Mancozeb: Banana agriculture & Resistance management

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EU Mancozeb Task Force Lead
Mancozeb – mode of action

**Protectant contact** multi-site fungicides (e.g. mancozeb) will prevent spores from germinating and infecting the plant if applied prior to spore release. Once the infection has occurred and the fungus has penetrated the leaf, these fungicides will no longer control the disease.

Updated 12/03/15
Mancozeb – molecule

\[
\text{Mn}^{2+} \quad \text{Zn}^{2+} \\
\text{mancozeb}
\]

chlorothalonil
Mancozeb – mode of action

MULTI-SITE
mancozeb

Fungal cell

A: Nucleic acid synthesis
A1 metalaxyl-M
benalaxyl-M

B: Mitosis and cell division
B3 zoxamide
B5 fluopicolide

C: Respiration, mitochondria
C3 famoxadone
fenamidone
C4 cyazofamid
amisulfuron
C5 fluazinam
C8 ametoctradin

F: lipid synthesis and membrane integrity
F4 propamocarb

H: cell wall biosynthesis
H5 dimethomorph
mandipropamid
benthiavalicarb
valifenalate

Mancozeb is an ethylene bisdithiocarbamate (EBDC) fungicide, belonging to the group of dithiocarbamates

Source: FRAC Code List 2017
Fungal disease in bananas

• Black Sigatoka, black leaf streak/spot caused by ascomycete *Mycosphaerella fijiensis*

• Pathogen produces conidia and ascospores, both are infective affecting photosynthesis indirectly

• High moisture conditions, dispersed via wind, for conidia, also by rain and irrigation water, yellow in cooler and black in warmer environment

• Untreated, yield can be affected by 35-50%, also shortening period between harvest & ripening - Most commercially important disease for export crop to control
Symptoms for Black Sigatoka
Fungicide control in bananas

- Large plantations reliant on chemical controls by aerial application
- Predominantly mancozeb due to solo or mixture use
- Mancozeb is often combined or rotated with morpholine, demethylation inhibitors (DMI), or strobilurin (QoI) fungicides to prolong the life other chemistries
- Resistance to other fungicides is widespread in many production areas eg: Strobilurins
Mancozeb – Importance for Bananas

• Mancozeb products control the broadest number of diseases in the greatest number of crops of any fungicide
  – Registered on >70 crops for >400 diseases globally
  – Including a large variety of minor uses in most Fruit & Vegetables, flowers, forest trees
  – New areas of development
• Fungus (Oomycetes primarily but not only) and some bacteria
• A cost effective key multi-site fungicide which can be utilized to protect key single site actives
• Mancozeb, as a dithiocarbamate, belongs to the “Low Risk” group under FRAC mixtures recommendations due to a proven history:
  – FRAC Recommendations for fungicide mixtures, 2010 & FRAC List 2017
  – FRAC codes Mode of Action – Mancozeb (M3)
Mancozeb Products

• Vondozeb 75 WG (mancozeb 750g/kg)
• Vondozeb 62 OD (mancozeb 620g/kg)
  • High compatibility with other products
  • Registered for control of black & yellow sigatoka
  • Economic solution to the farmer
  • Effective at low doses (1.5kg/ha solo/0.75 mix)
  • Nutrients like Zinc and Manganese enrich crop
  • Non volatile, short half life – quick degradation
  • Refined production process ensures good rainfastness
Mancozeb – micronutrients

Mancozeb is a complex of zinc and maneb containing 20% Mn and 2.5% Zn (ref. FAO Spec.)

- Boosts bulking
- Increases the yield of tubers
- Improves disease resistance
- Improves skin finish
- Increases tuber dry matter content
- Increases starch levels

Manganese deficiency in potato plant
FRAC Banana Recommendations

“Multi-site fungicides (Mancozeb...and other fungicides of low resistance risk) can be applied for control of black sigatoka in the following way:

Multi site fungicides can be used solo or in mixtures with partners at manufacturers’ recommended effective rates. There are no limitations or restrictions concerning the number of applications, the timing or the sequence as long as it is within the limits of the manufacturers' labels.”

