The order of benzimidazole and strobilurin applications in a spray programme for the control of citrus black spot

G.C. SCHUTTE
Citrus Research International, PO Box 28, Nelspruit 1200

ABSTRACT
The alternation of spray programmes consisting of tank mixtures of a benzimidazole, mancozeb and oil in November followed by a strobilurin, copper and spray oil (A+B; C+D sequence) in January show great potential for the control of citrus black spot even in orchards where the benzimidazole resistant citrus black spot population comprises 87% (2003/2004 season) and 63-66% (2004/2005 season). The future use of the benzimidazoles will depend on the outcome of a carbendazim residue study (study number 05/1119) that is currently underway on citrus in RSA as a result from the support of an EU import tolerance. If the study shows that a single benzimidazole application will enable compliance with the revised EU import tolerance, then no benzimidazoles had been sprayed on that Estate until the end of February 2002 when a single benomyl application at a rate of 75 g/hl water was advised and applied. With this application, good CBS control was achieved. It was therefore decided to investigate how a single curative benomyl application will perform in a benzimidazole-resistant environment and the placement thereof in a strobilurin spray programme, also consisting of two applications in November and January according to the label, but where a tank mixture with mancozeb is replaced with a copper fungicide.

INTRODUCTION
Fungal resistance development to strobilurins is an ever-existing possibility. Therefore, anti-resistance strategies using fungicides with different modes of action, that do not result in a loss in effective control with less spray rounds, must be investigated. Recently a consultant (I.J. Bruwer, personal communication) found that a single Benlate application gave good citrus black spot (CBS) control at Lisbon Estates where resistance towards benzimidazoles was reported in the 1980s (De Wet, 1987). Since then, no benzimidazoles had been sprayed on that Estate until the end of February 2002 when a single benomyl application at a rate of 75 g/hl water was advised and applied. With this application, good CBS control was achieved. It was therefore decided to investigate how a single curative benomyl application will perform in a benzimidazole-resistant environment and the placement thereof in a strobilurin spray programme, also consisting of two applications in November and January according to the label, but where a tank mixture with mancozeb is replaced with a copper fungicide.

MATERIALS AND METHODS
a) 2003 - 2004 field trial: Two orchards were selected. One was at Crocodile Valley Citrus Co. and one at Friedenheim Estates, both in the Nelspruit region. A randomised block design with 5 and 3 single-tree plots per treatment, respectively, was used. Fungicides were applied using a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Each treatment was replicated six times in single-tree plots arranged in a randomised block design. Guard trees were assigned between plots within rows. Trees in both groves were selected for uniformity in canopy density and tree size. Spray volumes varied according to the size and number of fruit per tree. Data were analysed by ANOVA, using Fisher’s Least significant difference test (P = 0.05).

b) 2004 - 2005 field trial: The same trial sites and the same fungicides were used as described above. All treatments were replicated six times in single-tree plots arranged in a randomised block design. Guard trees were assigned between plots within rows. Data were analysed by ANOVA, using Fisher’s Least significant difference test (P = 0.05).

c) Determination of benomyl resistance: Fruit infested with CBS were randomly sampled from all trees with visible CBS lesions throughout the orchard at Friedenheim Estates in 2002 and 2003 as well as at both Crocodile Valley Citrus Co. and Friedenheim Estates during 2005. About 150 isolates were made from the fruit samples and plated onto PDA and incubated at 25°C under artificial light. Radial growth was recorded after 14 days.

An aqueous stock suspension of benomyl was made up of sterile distilled water at a rate of 5 ppm. This was added to PDA and allowed to cool at 12°C for 15 minutes and cooled to 25°C for 14 days.

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Fungal plugs (5 mm in diameter) of 14-day old cultures were aseptically removed and placed onto each petri dish containing amended agar and incubated at 25°C under artificial light. Radial growth was recorded after 14 days. Fungal plugs were also taken from the same isolates and plated onto oatmeal-agar to distinguish between pathogenic and non-pathogenic cultures.

