Cercospora Leaf Spot in sugar beet: spread, crop protection strategies and resistances to fungicides in Serbia

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CLS resistance workshop, Sipcam-Oxon, October 20th, 2015, Milano
TOPICS:

1. Introduction: SB production parameters, damages from CLS, epidemiology, available control measures.

2. Research of *C. beticola* resistance to fungicides in Serbia.


Sugar beet areas and production in Serbia

2005-2013
- 70,000 ha
- 50 t/ha
-16%

PROFIT!

CLS resistance workshop, Sipcam-Oxon, October 20th, 2015, Milano
Economical losses

*Cercospora beticola* Sacc.

2014 – over 25 mio €

- 10%
- 40%
- +20 °C
- +80mm
CLS MONITORING IN SERBIA

1. State extension service
2. Private extension services
3. Plant Protection officers in chemical, sugar production, seed and other companies
4. University-Faculty of Agriculture – science (companies like Sunoko, Hellenic sugar, KWS, STRUBE, Sesvandehave etc co-finance research)
1st spots and conidia – usually mid to end of June

**EPIDEMIOLOGY**

First applications - first decade of July
Based on characteristics of each individual field (cultivar, canopy density, temperatures, rainfall, inoculum – 5% infection is a threshold)
Periods favorable for CLS infection and spread vary from year to year (no irrigation)
Good agricultural practice

INTEGRATED DISEASE MANAGEMENT

Fungicide application

Tolerant sugar beet cultivars
Good agricultural practice

- narrow crop rotation!!!
- excessive nitrogen fertilization
- reduced cultivation (diseased leaves not being properly handled)
-not all cultivars are of the same level of tolerance
-adapt fungicide applications to tolerance

Tolerant sugar beet cultivars
Fungicide application
Availability and use of fungicides that belong to different chemical groups are an important factor in controlling CLS.

Fungicides with specific mode of action possess high risk of resistance development.
2. Cercospora resistance to fungicides in Serbia

A stable, heritable pathogen adaptation that results in a reduced sensitivity to a fungicide (Gallian et al., 2001).

Enables individuals within the population to survive the application of fungicides (Brent & Holloomon, 2007).

- Benzimidazoles (Georgopoulous & Dovas, 1973; Marić et al., 1976),
- Tin containing fungicides (Giannopolitis, 1978; Bugbee, 1996; Campbell et al., 1998),
- DMI fungicides (Balaž et al., 1999; Karaoglanidis et al.; 2000, Budakov et al., 2014)
- Strobilurins (Kirk et al., 2012; Budakov-unpublished data).
Benzimidazoles

- Partially discontinued after the rise during 1970s
- In combinations with DMIs
- Monitoring of resistance (2007-2011) showed that frequency of resistant isolates was over 93% (Budakov et al, 2014; Trkulja et al., 2015).
- Fitness of resistant isolates – very stable even without fungicide selection pressure (Karaoganidis & Ioannidis, 2010).
- On fields with low frequency of resistant isolates can be used in management of *C. beticola* resistance to DMIs or Qols.
Restriction of β-tubulin gene with Bsh1236I

- 450 bp + 120 bp
- 250 bp + 200 bp + 120 bp
DMIs

• First changes of sensitivity noted in 1995 (flutriafol).

Polygenic nature of resistance  
+  
a large number of active substances available  
=  
slowly development of resistance

• Monitoring from 2007-2011 showed that frequency of resistant varied up to 42% (Budakov et al, 2014; Trkulja et al., 2015), but level of sensitivity also varied.

• Continuous sensitivity distribution.
Restriction of C-14 α demethylase gene with Alw26I

Flutriafol - DMIs

Tetraconazole - DMIs

Low Range

200 bp

100 bp + 80 bp
QoIs

- Monitoring in 2007 – no changes in sensitivity
- Monitoring in 2014 and 2015 – a large frequency of resistant isolates.

Media amended with azoxy+SHAM
Cercospora beticola (PA-A) on nutrient media amended with azoxystrobin + 0,5mM SHAM.
Field trial in 2011

- Preparation of inoculum
  - Conidia - Sugar Beet Leaf Extract Agar
  - Spore suspension – 500 spores/ml
  - 300 L H₂O/ha
  - Backpack sprayer

Favorable conditions for disease development
<table>
<thead>
<tr>
<th>Isolates</th>
<th>Active substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant to flutriafol &amp; carbendazim</td>
<td>Carbendazim</td>
</tr>
<tr>
<td></td>
<td>Flutriafol</td>
</tr>
<tr>
<td></td>
<td>Tetraconazole</td>
</tr>
<tr>
<td></td>
<td>Azoxystrobin</td>
</tr>
<tr>
<td>Sensitive to flutriafol &amp; carbendazim</td>
<td>Untreated control</td>
</tr>
</tbody>
</table>

2011
Sensitivity of *Cercospora beticola* isolates from Serbia to carbendazim and flutriafol

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c The Amalgamated Sugar Company, 1951 S. Saturn Way, Suite 100, Boise, ID 83709, USA
Fungicide trial in 2015:

To evaluate efficacy of different fungicides alone and in combination with multisite chlorothalonil in control of Cercospora population of known sensitivity to fungicides. Effect on disease severity and sugar content.
Detection of sensitivity of *C. beticola* before fungicide applications.

