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Postharvest fungicide treatments and cold storage control citrus black spot infections

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ABSTRACT

Citrus black spot (CBS) is caused by *Phyllosticta citricarpa*, which is regarded as a quarantine pathogen in certain countries. Pest risk assessments disagree on the risk of fruit as a pathway for introduction of the pest to new areas. Some countries accordingly regulate the movement of fruit from production regions where CBS occurs. Preharvest fungicide sprays are very effective in controlling CBS, but cannot consistently achieve complete control. The effect of postharvest treatments on CBS infections and on the reproductive ability of *P. citricarpa* in lesions that formed after these treatments was studied. Trials were conducted using naturally infected 'Eureka' lemon and Valencia orange fruit. Asymptomatic fruit were treated using commonly used packhouse sanitation and fungicide treatments, and cold storage (individually and combined), as well as alternative stand-alone treatments. After treatment, fruit were stored for 5 weeks (in the dark) at ambient temperature (20–22 °C) or −0.5–7 °C, whereafter they were incubated for a further 2 weeks at conditions conducive to expression of symptoms and formation of pycnidia. Individual treatments generally resulted in variable levels of control. The combination of packhouse treatments (including pre-packhouse drench with guazatine or propiconazole in combination with pyrimethanil, thiabendazole and 2,4-D; chlorine wash; dip treatment in imazalil; and brush application of a wax coating incorporated with imazalil, thiabendazole and 2,4-D) consistently showed moderate to high levels of control of CBS development from latent infections on lemons (32.4–43.4% for incidence and 61.6–68.3% for severity) and oranges (58.3–85.7% for incidence and 54.1%–88.8% for severity). However, cold storage subsequent to packhouse treatments (as is common shipping protocol) further improved the levels of control (44.0–58.3% for incidence and 78.1–82.5% for severity on lemons; 66.1–93.5% for incidence and 85.3–98.5% for severity on oranges). An average of 10% of new lesions on lemons and 15% on oranges formed pycnidia, indicating that *P. citricarpa* generally had a low reproductive capability in fruit lesions, which was in most cases further diminished by the combination of treatments followed by cold storage.

1. Introduction

Citrus black spot (CBS) is caused by *Phyllosticta citricarpa* (McAlpine) van der Aa. Leaf and fruit infections mostly remain latent, but a variety of CBS lesions may be expressed on the rind of maturing fruit. These lesions are not progressive postharvest decays, but heavy infections can make fruit aesthetically unsuitable for the fresh fruit market (Kotzé, 1981). In severe cases, and in highly suitable climates (Spósito et al., 2008, 2011), untreated or poorly treated fruit exposed to heavy infection can lead to premature fruit drop and crop loss (Araújo et al., 2013; Lanza et al., 2018). In South Africa, CBS rarely causes crop loss, but due to phytosanitary export restrictions it is economically important.

Phyllosticta citricarpa is regarded as a quarantine pathogen in certain countries and the presence of CBS in production regions (Carstens et al., 2012) or the presence of CBS lesions on fruit may limit access of fresh fruit exports to some markets (EFSA PLH Panel, 2014). In some countries, whole consignments may be rejected if one or more CBS lesions are observed during official inspection of fruit (Anonymous, 2000).

In most regions, the life cycle of *P. citricarpa* is monocyclic, with aerially dispersed ascospores being the main source of inoculum. These sexual spores are produced in pseudothecia that develop and mature in

Abbreviations: CBS, citrus black spot; IMZ, imazalil; FLU, fludioxonil; GZT, guazatine; OA, oatmeal agar; PDA, potato dextrose agar; PYR, pyrimethanil; PPZ, propiconazole; TBZ, thiabendazole; WA, water agar; 2,4-D, 2,4-dichlorophenoxyacetic acid

