence in 'Laetitia' plums treated curatively with orange oil showed a trend of decreased grey mould incidence with increasing concentrations, similar to that from fludioxonil (mean incidence = 68%). None of the treatments effectively reduced grey mould incidence when applied protectively in 'Laetitia' plums, with the most effective treatment (0.05% orange oil) reducing mean disease incidence to 74%, compared with 84% from the untreated control).

Disease severity data showed similar trends to the incidence data in both cultivars (Table 2). In 'African Delight' plums treated curatively with 0.5% orange oil, mean severity was reduced to 35%, lower than from fludioxonil (61%), but this difference was not statistically significant. Similarly, 0.5% OR007B and 0.5% OR79 reduced mean incidence to, respectively, 25 and 28%. Fludioxonil reduced disease mean severity to 47%, when applied protectively. Orange oil alone did not consistently lower grey mould severity from the protective applications and demonstrated variable efficacy across oil concentrations. In 'Laetitia', plums, curative application of fludioxonil reduced severity to 79%. Orange oil at the greatest concentration tested (1%) reduced mean severity to 60%, which was significantly less than from the fludioxonil treatment. OR007B and OR79 gave similar results to fludioxonil, with greatest efficacy in the curative experiments from the 0.5% OR79 treatment (mean = 67% severity). Overall, all protective treatments of 'Laetitia' gave high mean grey mould severities (92 to 100%), with no effective disease control.

Although there was no statistically significant interaction between treatment and the day of evaluation ($P = 0.32$) for the dip applications of strawberries followed by 14 d of storage at 4°C, these results are discussed. There was a significant interaction ($P < 0.001$) between the evaluation days (i.e., day 14, the evaluation conducted at the end of cold storage, and day 17, 3 d at ambient temperature). On day 14, mean grey mould incidence was

reduced from 82% in the untreated control to means of 55% for OR79, 21% for OR007B, and 45% for orange oil alone (Table 3). On day 17, fruit became severely affected by grey mould.

Influence of incubation period for plums and lemon fruit

The incubation period experiments conducted with 'Angelino' plums gave significant interactions ($P < 0.0001$) between incubation period and treatment, for both severity and incidence of sporulation incidence data. Only fludioxonil at 8 h reduced disease level (mean = 69%), while the other treatments gave a disease incidence of > 80%. For disease incidence, fludioxonil reduced sporulation incidence with shorter incubation period (mean incidence = 20% after 8 h, and 37% after 24 h. The shorter incubation period for the OR007B and OR79 formulations resulted in decreased sporulation incidence, with increasing formulation the concentrations (means = 69 to 59% for OR007B, and 61 to 35% for OR79).

Table 3. Mean percent incidence of grey mould after 14 d at 4°C and 17 d (14 d plus 3 d incubation at 25°C) on strawberries following curative treatments with orange oil only at 0.05, 0.1, 0.5 or 1%, OR007B (%% orange oil) and OR79 (10% orange oil adjusted to orange oil concentrations of 0.05, 0.1 or 0.5%.

Treatment	Mean grey mould incidence (%)	
	Day 14	Day 17
Control	82 b	98 a
Orange oil 0.05%	42 c	98 a
Orange oil 0.1%	48 c	98 a
Orange oil 0.5%	45 cd	94 a
Orange oil 1%	64 c-e	98 a
OR007B 0.05%	36 de	94 a
OR007B 0.1%	35 e	97 a
OR007B 0.5%	21 c-e	95 a
OR79 0.05%	58 cd	98 a
OR79 0.1%	58 c-e	98 a
OR79 0.5%	55 b-d	98 a

Means accompanied by the same letters are not significantly different ($P > 0.05$).

Figure 2. Brown discolouration and rind damage for 'Eureka' lemons following thyme oil applications (0.05 to 0.5%), using dip applications, against *Penicillium digitatum*. **A,** thyme oil at 0.05%. **B,** thyme oil at 0.5%.

Table 4. Mean percent green mould incidence and sporulation incidence after 4 d at 25°C, on wound inoculated Eureka' lemons with either imazalil sensitive or resistant *Penicillium digitatum* isolates, after 8 h or 24 h incubation ', following curative treatments with imazalil (IMZ) at 500 mg.L $^{-1}$, or orange oil (0.05, 0.1, 0.5 or 1.0%), OR007B (5% orange oil) and OR79 (10% orange oil) adjusted to orange oil concentrations of 0.05, 0.1 and 0.5%.

