Standard operating procedure for the detection of latent citrus black spot infection in immature citrus fruit

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Background

Paraquat was first used in 1984 to detect latent fungal infections in bean seed. It was also used to detect *Phyllosticta solitaria* in apples (Biggs, 1995). Paraquat dip treatments were however phytotoxic to green Valencia oranges, but replacing it with ethephon solved this problem (Schutte & Beeton, 1999). In Brazil, the same technique has been used in detecting latent CBS infections prior to harvest (Baldassari *et al*., 2007).

The EU CBS regulations specify that no CBS infected fruit may enter the EU. The ethephon technique can be used to detect latently CBS infected citrus fruit in a sample taken from the orchard prior to commencement of harvesting. This document provides a procedure for conducting such sampling and testing.

Timing

Sampling can commence in May. To cater for the 14 day incubation period of the sample and other pre-export inspections and procedures, sample collection and treatment should take place at least 4 weeks prior to commencement of harvest.

Sampling procedure

- To improve the sensitivity of the sampling strategy, the sample should be collected from sensitive indicator trees within each orchard.
- Sensitive indicator trees are trees in a poor condition and trees at row ends (where there is a concentration of risk of deficient spray application).
- The sample size should be 100 fruit per orchard.
- If an orchard exceeds 5ha in size, the sample size should be increased by 20 fruit per ha greater than 5 ha (for example a 6ha orchard would require a sample of 120 fruit).
- One half of the sample (50 fruit) should be collected from row end trees, evenly spread across both ends of the orchard at a rate of 1 fruit per selected tree, unless there are too few rows in which case the number of fruit per tree can be proportionately increased to provide the 50 fruit sample.
- The remaining 50 sample fruit should be collected from trees in poor condition, with no more than 5 fruit collected from any one such poor condition tree. If there

are less than 10 trees in poor condition, the shortfall in the 50 fruit sample should be made up by collecting fruit from trees evenly spaced in a diagonal transect across the orchard.

- From each sample tree, collect the fruit from the lower outside portion of the tree canopy on the sunny (North-westerly) side of the tree.

Treatment and incubation

- Prepare an ethephon (Ethrel®, 480 g/L SL, Bayer Cropscience; or Ethepon®, 480 g/L SL, Villa Crop Protection) suspension at a rate of 8 ml/L water in a suitable container. Each mixture can be used for a maximum of 7 days for the dipping of a maximum of 20 orchard samples.
- Dip the 100 fruit orchard sample into the mixture containing ethephon for 5 minutes; ensure that all fruit are submerged; remove and allow for run off of the excess mixture.
- Store fruit at ambient conditions, without cooling. Storage at temperatures above 20°C is ideal, but not essential. Humidity should be moderate, to avoid excessive drying out and decay.
- The maintenance of constant light is ideal, but not essential.

Inspection

- After 7 days, fruit should change in colour (ripen).
- Remove decayed fruit as they occur.
- Inspect each fruit after 14 days for typical CBS lesions.
- Latent CBS lesions will appear as reddish lesions, but not all reddish lesions will necessarily be indicative of CBS. Experience with CBS identification is required to reliably identify the symptoms as CBS.
- One or more CBS lesions constitute a positive CBS result for the orchard. In the event of uncertainty if the lesion is caused by CBS, have the fruit analysed by an accredited CBS identification lab by means of molecular identification techniques.

References

Baldassari, R.B., Brandimarte, I., Gustavo de Andrade, A.,Goncalves de Souza, D.C., Moretto, C. De Goes, A. 2007. Induction of the precoce expression of *Guignardia citricarpa* symptoms in fruits of Pera-Rio sweet orange. Rev. Bras. Frutic., Jaboticabal - SP, v. 29, n. 2, p. 269-275.

Biggs, A.R. 1995. Detection of latent infections in apple fruit with paraquat. Plant Dis. 79:1062-1067.

Schutte, G.C. & Beeton, K.V. 1999. Evaluation of paraquat and ethepon for monitoring latent *Phyllosticta citricarpa* infections in asymptomatic immature Valencia oranges. Deciduous Fruit Grower 49:S8 & S10.

Ethephon dip test to detect latent citrus black spot

Sample fruit from each orchard to be tested, add 8ml ethephon / L water, mix thoroughly and dip fruit for 5 minutes

Leave fruit at ambient conditions for 14 days

If present, CBS will show as reddish lesions