FRAC Banana Group, 2014
FRAC Banana - Research

- Research at Wageningen University, The Netherlands - Home of blight research
  - PromoBanana project looking at Panama Disease and Black Sigatoka for earlier detection to assist growers.
  - UPL collaborated with Wageningen to explore the role of mancozeb in resistance management for potato early and late blight
- Potential for new collaboration for banana research with a view to helping sustainability
Mancozeb — socio economic value of mancozeb

UK
France
Germany
Ireland
Italy
Netherlands
Spain
Greece
Mancozeb — socio economic value of mancozeb

- 90% of advisers were applying mancozeb to 100% of their crop at least once in the season.
- Average number of applications of mancozeb was 7 per season.
- Cost of production estimated to increase between €48 and €160 per ha, reducing the financial viability of potato enterprises.
- Yield losses from uncontrolled blight are reported at up to 40% which is equivalent to 2.2 M tonnes of potatoes.
- Loss of mancozeb will impact on resistant management strategies and increase risk of resistance developing to other active substances.
Mancozeb – socio economic value of mancozeb

EU 8
Cost €87-507M
Reduction in production up to 2,980,000 tonnes

UK
Cost €4-56M
Reduction in production up to 223,000 tonnes

Spain
Cost €3-34M
Reduction in production up to 108,000 tonnes

France
Cost €4-88M
Reduction in production up to 496,000 tonnes

Italy
Cost €0.6-4M
Reduction in production up to 16,000 tonnes

Germany
Cost €62-247M
Reduction in production up to 1,822,000 tonnes

Greece
Cost €6-38M
Reduction in production up to 83,000 tonnes

Ireland
Cost €3-14M
Reduction in production up to 61,000 tonnes

Netherlands
Cost €3-44M
Reduction in production up to 222,000 tonnes
Mancozeb - research

Potatoes
- Wageningen University (Netherlands)

Cereals
- ADAS (UK)
- Rothamsted Research (UK)

Various crops
- University of Bologna (Italy)
Mancozeb - working with Wageningen

- Testing the efficacy of fungicides to control different strains of potato blight.
  - August 2013
  - May 2014

- Testing the efficacy of fungicides to control early blight
- Project conducted through Hub Schepers - Head of Euroblight
- Results presented at Euroblight 2015 by Serge Devauchelle (Retired French ministry potato advisor)
# Mancozeb – resistance (potatoes)

<table>
<thead>
<tr>
<th>Mode of Action</th>
<th>Target site</th>
<th>Group name</th>
<th>Example of common names</th>
<th>Predicted FDR (yrs)</th>
<th>Resistance reported* &amp; strain colour</th>
<th>FRAC code</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>U</td>
<td>cyanoacetamide-oxime</td>
<td>cymoxanil</td>
<td>6.8</td>
<td>No (L to M)</td>
<td>27</td>
</tr>
<tr>
<td>A</td>
<td>A1</td>
<td>PA-fungicides</td>
<td>metalaxyl-M benalaxyl-M</td>
<td>5.9</td>
<td>Yes (H)</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>C4</td>
<td>Qil fungicides</td>
<td>amisulbrom</td>
<td>3.5</td>
<td>No (M to H)</td>
<td>21</td>
</tr>
<tr>
<td>C</td>
<td>C3</td>
<td>Qol-fungicides</td>
<td>famoxadone fenamidone</td>
<td>4.1</td>
<td>No (H)</td>
<td>11</td>
</tr>
<tr>
<td>C</td>
<td>C8</td>
<td>QoSI-fungicides</td>
<td>ametoctradin</td>
<td>7.8</td>
<td>No (M to H)</td>
<td>45</td>
</tr>
<tr>
<td>B</td>
<td>B3</td>
<td>benzamides</td>
<td>zoxamide</td>
<td>6.1</td>
<td>No (L to M)</td>
<td>22</td>
</tr>
<tr>
<td>B</td>
<td>B5</td>
<td></td>
<td>fluopicolide</td>
<td>5.5</td>
<td>No (?)</td>
<td>43</td>
</tr>
<tr>
<td>H</td>
<td>H5</td>
<td>CAA - fungicides</td>
<td>dimethomorph benthiavalicarb mandipropanid</td>
<td>4.2</td>
<td>No (L to M)</td>
<td>40</td>
</tr>
</tbody>
</table>

**SOURCE:** FRAC LIST

* Reported in *P. infestans* (late blight)

**Resistance risk**

FDR = First detection of resistance

? = unknown

H = High, M to H = Medium to High, L to M = Low to Medium
# Mancozeb - working with Wageningen