Treatment	Mean green mould incidence (%)		Sporulating fruit (%)	
	IMZ-sensitive	IMZ-resistant	IMZ-sensitive	IMZ-resistant
8 h				
Control	96.6 a-d	96.6 a-d	99.4 ab	99.4 ab
Imazalil 500 µg mL ^{1}	0.0 n	28.6 l	16.2 e	95.7 e
Orange oil 0.05%	96.6 a-d	91.1 a-d	99.4 ab	99.4 ab
Orange oil 0.1%	96.6 a-d	96.6 a-d	99.4 ab	99.4 ab
Orange oil 0.5%	96.6 a-d	96.6 a-d	99.4 ab	99.4 ab
Orange oil 1.0%	96.6 a-d	95.9 a-d	99.4 ab	99.4 ab
OR-007B 0.05%	96.6 a-d	96.6 a-d	99.4 ab	99.4 ab
OR-007B 0.1%	84.5 gh	96.6 a-d	99.4 ab	99.4 ab
OR-007B 0.5%	77.2 ij	77.9 ij	99.4 ab	99.4 ab
OR-79 0.05%	96.6 a-d	96.6 a-d	99.4 ab	99.4 ab
OR-79 0.1%	96.6 a-c	93.8 b-e	99.4 ab	99.4 ab
OR-79 0. %	81.3 f-h	92.4 c-e	96.7 a-c	99.4 ab
24 h				
Control	100.0 a	100.0 a	100.0 a	100.0 a
Imazalil 500 µg mL ^{1}	5.5 m	52.7 k	19.6 e	93.1 cd
Orange oil 0.05%	100.0 a	100.0 a	100.0 a	100.0 a
Orange oil 0.1%	100.0 a	100.0 a	100.0 a	97.2 ab
Orange oil 0.5%	100.0 a	100.0 a	100.0 a	100.0 a
Orange oil 1.0%	97.9 a-c	100.0 a	100.0 a	100.0 a
OR-007B 0.05%	75.3 j	100.0 a	100.0 a	100.0 a
OR-007B 0.1%	85.4 gh	99.3 ab	100.0 a	92.5 d
OR-007B 0.5%	93.4 e-g	100 a	100.0 a	100.0 a
OR-79 0.0 %	99.3 a	99 a	100.0 a	100.0 a
OR-79 0.1%	97.2 a-c	100 a	100.0 a	95.8 ab
OR-79 0.5%	85.7 f-h	100 a	100.0 a	100.0 a

Means accompanied by the same letters are not significantly different ($P > 0.05$).

Table 5. Mean percent incidence of green mould after 4 d at 25°C on 'Eureka' lemons and 'Midnight Valencia' oranges that were wound inoculated with a imazalil (IMZ) sensitive or resistant *Penicillium digitatum* isolates and incubated for 12 h following dip treatments (60, 90 and 120 s exposure time), including: IMZ at 500 mg.L $^{-1}$, orange oil (0.5 or 1%), OR007B (5% orange oil) and OR79 (10% orange oil) adjusted to orange oil concentrations of 0.5 and 1.0%.