The same trial sites and the same fungicides were also used except that trifloxystrobin (Flint 50% WG), carbendazim (Copstar 12% SC), copper hydroxide (Copstar 12% SC) was replaced with azoxystrobin (Ortiva, 25% SC) and the mineral spray oil, Criolco 40. The same trials were repeated in 2004 and 2005. About 150 isolates were made from the fruit samples and plated onto PDA and incubated at 25°C under artificial light. Radial growth was recorded after 14 days.

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Figure 1. Seven-day-old cultures of Guignardia citricarpa producing a distinct yellow pigment that is absent in cultures of G. mangiferae.

pathogenic strains as described by Baayen et al., (2002). Guignardia citricarpa isolates produce a distinct yellow pigment halo on the oatmeal-agar (Fig.1) that is absent from the G. mangiferae isolates.

RESULTS AND DISCUSSION

a) 2003-2004 field trial: Results at Friedenheim Estates (Table 1) showed that the alternation of the tank mix combination with either trifloxystrobin + copper hydroxide + oil; benomyl + mancozeb + oil (A+B; C+D) or benomyl + mancozeb + oil; trifloxystrobin + copper hydroxide + oil (C+D; A+B) sprayed in mid-November and mid-January, only resulted in 86% clean exportable fruit that was significantly different (P < 0.05) from the standard mancozeb treatment. The mancozeb treatment resulted in 10% more clean, exportable fruit. However, where benomyl was replaced with its breakdown product, carbendazim, also in tank mixtures with mancozeb and oil, these treatments sprayed as either trifloxystrobin + copper hydroxide + oil; carbendazim + mancozeb + oil (A+B; C+D) or carbendazim + mancozeb + oil; trifloxystrobin + copper hydroxide + oil (C+D; A+B) resulted in more clean exportable fruit for export of 99.33%, that is 3% better than the standard mancozeb treatment. All these treatments were significantly different from the control that had only 19.33% clean exportable fruit (Table 1).

The trial site at Crocodile Valley Citrus Co. showed that there were no significant differences (P > 0.05) between any of the treatments. However, they were all significantly different from the control that resulted in 39.60% clean exportable fruit (Table 1). In both trials, the trifloxystrobin + copper hydroxide + oil; carbendazim + mancozeb + oil (A+B; C+D) sequence at Friedenheim Table 1. Evaluation of spray programmes consisting of one tank mixture of trifloxystrobin (Flint), copper hydroxide (Copstar) and spray oil and another of benomyl (Benlate) or carbendazim (Bavistin) and mancozeb (Sancozeb) and mineral spray oil during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Friedenheim Estates and Crocodile Valley Citrus Co. during 2003 and 2004.

<table>
<thead>
<tr>
<th>Treatments (A+B; C+D)</th>
<th>Rate / 100 litre water</th>
<th>Friedenheim Estates, Nelspruit</th>
<th>Crocodile Valley Citrus Co., Nelspruit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Clean exportable fruit a</td>
<td>% Fruit with 1-3 CBS lesions b</td>
<td>% Fruit with four and more CBS lesions c</td>
</tr>
<tr>
<td>Carbendazim+mancozeb+oil; Trifloxystrobin+copper hydroxide+oil</td>
<td>55ml + 150g + 0.25%; 10g + 300ml+ 0.25%</td>
<td>99.33a</td>
<td>0.33a</td>
</tr>
<tr>
<td>Trifloxystrobin+copper hydroxide+oil; Carbendazim+mancozeb+oil</td>
<td>10g + 300ml + 0.25%; 55ml + 150g + 0.25%</td>
<td>99.33a</td>
<td>0.67ab</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>200 g</td>
<td>96.33a</td>
<td>0.66ab</td>
</tr>
<tr>
<td>Trifloxystrobin+copper hydroxide+oil; Benomyl+mancozeb+oil</td>
<td>10g + 300 ml + 0.25%; 50 g + 150g + 0.25%</td>
<td>86.00b</td>
<td>7.00bc</td>
</tr>
<tr>
<td>Benomyl+mancozeb+oil; Trifloxystrobin+copper hydroxide+oil</td>
<td>50g + 150g + 0.25%; 10g + 300ml + 0.25%</td>
<td>86.00b</td>
<td>8.00c</td>
</tr>
<tr>
<td>Control</td>
<td>19.33c</td>
<td>20.33d</td>
<td>60.33b</td>
</tr>
</tbody>
</table>

a) Means in a column, based on 3 replicates, followed by the same letter are not significantly different (P > 0.05) according to Fisher's least significant difference test.

