Bitter rot of cherries in Poland – genetic diversity of its causal agent *Colletotrichum acutatum*

Monika Michalecka, Joanna Puławska
Research Institute of Horticulture, Skierniewice, Poland

Sustainable production of high quality cherries for the European market
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Bordeaux
Colletotrichum spp.

- Fruits: **anthracnose** (eg. strawberry, mango) or **bitter rot** (eg. sour cherry, sweet cherry, apple, pear)
- On sour cherry – common severe problem
- On sweet cherry – less disease severity

- Causal agent of bitter rot on many plants (eg. strawberry, tropical fruits, sour cherry): **Colletotrichum gloeosporioides** (Penz.) Penz. and Sacc.
- Further analysis using molecular tools: **Colletotrichum acutatum** (Simmonds ex. Simmonds) (Gunnell and Gubler, 1992; Agostini et al., 1992; Brown et al., 1996; Sreenivasaprasad and Talhinas, 2005)

**In POLAND:**
- wide dissemination of pathogenic **Colletotrichum** spp. on various small fruits and fruit trees
- severe damage and losses in the crop yield
- On sour and sweet cherries – the causal agent needs reclassification
• **Aim of the study:**

Identification and characterisation of causal agent of bitter rot on cherries
Material:

- 2012-2013: 29 orchards were monitored and diseased fruits were collected
- Obtained isolates: 93 - sour cherry and 18 – sweet cherry
  + 10 isolates from pear, apple, strawberry, quince, cranberry
  + 1- *C. gloeosporioides*, 1 - *C. coccodes*

TOTAL: 123 isolates

Identification:
- conventional methods: pure cultures on PDA (not sufficient for clear determination of species affiliation of isolates)
- molecular diagnostic with primers specific to ITS rDNA region: ITS4 (White et al., 1990) and CaInt2 (Sreenivasaprasad et al. 1996)

Results:

ONLY *C. acutatum* on sour & sweet cherries, and also on other hosts

Remark:

*C. acutatum* species complex (Damm et al., 2012)
Analysis of genetic diversity using fingerprinting techniques

- **ISSR, RAPD** and **PCR MP** (Masny and Płucienniczak, 2003) assays were performed

- on the basis of scored amplification bands - for each technique separately - dendrograms were constructed, using formula of Nei & Li (1979) and clustering by UPGMA
RAPD

- Random primers:
- OPT-07, OPC-05, OP-U19
  (Operon Technologies)

Similar results were obtained for PCR MP assay and 4 ISSR primers
Fingerprinting assays

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<thead>
<tr>
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<th>RAPD x 3</th>
<th>ISSR x 4</th>
<th>MP PCR x 2</th>
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<tbody>
<tr>
<td>Number of haplotypes of <em>C. acutatum</em></td>
<td>50</td>
<td>67</td>
<td>65</td>
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<td>Genetic distance between cherry isolates</td>
<td>70%</td>
<td>50%</td>
<td>52%</td>
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Pathogenicity tests on fruits & plants

• If isolates from cherries are pathogenic for strawberry, quince, apple, pear or cranberry?
• If isolates from other hosts are pathogenic for cherries?
• Preparation:
  – Inoculation: $1 \times 10^6$/ml conidial suspension, incubation at room temperature, 100% of relative humidity
  – Observations: 3-6th day on fruits, 3-4th week on plants
✓ from diseased fruits fungi were isolated and their identity was confirmed in order to fulfill Koch’s postulates
On sweet cherry

Isolates from:

- cranberry
- strawberry
- quince
- pear
- apple
- sour & sweet cherry

Results:
- All isolates from other hosts caused symptoms on sweet cherry fruits (3rd day)
On sour cherry

Isolates from:

apple  pear  cranberry  strawberry  quince  sour & sweet cherry

Results:

• Isolates from other hosts caused symptoms on sour cherry fruits (3rd day)
Strawberry

Injection of 0.1 ml of inoculum

Isolates from: sour cherry and sweet cherry

Results:
• Isolates from cherries caused anthracnose symptoms on strawberry fruits (5th day)
• On whole plants: symptoms only in the injection site
On pears

Results:
- Isolates from cherries caused anthracnose symptoms on pear fruits (4th day)
On apples

Results:
- Isolates from cherries caused anthracnose symptoms on apple fruits (6th day)
Summary

• **Identification:**
  – isolates belonging to *C. acutatum species complex* on sour & sweet cherry, and also on other hosts

• **Fingerprinting assays:**
  – isolates of *C. acutatum* were grouped by culture – color and host plant
  – for isolates from sour & sweet cherry: no relation between obtained amplification patterns and geographical origin or host plant was observed
  – other *Colletotrichum* species – clearly separated

• **Pathogenicity tests:**
  - No pathogenic specialization was observed among isolates from cherries and strawberry, apple and pear
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Thank you for your attention