Treatment	Exposure time (s)	Mean green mould incidence (%)			
		'Eureka' lemon		'Midnight Valencia' orange	
		IMZ-sensitive	IMZ-resistant	IMZ-sensitive	IMZ-resistant
Control	0	100 a	100 a	99.3 a	100 a
IMZ	60	3.1 n	40.9 m	0.2 p	0.2 p
Orange oil 0.5%	60	99.6 ab	97.9 ab	99.1 a	96.9 a-c
Orange oil 1.0%	60	96.6 a-d	95.9 a-c	99.4 a	99.4 a
OR-007B 0.5%	60	93.4 a-g	82.9 d-i	77.3 j-m	90.1 a-g
OR-007B 1.0%	60	73.9 i-k	73.2 i-k	79.8 d-j	72.3 f-j
OR-79 0.5 %	60	94.7 a-d	81.5 e-j	84 g-k	77.4 i-n
OR-79 1.0 %	60	73.9 i-k	58.6 l	67.4 m-o	64.6 no
Orange oil 0.5%	90	99.6 ab	90.9 a-g	97.7 ab	95.5 a-e
Orange oil 1.0%	90	91.6 a-f	93.7 a-g	94.8 a-f	99.1 a
OR-007B 0.5%	90	87.2 b-h	80.9 g-j	90.2 a-h	85.3 c-i
OR-007B 1.0%	90	78.1 h-k	69.0 kl	68.9 g-l	70.5 j-n
OR-79 0.5 %	90	94.1 a-e	81.1 i-j	77.1 i-m	83.5 d-j
OR-79 1.0 %	90	76.7 i-k	45.5 m	64.2 no	61.1 o
Orange oil 0.5%	120	90.9 a-g	94.7 a-g	98.7 a	98.4 ab
Orange oil 1.0%	120	94.4 a-d	95.5 a-d	99.8 a	91.3 a-g
OR-007B 0.5%	120	84.0 f-j	81.2 c-i	84.2 g-k	83.1 ab
OR-007B 1.0%	120	87.3 a-h	66.8 j-l	80.5 k-o	75.5 k-o
OR-79 0.5 %	120	95.1 a-d	72.2 i-k	83.1 f-j	85.9 b-i
OR-79 1.0 %	120	76.3 h-k	70.0 j-l	74.7 i-n	68.0 l-o

Means accompanied by the same letters are not significantly different ($P > 0.05$).

Data for 'Eureka' lemon disease incidence data showed a significant disease incidence interaction ($P < 0.001$) between isolate, treatment, and incubation period. In preliminary tests, thyme oil was also assessed, to compare the effect on green mould with that from orange oil. Thyme oil was subsequently excluded from the main experiments due to host tissue damage observed immediately after treatment (Figure 2, A and B).

Imazalil showed reduced green mould control caused by the sensitive strain of *P. digitatum*, at both 8 h (mean = 0%) and 24 h (5%), while the resistant strain of the fungus was reduced to 29% at 8 h and 53% at 24 h (Table 4). Although disease levels were high from all the other treatments, the OR007B formulation showed decreasing mean levels of disease incidence (97 to 77%) with increasing concentration, for the sensitive strain after 8 h incubation. Greater amounts of sporulation incidence were observed following imazalil treatment except for the sensitive isolate.

Influence of exposure time (lemons and sweet oranges)

A statistically significant interaction for disease incidence ($P < 0.001$) was measured in 'Eureka' lemons between *P. digitatum* strains (sensitive or resistant) and

treatment type. In contrast, no significant interaction ($P = 0.83$) was detected for 'Valencia' oranges.

On 'Eureka' lemons, the untreated control fruit inoculated with the sensitive or resistant strains resulted in 100% disease incidence (Table 5). Disease symptomatic fruit inoculated with the sensitive strain and treated with imazalil were found at reduced disease incidence (mean = 3%), and the fruit inoculated with the resistant strain had mean incidence of 41%. While the resistant strain gave greater disease incidence, this was less from the orange oil and orange oil-based formulations 1% concentrations. The OR007B formulation resulted in decreasing mean disease incidence levels (73 to 66%) with increasing exposure times in the treatment bath. Similarly, the OR79 formulation gave disease incidence levels between 45 and 70%. However, this trend was not observed for the sensitive strain. 'Valencia' oranges showed comparable results to the OR79 formulation. The resistant strain resulted in reduced mean level of disease incidence, between 61 and 68%, and some reduction was also detected (64 and 74%) for the sensitive isolate treatments.

DISCUSSION

This study is the first comprehensive investigation of the efficacy of orange essential oil products as options for postharvest disease management in plums, strawberries, and citrus. The manufacturer of these products indicated that the orange oil batch used in this study contained 97% d-limonene. This corresponded with the previous studies that have investigated composition of citrus oil, including orange oil (Caccioni *et al*., 1998; Sharifi-Rad *et al*., 2017; Ramírez-Gómez *et al*., 2020). The results obtained here have demonstrated the potential of orange oil for management of grey mould on plums and strawberries, using the fruit dip applications. Although these experiments were conducted *in vitro*, several studies demonstrated comparable results following applications of orange oil against several pathogens, including *B. cinerea* and *Aspegillus niger* Tiegh (Sharma and Tripathi, 2006; Viuda-Martos *et al*., 2008). Lemon oil was also reported to induce reductions of grey mould severity when evaluated on tomatoes, strawberries, and cucumbers (Vitoratos *et al*., 2013).