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leaf litter on the orchard floor (Fourie et al., 2013; Kiely, 1948; Kotzé, 1981; McOnie, 1965); pseudothecia and ascospores have never been reported from fruit or twigs. Asexual pycnidia with pycnidiospores can also form on leaf litter, twigs, as well as in certain fruit lesions. Of the different lesion types that are found on fruit, *viz.* hard spot, freckle spot, false melanose or speckled blotch, virulent spot, lacy and tan spot, pycnidia are only formed in hard spot (Brentu et al., 2012; FAO, 2014; Kiely, 1948; Kotzé, 2000; Marques et al., 2012; Wager, 1952), freckle spot (Kotzé, 2000; Lourenço et al., 2012), and virulent spot (FAO, 2014; Kiely, 1948; Kotzé, 2000). Pycnidiospores ooze from mature pycnidia in a gelatinous mass (Kiely, 1948; McOnie, 1965, 1967) and are dispersed by water to cause new infections short distances (less than 1 m) downward from the source (Kiely, 1948; Spósito et al., 2008, 2011; Wager, 1952). Given this dispersal limitation, as well as their short-lived nature, the epidemiological contribution of pycnidiospores is regarded as minor (Kiely, 1948; McOnie, 1965), except under highly CBS conducive climatic conditions such as in Florida and Brazil (Spósito et al., 2008, 2011; Hendricks et al., 2017).

Most citrus varieties grown globally are susceptible to *P. citricarpa* infection, with 'Eureka' lemons and Valencia oranges regarded as being more susceptible (Brodrick, 1969; Kiely, 1948, 1970). In South Africa and Australia, CBS is primarily controlled in the orchard through the application of protective and/or curative fungicidal sprays to protect young fruit during the susceptibility period (the first 4–5 months after petal drop) (Kiely, 1948; Miles et al., 2004; Schutte, 2002; Schutte et al., 2003), while Lanza et al. (2018) demonstrated that longer periods of fungicide protection of fruit were required under highly conducive conditions in Brazil. In South Africa, preharvest spray programmes generally consists of 3–4 applications, combining and alternating between mancozeb, benzimidazoles, strobilurins and copper (Kellerman and Kotzé, 1977; Lanza et al., 2018; Miles et al., 2004; Schutte et al., 2003, 2012). Whilst very high levels of control can be achieved (Makowski et al., 2014), even timely applied fungicides can result in variable level of protection due to climatic conditions, variable spray coverage, and cultivar susceptibility (Calavan, 1960; Kiely, 1969, 1970, 1971; Schutte et al., 2003, 2012). High levels of control are required as whole consignments will be rejected when one or more CBS lesions are detected during official inspection for export to certain CBS sensitive markets and repeated non-compliance may jeopardise sustained access to certain markets (Anonymous, 2000; WTO, 1993).

South African fresh fruit travel along some of the longest export routes: the time from harvest until fruit reaches the consumer can be up to 6 weeks, depending on the place of production and the export destination (Pelser, 1977). Due to the extended time from harvest to consumption, producers and packhouses are required to apply effective measures to maintain product quality and limit postharvest decay. These measures include application of postharvest fungicides, wax coatings, and sanitation practices (Erasmus et al., 2011; Njombolwana et al., 2013).

Storage temperatures influence the rate of symptom development. Low temperatures (~8 °C) delay symptom development (Agostini et al., 2006), while high storage temperatures (~27 °C) increase the rate of symptom development (Baldassari et al., 2007; Korf, 1998). Viability of *P. citricarpa* in CBS lesions was reduced by 3- to 7-fold, compared with untreated fruit, by fruit wash and postharvest fungicide treatments, and storage conditions (8–11 °C, depending on citrus type) that are commonly used for export fruit (Korf et al., 2001). Moreover, none of the pycnidiospores harvested from hard spot lesions on treated fruit germinated (Korf et al., 2001). Seberry et al. (1967) also reported that applying a wax coating to Valencia orange fruit significantly reduced the expression of CBS lesions on stored fruit. Several other studies also reported control of latent infections through application of postharvest treatments (Lucon et al., 2010; Rappussi et al., 2009, 2011; Yan et al., 2016). To the contrary, Agostini et al. (2006) reported that none of the postharvest fungicides evaluated in their study had a significant effect on lowering postharvest CBS incidence.