<table>
<thead>
<tr>
<th>Strains</th>
<th>Background</th>
<th>Trial detached leaf</th>
<th>Trial pot in greenhouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue 13 (13-A2)</td>
<td>Detected in NL and DE in 2004, in UK and FR in 2005, in Ireland in 2007, also present in many other EU countries since 2012. Strain more aggressive at low temperatures (8°C), resistant to phenylamides</td>
<td>Tested</td>
<td>Tested</td>
</tr>
<tr>
<td>Green 33 (33_A2)</td>
<td>Detected in NL in 2011, also present in BE and PL. Fluazinam is less efficacious on this strain. Expresses a weak fitness, therefore regresses when fluazinam solo is not any more used in a systematic way. Represented more than 20% of the strains in 2010 and 2011 but only 6% in 2012</td>
<td>Tested</td>
<td>Tested</td>
</tr>
<tr>
<td>Pink 6 (6_A1)</td>
<td>Detected in NL in 2002, in UK and FR in 2004. Seems very aggressive on leaf at temperatures around 10°C. Dominant in UK in 2011 and 2012, tends to regress during the last years</td>
<td>Tested</td>
<td>Tested</td>
</tr>
<tr>
<td>Orange</td>
<td>Old strain of A1 type, still present today but in a minority proportion</td>
<td>Not tested</td>
<td>Tested</td>
</tr>
<tr>
<td>Population VK98014</td>
<td>Old strain stored in the lab, supposed to be fully susceptible to fungicides</td>
<td>Tested</td>
<td>Not tested</td>
</tr>
</tbody>
</table>
## Mancozeb - working with Wageningen

<table>
<thead>
<tr>
<th>Type of strain</th>
<th>Untreated</th>
<th>Mancozeb 1500 g/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% necrotic surface 7 days after inoculation</td>
<td>% necrotic surface 7 days after inoculation (efficacy)</td>
</tr>
<tr>
<td>Blue 13</td>
<td>99.8</td>
<td>17.3* (82.7)</td>
</tr>
<tr>
<td>Green 33</td>
<td>96.8</td>
<td>2.5* (97.4)</td>
</tr>
<tr>
<td>Pink 6</td>
<td>95.5</td>
<td>5.0* (94.8)</td>
</tr>
<tr>
<td>VK 98074</td>
<td>91.2</td>
<td>2.5* (97.3)</td>
</tr>
</tbody>
</table>

* values statistically different from untreated at 5% threshold in the 2 trials

Comparatively - Table 4 from August 2013 report:
Metalaxyl gave poor control of Blue 13 proving that it is metalaxyl resistant
Mancozeb - working with Wageningen

Greenhouse trial: protocol

<table>
<thead>
<tr>
<th>Code</th>
<th>Fungicide</th>
<th>Dose rate g ai / ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>water (untreated control)</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>mancozeb 75% WG</td>
<td>1500</td>
</tr>
<tr>
<td>C</td>
<td>mancozeb 64% + metalaxyl-M 8% WG</td>
<td>1600 + 200</td>
</tr>
<tr>
<td>D</td>
<td>mancozeb 75% WG + fluazinam 500g/L SC</td>
<td>1500 + 200</td>
</tr>
<tr>
<td>E</td>
<td>metalaxyl-M 25% WG</td>
<td>200</td>
</tr>
<tr>
<td>F</td>
<td>fluazinam 500g/L SC</td>
<td>200</td>
</tr>
</tbody>
</table>

Percentage necrotic foliage assessed 7, 9, 10, 13 and 15 days after inoculation. A Standard Area Under Disease Progress Curve (stAUDPC) was calculated.

4 replicates (1 replicate = 1 potato plant)

Analysis of variance based on the percentage of necrotic foliage

Rates comparable to those used for Bananas
Mancozeb - working with Wageningen

Greenhouse trial: results (2/2)

All strains controlled better where mancozeb is included
Mancozeb - working with Wageningen

- Mancozeb is effective against each type of strain.
- Mancozeb strengthens significantly the efficacy of fluazinam and metalaxyl-m, even on strains sensitive to these fungicides.
  - Stability of performance ensured whatever the type of strains met in the field.
  - Essential for resistance management program.
Mancozeb - working with Wageningen

- Early blight (*Alternaria solani*)
- Global disease
- Yield penalties of 30% plus