The present study showed that orange oil and orange oil-based formulations were not capable of reducing green mould caused *P. digitatum* on citrus, similar to results of Rodríguez *et al*. (2015), Wuryatmo *et al*. (2014), or the *in vitro* study by Droby *et al*. (2008), which found the adverse effect of growth stimulation of *P. digitatum* by volatiles emitted from citrus rind. Rahman (2020) on the other hand found orange oil only effectively controls *P. digitatum* on citrus, provided it was UV irradiated. However, the orange oil formulated products used here (OR007B and OR79), at 1% adjusted oil concentration, exhibited an interesting phenomenon during the evaluation of exposure times. When these products were tested against the imazalil resistant isolate of *P. digitatum,* a reduced disease incidence on lemons and sweet oranges was found compared to results of trials with the sensitive isolate. This could have been due to synergism within the formulations. For instance, apart from surfactants and inert compounds that are incorporated in the formulation, orange oil contains terpenes that have been individually extracted and tested against an imazalil resistant *P. digitatum* strain. Ouyang *et al*. (2022) showed that α-Terpineol inhibited disease by this fungus at a minimum concentration of 4.00 $\mu L\ mL^{-1}$, but also disrupted cell membrane integrity, reduced ergosterol content, and inhibited squalene synthase (ERG9) gene expression. Additionally, aldehydes such as Trans-2-hexenal-β-cyclodextrin, which are present in most essential oils, could also overcome genetic mutation of *P. digitatum*. Yuan *et al*. (2023) showed that trans-2-hexenal-β-cyclodextrin interfered with the gene expression mediating the tricarboxylic acid cycle (an energy metabolism associated gene), glycolysis, and oxidative phosphorylation of an imazalil resistant strain. Since these results were not observed from the orange oil only treatments in the present study, it is likely that presence of surfactants within the formulations triggered the responses of the orange oil component compounds that were responsible for inhibition of the imazalil resistant pathogen strain. However, this concept requires further assessment to establish the mechanisms that are involved.

The attempt to incorporate thyme oil in the present study, by conducting pilot tests prior to the detailed investigation of potential bioactivity against green mould on citrus and grey mould on plums, was interrupted by elevated levels of phytotoxicity from thyme oil observed on the fruit, either directly after dip applications for citrus, or the day after treatment for plums. Although essential oils can cause phytotoxicity (Plaza *et al*., 2004), the present study is not the first to report phytotoxicity from thyme oil applications on fruit. Lopez-Reyes *et al*. (2013) demonstrated effects of thyme, oregano, savory oils against *B. cinerea* and *Monilinia laxa* on stone fruit. Although these treatments were highly effective, at 1% concentrations, phytotoxicity symptoms were observed, and at 10% the symptoms were exacerbated. Regnier *et al*. (2014) investigated seven essential oils (including thyme oil) for phytotoxicity, showing that of all the

oils, only those from *Mentha spicata* and *Geranium graveolens roseum* did cause browning or desiccation of the fruit rinds, and incorporation of essential oils with edible coatings did not result in rind damage.

Dip application was selected to apply the treatments in the present study. This method was used as a primary first step, to establish if orange oil was a potential postharvest disease management strategy against *B. cinerea* and *P. digitatum*. The fungicides fludioxonil and imazalil are registered for dip applications to, respectively, stone fruit and citrus. The industry norm in South Africa is to use atomiser sprays as postharvest applications of fludioxonil to plums. However, with the spray applications being preferred, it was important to assess whether dip application was a viable method for oranges. Erasmus *et al*. (2011) showed the superiority of dip application against green mould compared to spray application, and confirmed that the approved maximum residue limit for fludioxonil on plums (5 mg g^{-1}; DALRRD, 2021) was not exceeded. The residue level obtained in the present study was 1.03 mg g^{-1}. These results corresponded with those from previous studies on effectiveness of spray and dip applications, where spray application gave greater fruit residues than dip applications (Erasmus *et al*., 2011).

It is important to evaluate both curative and protective treatments when investigating postharvest disease management strategies, as fruit becomes injured during handling and packaging stages. Although disease incidence was high after the curative treatments, the orange oil gave similar control to that achieved with the registered standard, fludioxonil. The reduced performance of fludioxonil and orange oil treatments could have been due to the high inoculum levels used, meaning that the study was not a true depiction of commercial packhouse realities. Reducing the inoculum loads should be considered in future investigations.