Postharvest treatment and handling practices have changed considerably from those evaluated by these studies (Agostini et al., 2006; Korf et al., 2001; Seberry et al., 1967), and include different active ingredients used as sanitising agents or fungicides, different application methods, and storage regimes (Dodd et al., 2010; Erasmus et al., 2011; Kellerman et al., 2014, 2018; Njombolwana et al., 2013). In addition, pre-packhouse drenching before degreening to minimize postharvest decay, primarily caused by *Penicillium* spp. and *Galactomyces* spp., is a more recent practice that is increasingly being used in South Africa. The aim of this study was therefore to evaluate the effect of commonly used postharvest treatment and handling practices on CBS fruit infections, focusing on *P. citricarpa* viability and reproductive capability from lesions.

2. Materials and methods

2.1. Collection of fruit

'Eureka' lemons and Valencia oranges were used in all the trials, and were chosen for their high susceptibility to *P. citricarpa* (Kiely, 1948). Fruit were harvested from farms located in Gauteng, Mpumalanga and Eastern Cape provinces of South Africa, where CBS does occur. Lesion-free fruit were collected from eight unsprayed or abandoned orchards (four 'Eureka' lemon and four Valencia orange orchards) that were inspected for CBS symptoms (on out-of-season fruit, and leaves) to verify the presence of CBS. Fruit were harvested into plastic fruit crates (325 × 505 × 245 mm) and transported to the laboratory and used within 48 h after harvest.

2.2. Postharvest treatments

Commonly used postharvest fungicides in South Africa, as well as newly registered alternatives, were used in the treatments for the different trials and are discussed in detail in following separate sections. These were evaluated as pre-packhouse drench, chlorine wash, fungicide dips, and wax application as individual treatments, as well as the complete packhouse treatment regime (consisting of all of the aforementioned treatments; termed combination treatment), with and without cold storage. A commercial product that allegedly acts as a rind fortifier (Fortisol Ca Plus, Citrosol, Potríes, Valencia, Spain) was also included to determine its value in the suppression of CBS lesion development. Per trial, each of the treatments consisted of four replicates, with each replicate consisting of 12 fruit. In order to establish a more accurate level of initial infection, which was used as reference value to calculate percentage control obtained by the various treatments, the sample size of the untreated control was tripled, and consisted of 36 fruit per replicate. Untreated control fruit were incubated at ambient temperatures (20–22 °C) for 5 weeks in the dark to simulate postharvest handling and shipping without packhouse treatments and cold storage.

2.2.1. Pre-packhouse drench

Harvested fruit is often drenched before degreening to minimize postharvest decay, primarily caused by *Penicillium* spp. and *Galactomyces* spp. (Erasmus et al., 2011; Kellerman et al., 2014, 2018; Ladaniya, 2010). A typical drench treatment contains thiabendazole (TBZ; 1000 mg L^{-1}), pyrimethanil (PYR; 1000 mg L^{-1}), guazatine (GZT; 1000 mg L^{-1}) and 2,4-D (250 mg L^{-1}) (Kellerman et al., 2018). The drench mixture used in these trials was prepared with 120 L of tap water and adding fungicides in the following order: 220 mL TBZ (Thiabendazole, 500 g L^{-1} SC, ICA International Chemicals, Stellenbosch, South Africa), 275 mL PYR (Protector, 400 g L^{-1} SC, ICA International Chemicals, Stellenbosch, South Africa), 528 mL GZT (CitriCure, 210 g L^{-1} SL, ICA International Chemicals, Stellenbosch, South Africa), and 1100 mL 2,4-D [(2,4-dichlorophenoxy acetic acid) Deccomone, 25 g L^{-1} SL, Citrashine, Johannesburg, South Africa]. The final mixture was stirred constantly to keep all the fungicides in suspension.