UPL commissioned Wageningen University to study the efficacy of mancozeb against *Alternaria* (early blight), especially against strains resistant to strobilurins – ongoing

- mancozeb, azoxystrobin and boscalid + pyraclostrobin
- Similar results found – mancozeb controlled all strains where included
Mancozeb – working with Rothamsted

Dr Bart Fraaije (FRAG UK)

*In vitro* sensitivity testing of *Zymoseptoria tritici* field strains and mutants with resistant phenotypes to site-specific fungicides used for Septoria leaf blotch control
Mancozeb – working with Rothamsted

Objective

- To test the efficacy and cross resistance patterns between a selection of fungicides on *Z. tritici* field strains (13) and lab strains (3)
- Fungicides tested were:
  - Epoxiconazole, azoxystrobin, bixafen and multi-sites
- Approximately 2500 spores of *Z. tritici* added to each well which was filled with appropriate fungicide. Plates were incubated for four days and then measured
- Growth measured and reported as EC$_{50}$
# Mancozeb – working with Rothamsted

Completed March 2014

<table>
<thead>
<tr>
<th>Mancozeb</th>
<th>EC₅₀ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ip6</td>
<td>0.01</td>
</tr>
<tr>
<td>G303</td>
<td>0.1</td>
</tr>
<tr>
<td>AF13UR1.1</td>
<td>1</td>
</tr>
<tr>
<td>MM20</td>
<td>10</td>
</tr>
<tr>
<td>F011.01</td>
<td>100</td>
</tr>
<tr>
<td>MIA2</td>
<td>0.001</td>
</tr>
<tr>
<td>MS12/07/08</td>
<td>0.01</td>
</tr>
<tr>
<td>JRE30</td>
<td>0.01</td>
</tr>
<tr>
<td>NZ31.1</td>
<td>0.01</td>
</tr>
<tr>
<td>POL27</td>
<td>0.01</td>
</tr>
<tr>
<td>C10.5</td>
<td>0.01</td>
</tr>
<tr>
<td>MM24</td>
<td>0.01</td>
</tr>
<tr>
<td>IPO23</td>
<td>0.01</td>
</tr>
<tr>
<td>RD13</td>
<td>0.01</td>
</tr>
<tr>
<td>441(R12)</td>
<td>0.01</td>
</tr>
<tr>
<td>TAG743</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Azoxystrobin</th>
<th>EC₅₀ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM24</td>
<td>0.001</td>
</tr>
<tr>
<td>MM20</td>
<td>0.01</td>
</tr>
<tr>
<td>NZ31.1</td>
<td>0.01</td>
</tr>
<tr>
<td>F011.01</td>
<td>0.01</td>
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<tr>
<td>C10.5</td>
<td>0.01</td>
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<td>JRE30</td>
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<td>MS12/07/08</td>
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<td>G303</td>
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<td>POL27</td>
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<tr>
<td>441(R12)</td>
<td>0.01</td>
</tr>
<tr>
<td>TAG743</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Consistent level of control where mancozeb is used
Mancozeb – working with Rothamsted

- Resistant mutants (SDHI fungicides) and field strains (MBC, Qol and DMI fungicides) were all well controlled by mancozeb
- *In vitro* susceptibility testing of *Z. tritici* field strains showed no cross resistance between mancozeb and the single-site fungicides epoxiconazole, azoxystrobin, carbendazim and bixafen
- EC₅₀ values were narrow for the multi-sites
- EC₅₀ values were large for the other fungicides
- All multi-site fungicides were able to control strains with high levels of insensitivity to one or more classes of site-specific fungicides
Mancozeb – working with Rothamsted

- The sensitivity of European field strains of *Zymoseptoria tritici* (Septoria tritici) to mancozeb
- Sensitivity of epoxiconazole (triazole) was included in the project
- 11 concentrations of the test products were used to determine EC$_{50}$ values
## Mancozeb – working with Rothamsted

<table>
<thead>
<tr>
<th>Site name</th>
<th>Number of strains tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Mecklenburg</td>
<td>26</td>
</tr>
<tr>
<td>2 Osnabruck</td>
<td>19</td>
</tr>
<tr>
<td>3 Reims</td>
<td>44</td>
</tr>
<tr>
<td>4 Le Mans</td>
<td>29</td>
</tr>
<tr>
<td>5 Rothamsted</td>
<td>53</td>
</tr>
</tbody>
</table>