Curative treatments of plums gave greater control compared to protective treatments. Du Plooy *et al*. (2009) reported similar results, following the application of several essential oils on citrus for control of *P. digitatum* causing green mould. Jenneker *et al*. (2024) also demonstrated efficacy against grey mould of thymol (the active compound in thyme oil) in edible coatings on plums. Thyme oil has also been reported to be fungistatic for *P. digitatum*, and has been successfully incorporated into packaging materials for oranges (Plaza *et al*., 2004). On citrus fruit in the present study, however, phytotoxicity of thyme oil was observed at all the tested concentrations in two separate experiments, so thyme oil was not used in the dip application investigations.

In a study on plums, preventive applications of fludioxonil gave poor control of postharvest decay compared to curative treatments with this fungicide (Förster *et al*., 2007). Edible fruit coatings are hydrophobic, and must act as barriers against moisture loss and entry by fungal pathogens (Njombolwana *et al*., 2013). Orange oil is lipophilic and hydrophobic, and should act as a barrier against *B. cinerea* when used as a protective treatment, although this was not assessed in the present study.

The 'African Delight' cultivar was less susceptible to grey mould than 'Laetitia'. Lopez-Reyes *et al*. (2013) also showed varying degrees of susceptibility of plum cultivars to grey mould. 'African Delight' is more prone to shrivelling than to grey mould (P. Rossouw, pers. comm.), and could have elevated epidermis resistance owing to a thicker cuticle, and increased levels of phenolics and pectin, differences which often occur between cultivars of the same species (Gradziel *et al*., 2003).

For strawberries, the industry heavily relies on synthetic fungicides for the control of *B. cinerea* during crop growth stages (Feliziani and Romanazzi, 2013; Fan *et al*., 2017). The available means for maintaining and preserving postharvest fruit quality are by direct cooling to 0 to 5°C directly after picking. (Haffner, 2002). The shelf life of strawberries is usually approx. 5 d, provided that temperatures are maintained between 0 and 4°C (Parvez and Wani, 2018). In the present study, beneficial effects were observed for orange oil and orange oil-based products to increased strawberry shelf-life period up to 14 days. Orange oil and commercial orange oil products could be recommended, as they pose no residue threats and are generally regarded as safe products. The 1% concentration of orange oil probably predisposed strawberries to postharvest disease, as the treated fruits y were generally softer than untreated fruit, a symptom that was suspected to be due to phytotoxicity. These results were similar to those of Lopez-Reyes *et al*. (2010; 2013) on long-term and cold storage evaluations of essential oils. They showed that these compounds could be useful for short storage periods. The present study evaluation conducted immediately after 14 d of cold storage at 4°C aligned closely with standard industry practices for maintaining strawberry quality. The observed loss of control during later evaluations (day 17) was anticipated, as strawberries are not typically exposed to ambient temperatures during supply chains.

This study adds to an expanding research database on the benefits of incorporating essential oils in postharvest disease management strategies for fruit products. However, their bioactivity is hampered by their volatility, which leads to oxidative deterioration especially when exposed to oxygen, light, moisture, and/or heat (Botrel *et al*., 2015; Maes *et al*., 2019). The next research step should focus on exploring fruit encapsulation, which

ensures that bioactivity of active ingredients is preserved, and less amounts are released, without causing harm to host tissues, but invasions by target fungi or bacteria are controlled (Pothakamury and Barbosa-Cánovas, 1995). Organoleptic characteristics of essential oil-based products and their volatile actives need to be evaluated through tasting panels of the treated fruit, unless methods are employed to remove strongly flavoured compounds, provided this does not reduce the antifungal activity of the respective essential oils. Once an appropriate formulation has been developed for oil application, trials can be upscaled to test large amounts of fruit (i.e., 500 fruit per treatment, and at commercial scales), including storage trials to simulate export conditions.

The present study forms part of a continuing effort to assess effects of orange oil against postharvest pathogens on selected fruit types. Orange oil has potential for post-harvest disease management. Future investigations should cover other plum cultivars, and for strawberries, should incorporate potential protective effects of orange oil and orange oil-based products.

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