b) Means in a column, based on 5 replicates, followed by the same letter are not significantly different (P > 0.05) according to Fisher's least significant difference test.

c) Spray dates were 16 October 2003, 13 November 2003, 11 December 2003, 8 January 2004 for mancozeb alone and 13 November 2003 and 8 January 2004 for the other treatments sprayed in a tank mixture.

d) Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004 for mancozeb alone and 11 November 2003 and 6 January 2004 for the other treatments sprayed in a tank mixture.

ND = Not determined.
Estates and the trifloxystrobin + copper hydroxide + oil; benomyl + mancozeb + oil (A+B; C+D) sequence at Crocodile Valley Citrus Co., was the only spray programme that resulted in fruit with no lesions in the category of four and more CBS lesions. The latter spray programme applied at Friedenheim Estates had 7% fruit with four and more CBS lesions. This can be attributed to the poor spraying and coverage of one particular tree.

b) 2004-2005 field trial: Results at Friedenheim Estates (Table 2) showed that the alternation of the tank mix combination with either azoxystrobin + copper oxychloride + oil; benomyl + mancozeb + oil or azoxystrobin + copper oxychloride + oil; carben-dazim + mancozeb + oil (A+B; C+D) or benomyl + mancozeb + oil; azoxystrobin + copper hydroxide + oil or carben-dazim + mancozeb + oil; azoxystrobin + copper hydroxide + oil (C+D; A+B) sprayed in mid-November and mid-January, resulted in 92-98% clean, exportable fruit that was not significantly different (P > 0.05) from the standard mancozeb treatment. A similar result was evident using the other fruit infestation criteria. All these treatments were significantly different from the control that had only 31.67% clean exportable fruit.

The trial site at Crocodile Valley Citrus Co. showed that there were no significant differences (P > 0.05) between any of the sprayed treatments (Table 2). The standard mancozeb treatment resulted in the most clean, exportable fruit of 98.2%. However, all treatments were significantly different from the control that resulted in 33.8% clean exportable fruit and the same scenario was experienced with the other criteria as well.

c) Determination of benomyl resistance: From the initial 150 CBS isolates made in 2004 from the fruit collected at Friedenheim Estates, only 49 grew and 87% of these CBS isolates were resistant to benomyl. Furthermore, 85% of the same isolates were G. citricarpa and the rest were G. mangiferae.

From the initial 100 CBS isolates made in 2005 from the fruit, only 62.9% grew and were resistant to benomyl and 100% of all the isolates were G. citricarpa from the Friedenheim Estates site, while 100% of the isolates were G. citricarpa from the Crocodile Valley Citrus Co. orchard of which 66% were resistant to benomyl.

**CONCLUSION**

It appears from the 2003/2004 trial results that the residual action of the strobilurin, trifloxystrobin, is long enough to protect fruit for 8 weeks if mixed with copper hydroxide and spray oil. Benomyl on the other hand, although it was sprayed where the benzimidazole population was 87% (2003/2004 season) and 63-66% (2004/2005 season) resistant, still maintained clean fruit and could still provide a curative action perhaps in a synergistic

**Table 2. Evaluation of spray programmes consisting of one tank mixture of azoxystrobin (Ortiva), copper oxychloride (Fynam) and mineral spray oil and another of benomyl (Benlate) or carben-dazim (Bavistin) and mancozeb (Sancozeb) and mineral spray oil during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Friedenheim Estates and Crocodile Valley Citrus Co. during 2004 and 2005.**

