

Controlling CBS and inhibiting spore release

Standard postharvest treatments control CBS and inhibit spore release. By Providence Moyo, Siyethemba L. Masikane, Lindokuhle C. Mamba, Régis de Oliveira Fialho, Paul H. Fourie, and Vaughan Hattingh

Background

Phyllosticta citricarpa causes citrus black spot (CBS) and is listed as a quarantine organism in the European Union (EU). Contrary to most other countries importing citrus fruit, the EU upholds an opinion that CBS-infected fruit may be a pathway for introduction of the CBS pathogen. Accordingly, the ability of pycnidia on fruit to produce pycnidiospores after exposure to standard packhouse treatments and shipping conditions, and the effect of these treatments on natural CBS infections were investigated. This was performed under simulated supply chain conditions culminating in the whole fruit, peel segments, or citrus pulp with CBS lesions being discarded under natural sunlight conditions.

Experimental approach

Naturally infected 'Midnight' Valencia orange and 'Eureka' lemon fruit either untreated or treated using standard packhouse treatments, were placed into cold storage for five weeks (oranges at 4 °C and lemons at 7 °C). The packhouse treatment regime consisted of a drench mixture containing thiabendazole (1000 mg L⁻¹), pyrimethanil (1000 mg L⁻¹), propiconazole (600 mg L⁻¹) and 2,4-dichlorophenoxy acetic acid (250 mg L⁻¹), followed by a fungicide dip consisting of 500 mg L⁻¹ imazalil sulphate and a polyethylene-based wax (18% solids) mixed with imazalil (2000 mg L⁻¹). Thereafter, treated and untreated fruit was incubated for a further two weeks at conditions favourable for CBS symptom expression and formation of pycnidia. New CBS lesions expressed during incubation and the number of new lesions that

developed pycnidia were recorded. Fruit with new lesions containing pycnidia was selected for use in further experiments. These experiments evaluated the ability of pycnidia to secrete viable pycnidiospores after whole fruit, peel segments or peel pieces from citrus pulp were exposed to sunlight at warm temperatures (± 28 °C) and $\pm 75\%$ relative humidity. No rain was recorded during the trials.

To stimulate pycnidiospore release from pycnidia contained in lesions, a 20 μ L drop of a Valencia juice and citric acid solution (Moyo et al., 2020) was placed on each



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Figure 1. Citrus pulp generated from processing 'Midnight' Valencia oranges



Treatments	Mean number of new CBS lesions per fruit		New lesions with pycnidia (%)
	2019	2020	
'Eureka' lemons			
Untreated fruit	17.60 a	7.90 b	6.61 a
Treated fruit	2.10 c	1.30 c	1.40 b
'Midnight' Valencia oranges			
Untreated fruit	14.21 a	-	4.43 a
Treated fruit	2.33 b	-	0.77 b

Table 1. Mean number of new CBS lesions and percentage of pycnidia-forming lesions per fruit on 'Eureka' lemons and 'Midnight' Valencia oranges after exposure to postharvest fungicide treatments as well as a 5-week period of cold storage at 4 °C (oranges) and 7 °C (lemons) and 2 weeks at 25-27 °C under constant light and > 80% humidity

selected lesion and left for 10 minutes to allow spores to be released into the droplet. The droplet was then drawn up using a pipette and spread onto water agar plates that were examined using a stereo microscope after 48 h of incubation at ± 25 °C for the presence of *P. citricarpa* pycnidiospores. A lesion was considered

viable if one or more germinating spores were observed on water agar.

Different sets of treated and untreated whole fruit and peel segments (lemons and oranges) were examined for pycnidiospore release and germination after zero, two, four and six hours of exposure to natural conditions in direct sunlight. Treated and

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untreated fruit was also processed for juice extraction to obtain citrus pulp, which was in the form of peel segments, rag, and seeds (Figure 1). The pulp was spread flat in open trays and exposed to sunlight conditions for either zero, one, two or three days before lesions with pycnidia were investigated for pycnidiospore release and germination. Fruit pulp from processing plants that is typically spread out on concrete slabs to dry (and later used as animal feed) was regarded by the EU as posing a high risk of CBS spread to nearby citrus orchards.

The poor reproductive capability of CBS fruit lesions was clearly demonstrated in the study.



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Results

New CBS lesions were observed on both treated and untreated fruit after incubation at optimum conditions for symptom expression. However, the combination of packhouse treatments and cold storage significantly reduced the number of new CBS lesions and effectively controlled CBS

latent infections and pycnidium formation by around 80% (Table 1).

Means followed by the same letter (Table 1) are not significantly different per Tukey's HSD test at a confidence interval of 95%.

Spore release from whole fruit and peel segments did not differ significantly ($p = 0.085$). Thus, results were combined during analysis. Packhouse treatments and cold storage completely inhibited pycnidiospore release as none of the pycnidia on treated whole fruit and peel segments released spores. Microscopic observations revealed that pycnidia on both treated lemon and orange fruit were covered with wax coating. Pycnidiospores were only released from pycnidia on untreated lemon and orange fruit, but sun exposure led to a significant reduction in the proportion of pycnidia releasing spores after two, four, and six hours (Table 2). Spores recovered from untreated whole fruit were significantly more viable compared with those from peel segments for both lemons and oranges. Spores from fruit and peels exposed to sunlight were significantly less viable than those from fruit or peel not exposed to sunlight.

Means for each fruit type followed by the same letter (Table 2) are not significantly different ($p < 0.05$).

Low levels of viable pycnidiospore release were observed from pycnidia in lesions on peel pieces in citrus pulp from treated fruit (<8.6%) and spore release and viability, rapidly declined when exposed to sunlight conditions (Table 3).

Means for each fruit type followed by the same letter (Table 3) are not significantly different ($p < 0.05$).

Discussion

Standard packhouse treatments, commonly employed for fresh citrus fruit, consist of a combination of postharvest sanitation and fungicide treatments, as well as wax application and cold storage. These treatments were shown to effectively control CBS latent infections and pycnidia formation.

Furthermore, the wax coating completely inhibited pycnidiospore release in fruit and peel segments. This clearly indicates that infected packhouse-treated fruit is not a viable pathway for *P. citricarpa* spread.

The poor reproductive capability of CBS fruit lesions was clearly demonstrated in the study, as viability of CBS lesions rapidly declined after sunlight exposure of whole fruit, peel segments, and citrus pulp. Low levels of viable pycnidiospore release were observed from pycnidia in lesions on peel pieces in citrus pulp from treated fruit, but spore release and viability rapidly declined when exposed to sunlight conditions. Infected peel pieces in citrus pulp cannot, therefore, be considered as an epidemiologically probable pathway for *P. citricarpa* spread. Pycnidiospores are effectively water-dispersed

Citrus fruit without leaves do not represent an epidemiologically significant pathway for the entry, establishment, and spread of *P. citricarpa*.

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for short and downward distances (<1 m) from the source (Spósito et al., 2011) and effective dispersal from discarded citrus pulp to a susceptible host is highly unlikely. Therefore, the study supports the conclusion that citrus fruit without leaves do not represent an epidemiologically significant pathway for the entry, establishment, and spread of *P. citricarpa* (USDA-APHIS, 2010; CBS Expert Panel, 2013).

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