Inter- and intraspecific diversity of *Monilinia* spp. causing brown rot of sour and sweet cherry fruits in Poland

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Sustainable production of high quality cherries for the European market
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• Poland is one of the leading producer of cherry fruits in the world
• Brown rot is a common and destructive disease of stone fruits
• Typical brown rot symptoms include blossom and twig blight, cankers and fruit rot
Monilinia spp. causing brown rot of stone fruits in Europe

M. laxa (Aderhold and Ruhland) and M. fructicola (Winter)
– infects blossoms, twigs and fruits

M. fructigena (Honey) and M. polystroma (van Leeuwen) – infects mainly fruits, can cause blossom and twig blight

M. fructicola and M. polystroma – were detected on pome and stone fruits during last few years in several countries in Central and Western Europe
**Aim of the study:**
Identification and characterization of causal agent of brown rot on cherries

**Material:**
2012-2013: 26 orchards were monitored and brown rot symptomatic fruits were collected

**Identification:**
- **conventional methods**: morphology of pure cultures on PDA medium (not sufficient for clear determination of species affiliation of isolates)
- **molecular diagnostic** with primers: MO368-5, MO368-8R, MO368-10R and R2 (Côté et al. 2004), in order to amplify the non–coding region of *Monilinia* spp. with unknown function
Analysis of genetic diversity using fingerprinting techniques

ISSR, RAPD and PCR MP (Masny and Płucienniczak, 2003) assays were performed for 91 Monilinia spp. isolates from cherries, 25 Monilinia spp. isolates from other host plants and 4 reference isolates of Monilinia spp. from the Centraalbureau voor Schimmelmelcultures (CBS) collection.

On the basis of data obtained with fingerprinting techniques genetic similarity between all pairs of isolates were calculated according to Nei & Li (1979) coefficient and for each technique separately dendrograms were constructed using UPGMA.
RAPD

- Random primers used in this study: OP-A10, OPAR-03, OP-C06, OP-A11 (Operon Technologies)
- *Monilinia* spp. were grouped according to their species classification
- *Monilinia* spp. isolates formed two distinct major clades
- *M. fructigena, M. polystroma* and *M. laxa* clustered together, while *M. fructicola* isolates clustered separately
- Results obtained from the dendrogram showed that isolates were not grouped by geographical origin, host plant and the year of isolation
- Similar results were obtained for PCR MP and 3 ISSR primers
  - indicating *Monilinia* spp. isolates from sweet cherries
  - indicating *Monilinia* spp. isolates from sour cherries
  - indicating *Monilinia* spp. isolates from other hosts
### Fingerprinting assays

<table>
<thead>
<tr>
<th></th>
<th>RAPD x 4</th>
<th>ISSR x 3</th>
<th>PCR MP x 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of species specific clusters</td>
<td>3 + 1</td>
<td>3 + 1</td>
<td>2 + 2</td>
</tr>
<tr>
<td></td>
<td><strong>M. fructigena</strong></td>
<td><strong>M. fructicola</strong></td>
<td><strong>M. fructigena</strong> <strong>M. fructicola</strong></td>
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<tr>
<td></td>
<td><strong>M. polystroma</strong></td>
<td><strong>M. polystroma</strong></td>
<td><strong>M. polystroma</strong> <strong>M. polystroma</strong></td>
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<tr>
<td></td>
<td><strong>M. laxa</strong></td>
<td><strong>M. laxa</strong></td>
<td><strong>M. laxa</strong> <strong>M. laxa</strong></td>
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<tr>
<td>Number of haplo types</td>
<td><strong>33</strong></td>
<td><strong>30</strong></td>
<td><strong>21</strong></td>
</tr>
<tr>
<td><strong>M. fructigena</strong></td>
<td>33</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td><strong>M. laxa</strong></td>
<td>17</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td><strong>M. polystroma</strong></td>
<td>9</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td><strong>M. fructicola</strong></td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Level of genetic similarity (Nei &amp; Li’s Coefficient) from:</td>
<td></td>
<td></td>
<td>0,36</td>
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<tr>
<td></td>
<td>0,23</td>
<td>0,16</td>
<td>0,36</td>
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</table>
Pathogenicity tests

Preparation:

- **26 representative** cherry isolates comprising **four Monilinia spp.**
  tested on mature cherries and other host plants (blueberry, chuckleberry, apple, pear, plum)
- inoculation by 1x10^4/ml conidial suspension or with a plug of actively growing mycelium, incubation at room temperature, 100% of relative humidity
- observations after 7 days
- reisolation of fungi from inoculated fruits and confirmation of identity in order to fulfill Koch’s postulates
<table>
<thead>
<tr>
<th>Pathogenicity on:</th>
<th>M. fructicola isolates from cherries (6)</th>
<th>M. laxa isolates from cherries (8)</th>
<th>M. fructigena isolates from cherries (7)</th>
<th>M. polystroma isolates from cherries (5)</th>
<th>Monilinia spp. isolates from blueberry (2)</th>
<th>Monilinia spp. isolates from chuckleberry (2)</th>
<th>Monilinia spp. isolates from pear (2)</th>
<th>Monilinia spp. isolates from apple (2)</th>
<th>Monilinia spp. isolates from plum (2)</th>
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<tbody>
<tr>
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</table>
Summary

Identification:

- Four *Monilinia* spp.: *M. fructicola*, *M. laxa*, *M. fructigena* and *M. polystroma* were identified as the causal agents of brown rot disease of cherries in Poland.

- *M. polystroma* were isolated and identified as the causal agent of brown rot of cherry fruits. This is to our knowledge the first report of *M. polystroma* on cherry fruits.

Pathogenicity tests:

- Isolates of *Monilinia* spp. obtained from cherries and other host plants were pathogenic to all tested fruits – no host specialization observed.
Fingerprinting assays:

- *Monilinia* spp. isolates were grouped according to their species classification.
- The highest number of different amplification patterns was observed in RAPD technique.
- In all analyses, the highest genetic heterogeneity was found among *M. fructigena* and *M. laxa* isolates, while *M. polystroma* and *M. fructicola* created more homogeneous clusters.
- Based on DNA fingerprinting techniques, *Monilinia* spp. isolates from cherries were intermingled with the *Monilinia* spp. isolates from blueberry, chuckle berry, apple, plum, pear, apricot, and peach.
- Isolates were not grouped by geographical origin and the year of isolation.
Acknowledgements

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Thank you for your attention