<table>
<thead>
<tr>
<th>Treatments (A+B; C+D) or (C+D; A+B)</th>
<th>Rate / 100 litres water</th>
<th>Friedenheim Estates, Nelspruit</th>
<th>Crocodile Valley Citrus Co., Nelspruit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Clean exportable fruit</td>
<td>% Fruit with 1-3 CBS lesions</td>
</tr>
<tr>
<td>Azoxystrin + copper oxychloride + oil; Carben-dazim + mancozeb + oil</td>
<td>20ml + 150g + 0.25%</td>
<td>98.33a</td>
<td>1.67a</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>200g</td>
<td>95.33a</td>
<td>3.0a</td>
</tr>
<tr>
<td>Azoxystrin + copper oxychloride + oil; Benomyl + mancozeb + oil</td>
<td>20ml + 150g + 0.25% 50g + 150g + 0.25%</td>
<td>94.67a</td>
<td>3.0a</td>
</tr>
<tr>
<td>Benomy + mancozeb + oil; Azoxystrin + copper oxychloride + oil</td>
<td>10g + 150g + 0.25% 50g + 150g + 0.25%</td>
<td>94.0a</td>
<td>3.33a</td>
</tr>
<tr>
<td>Carben-dazim + mancozeb + oil; Azoxystrin + copper oxychloride + oil</td>
<td>55ml + 150g + 0.25% 20ml + 150g + 0.25%</td>
<td>91.67a</td>
<td>3.67a</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>31.67b</td>
<td>11.67b</td>
</tr>
</tbody>
</table>

*W Means in a column, based on 3 replicates, followed by the same letter are not significantly different (P > 0.05) according to Fisher's least significant difference test.

*A Means in a column, based on 5 replicates, followed by the same letter are not significantly different (P > 0.05) according to Fisher's least significant difference test.

Spray dates were 15 October 2003, 12 November 2003, 10 December 2003, 7 January 2004 for mancozeb alone and 12 November 2003 and 7 January 2004 for the other treatments sprayed in a tank mixture.

Spray dates were 14 October 2003, 10 November 2003, 8 December 2003, 5 January 2004 for mancozeb alone and 10 November 2003 and 5 January 2004 for the other treatments sprayed in a tank mixture.*
way with the strobilurins. The addition of copper hydroxide or copper oxychloride to a tank mixture of either trifloxystrobin or azoxystrobin and mineral spray oil, showed no phytotoxic symptoms like stippling and could play a role in the extended protection period required and could be considered as an alternative to mancozeb in such treatment mixtures. Therefore, the alternation of strobilurins with benzimidazoles is not only effective against CBS, but can also result in less residues on fruit. The sequence of spraying will depend on the outcome of a carbendazim residue decline study (study number 05/1119) on citrus conducted by CRI and the SABS for support of an EU import tolerance. If the residue trials indicate that a single carbendazim spray does not result in residues in excess of the revised EU import tolerance, then the alternation of strobilurins with benzimidazoles + mancozeb + oil; strobilurin + copper fungicide + oil sequence (A+B; C+D) would be suitable for CBS control.

OPSSOMMING
Afwisselende spuitprogramme bestaande uit tenkmengsels met 'n benzimidazool, mancozeb en olie in Novembermaand opgevolg met 'n strobilurine, koper en olie (A+B; C+D volgorde) in Januarie maand, toon groot potensiaal vir die beheer van swartvlekselfs in sitrusboorde waar die benzimidazool weerstandbiedende sitrusswartvlekpopdasie tussen 87% (2003/2004 seisoen) en 63-66% (2004/2005 seisoen) beslaan. Die toekomstige gebruik van die benzimidazool sal afhang van die uitkoms van die carbendazim residustudie (studienummer 05/1119) wat tans op sitrus in die RSA uitgevoer word deur die CRI en die SABS ter ondersteuning van 'n EU invoertoleransie. Indien die studie toon dat een benzimidazoolbespuiting aanvaarbare lae residu tot gevolg het, sal dit die spuitproggam behels waar die benzimidazool in Novembermaand gespuit word (A+B; C+D volgorde).

REFERENCES CITED