5 sites across Europe to give a representative spread
**Mancozeb – working with Rothamsted**

Mancozeb sensitivity test results (EC$_{50}$ values, ppm)

- Limited variation in sensitivity within sites, between 0.26 and 2.3 ppm for all strains
- Similar sensitivity distributions between sites
- No evidence for emergence of insensitive strains
Mancozeb – working with Rothamsted

Epoxiconazole sensitivity test results ($EC_{50}$ values, ppm)

- Wide range of sensitivity within sites, between 0.01 and 6.4 ppm for all strains
- Similar sensitivity distributions between sites
- Evidence of resistance shifts and emergence of specific genotypes (e.g. CYP51 variants and strains over-expressing CYP51 and/or efflux pumps)
Mancozeb – working with Rothamsted

Combined sensitivity profiles – a resistant management tool in action

Adding a multi-site inhibitor (mancozeb), with a low resistance risk, as mixing partner to a fungicide that is at-risk of resistance (epoxiconazole) reduces the rate of selection for fungicide resistance
Mancozeb – working with Rothamsted

Shifts in epoxiconazole sensitivity measure for *Zymoseptoria tritici* populations sampled at Rothamsted

Long-term exposure to a fungicide that is at-risk of resistance leads to an increased rate of selection for fungicide resistance
Mancozeb – Working with University of Bologna

Apples
- Apple scab (*Venturia inaequalis*)
- Alternaria (*Alternaria spp.*)

Pears
- Pear brown spot
  (*Stemphylium vesicarium*)

Grapes/vines
- Grape Downy mildew
  (*Plasmopara viticola*)
- Phomopsis Cane and Leaf spot
  (*Phomopsis viticola*)
- Black rot
  (*Guignardia bidwellii*)
Mancozeb – Working with University of Bologna

Apple scab (Venturia inaequalis) – conidial germination

- mancozeb was highly active against two tested isolates analysed, one was sensitive and the other resistant to strobilurins

Pear brown spot (Stemphylium vesicarium) and Alternaria spp. from apple – conidial germination

- Mancozeb was highly selective against the tested isolates

Grape Downy mildew (Plasmopara viticola), Phomopsis Cane and Leaf spot (Phomopsis viticola) and Black rot (Guignardia bidwellii)

- Mancozeb was highly selective against the tested isolates
Petri dishes Assay in order to assess activity of mancozeb @ 6 concentrations (0-10-20-50-100 mg/L) on mycelial growth of *Phomopsis viticola* and *Guignardia bidwellii*

Efficacy following Abbott’s Formula in term of reduction of mycelial growth vs untreated from 6 to 15 days

<table>
<thead>
<tr>
<th>Active</th>
<th>Pathogen Isolate</th>
<th>Concentrations of mancozeb in mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>After 6 days</td>
<td><em>Phomopsis viticola</em></td>
<td>51,4</td>
</tr>
<tr>
<td></td>
<td><em>Guignardia bidwellii</em></td>
<td>18,5</td>
</tr>
<tr>
<td>After 8 days</td>
<td><em>Phomopsis viticola</em></td>
<td>44,0</td>
</tr>
<tr>
<td></td>
<td><em>Guignardia bidwellii</em></td>
<td>4,1</td>
</tr>
<tr>
<td>After 11 days</td>
<td><em>Phomopsis viticola</em></td>
<td>38,3</td>
</tr>
<tr>
<td></td>
<td><em>Guignardia bidwellii</em></td>
<td>1,5</td>
</tr>
<tr>
<td>After 15 days</td>
<td><em>Phomopsis viticola</em></td>
<td>21,2</td>
</tr>
<tr>
<td></td>
<td><em>Guignardia bidwellii</em></td>
<td>1,3</td>
</tr>
</tbody>
</table>
Petri dishes Assay in order to assess activity of mancozeb @ 6 concentrations (0-10-20-50-100 mg/L) on mycelial growth of *Phomopsis viticola* and *Guignardia bidwellii*.

Efficacy following Abbott’s Formula in terms of reduction of mycelial growth vs untreated from 6 to 15 days.
Conclusions and final summary

- Effective and economic option for the farmers toolbox
- No resistance after over 60 yrs
- Highly compatible with other products
- UPL products show good rainfastness
- Quick degradation, safer to environment
- Key in resistance management
- Multisite
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