**DISCRIMINATIVE CONCENTRATIONS**

- Carbendazim 5 μg/mL
- Tetraconazole 0.6 μg/mL
- Azoxystrobin 0.1 μg/mL

Cca. 900 isolates per fungicide
## Field trial treatments

<table>
<thead>
<tr>
<th>No</th>
<th>Active ingredient</th>
<th>Fungicide</th>
<th>Dose per ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Azoxystrobin (250 g/L)</td>
<td>Azbany 250SC</td>
<td>1L</td>
</tr>
<tr>
<td>2</td>
<td>Carbendazim (500 g/L)</td>
<td>Galofungin</td>
<td>0.6L</td>
</tr>
<tr>
<td>3</td>
<td>Tetraconazole (125 g/L)</td>
<td>Eminent 125ME</td>
<td>0.8L</td>
</tr>
<tr>
<td>4</td>
<td>Flutriafol (125 g/L)</td>
<td>Takt</td>
<td>0.5L</td>
</tr>
<tr>
<td>5</td>
<td>Azoxystrobin (250 g/L) + Chlorothalonil (500 g/L)</td>
<td>Azbany 250SC+ Bevetikola</td>
<td>1L+ 0.75L</td>
</tr>
<tr>
<td>6</td>
<td>Carbendazim (500 g/L)+ Chlorothalonil (500 g/L)</td>
<td>Galofungin+ Bevetikola</td>
<td>0.6L+ 0.75L</td>
</tr>
<tr>
<td>7</td>
<td>Tetraconazole (125 g/L)+ Chlorothalonil (500 g/L)</td>
<td>Eminent 125ME+ Bevetikola</td>
<td>0.8L+ 0.75L</td>
</tr>
<tr>
<td>8</td>
<td>Flutriafol (125 g/L)+ Chlorothalonil (500 g/L)</td>
<td>Takt+ Bevetikola</td>
<td>0.5L+ 0.75L</td>
</tr>
<tr>
<td>9</td>
<td><strong>FUNGICIDE ROTATION:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Untreated control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**1.** Difenoconazole (150 g/L) + Propiconazole (150 g/L) + Chlorothalonil (500 g/L)  
Rias 300EC + Bevetikola  
0.3L + 0.75L

**2.** Azoxystrobin (200 g/L) + Ciproconazole (80 g/L) + Chlorothalonil (500 g/L)  
Amistar Extra + Bevetikola  
0.75L + 0.75L

**3.** Tetraconazole (125 g/L) + Chlorothalonil (500 g/L)  
Eminent 125ME + Bevetikola  
0.8L + 0.75L

**4.** Trifloxystrobin (375 g/L) + Ciproconazole (160 g/L) + Chlorothalonil (500 g/L)  
Sphere + Bevetikola  
0.35L + 0.75L
RESULTS

Frequency of sensitive and resistant isolates

- Carbendazim: 67.2% sensitive, 32.8% resistant
- Tetraconazole: 74.8% sensitive, 25.2% resistant
- Azoxystrobin: 71.4% sensitive, 28.6% resistant
Disease intensity (AUDPC) and sugar content (%)
Azoxystrobin alone

Fungicide rotation
Availability and use of fungicides with different mode of action.

**Always use fungicides in combinations.**

**Use of multisite protective fungicides.**

**FRAC – Fungicide Resistance Action Committee**
Conclusions

Isolates that showed resistance to flutriafol and carbendazim in in vitro experiments, were successfully controlled in field trials with azoxystrobin and tetraconazole, which provided a high yield and sugar content.

Carbendazim and flutriafol were not sufficiently effective in control of isolates with corresponding resistance. These two fungicides were as successful as others in disease control caused by sensitive isolates.
Importance of monitoring changes in the sensitivity of *Cercospora beticola* populations to the fungicides used in our sugar beet growing area.

Make a selection of fungicides according to the frequency of resistant isolates.

Prevalence of resistant individuals in the field population can lead to significant losses in sugar beet yield and sugar content, but also to major economic losses due to fungicide application in which lacks the necessary biological efficacy.
Thank you for your attention.

Questions?