Sensitivity to fungicides and mating types of *Phytophthora infestans* populations collected in North-Eastern and Central Italy

Galletti B., Collina M., Bugiani R., D’Ascenzo D., Salvatore C., Landi L., Romanazzi G., Brunelli A.
A short overview

Not many studies had been carried out in Italy about sensitivity to fungicides and determination of mating types of *P. infestans* until the years 1995 and 1996 when the first important data on these aspects were obtained by Cristinzio & Testa - University of Naples.

Among the reasons of this situation is the medium-low infective pressure that normally characterizes the Italian tomato and potato crops during the traditional crop cycles (from April to September) because of the hot and dry climatic conditions typical in the majority of cultivated areas.

Some rainy Springs (especially between 1999 and 2002) unexpectedly increased the pressure of the disease in the North of Italy.

Our studies started in 2002 to clarify this new situation that could be also linked to a decrease of fungicide activities and/or to a spread of A2 mating type.
**ORIGIN OF *P. INFESTANS* POPULATIONS**

Seventy four *P. infestans* populations were collected from potato and tomato crops and analysed for their mating types and sensitivity to fungicides since 2002.

![Map of Italy highlighting regions](image)

<table>
<thead>
<tr>
<th>Region</th>
<th>Nr of populations from potato fields</th>
<th>from tomato fields</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lombardia</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Emilia Romagna</td>
<td>11</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>Marche</td>
<td>1</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Abruzzo</td>
<td>4</td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>16</strong></td>
<td><strong>58</strong></td>
<td></td>
</tr>
</tbody>
</table>
Materials and methods

MATING TYPE

Each unknown isolates was paired with two isolates of known A1 and A2 mating types in petri dishes containing 10% V8 medium.

After 10 days the cultures were observed under microscope to check the probably presence of oospores in the area where the mycelia crossed.
Materials and methods

**SENSITIVITY TO FUNGICIDES**

V8 medium amended with several fungicides at different concentrations; tests were carried out using technical grade of:

- **METALAXYL**
  
  0.001/0.005/0.01/0.05/0.1/1/5/10 mgL⁻¹

- **CYMOXANIL**
  
  0.5/0.75/1/1.5/2 mgL⁻¹

- **DIMETHOMORPH**
  
  0.1/0.15/0.2/0.25/0.5/1 mgL⁻¹

- **AZOXYSTROBIN**
  
  0.05/0.75/0.1/0.5/1 mgL⁻¹

- **ZOXAMIDE**
  
  0.025/0.05/0.1/0.25/0.5/1 mgL⁻¹

- **IPROVALICARB**
  
  0.1/0.15/0.2/0.3/0.4/0.5/1 mgL⁻¹

The mycelial growth was evaluated after 7 days of incubation at 20°C and 12 hours of photoperiod, measuring and averaging the diameters of colonies. EC₅₀ were determined by probits analysis for each compound.
Materials and methods

SENSITIVITY TO FUNGICIDES:

ONLY FOR METALAXYL

All the populations taken from Abruzzo and 5 taken from Marche were tested comparing radial mycelial growth between amended and control colonies at 0.1/1.5/10 mgL\(^{-1}\)

according to the formula suggested by Shattock (1988):

\[
\text{% growth} = \frac{\text{avg diam (- 9 mm) on V8 + metalaxyl}}{\text{avg diam (- 9 mm) on V8}} \times 100
\]

isolates were then classified as

**Metalaxyl Resistant (MR)**

% growth > 60%

**Metalaxyl Intermediate (MI)**

% growth 10-60%

**Metalaxyl Sensitive (MS)**

% growth < 10%
**Results**

**MATING TYPE**

<table>
<thead>
<tr>
<th>MATING TYPE</th>
<th>from tomato (Nr of isolates)</th>
<th>from potato (Nr of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td>A2</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>A1A2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**A2 populations:**

- On Tomato about the 45%
- On Potato about the 38%
## Results and Conclusions

### Mating Type

<table>
<thead>
<tr>
<th>Region</th>
<th>Nr of populations from potato fields</th>
<th>from tomato fields</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
</tr>
<tr>
<td>Lombardia</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Emilia-Romagna</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Marche</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Abruzzo</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

- The first occurrence of A2 in Italy was in 1996 by Cristinzio and Testa (University of Naples)

- Self fertility *in vitro* seems to be affected by genetic and external factors such as medium composition, pH, etc.
Results

SENSITIVITY TO METALAXYL

$0.001 \leq EC_{50} \leq 0.3 \text{ mg/l}$

Referring values: $0.005 \leq EC_{50} \leq 0.6 \text{ mg/l}$

(Deahl et al., 1993)
Results

SENSITIVITY TO DIMETHOMORPH

$0.008 \leq \text{EC}_{50} \leq 0.5 \text{ mg/l}$

Referring values: $0.13 \leq \text{EC}_{50} \leq 0.80 \text{ mg/l}$

(Stein and Kirk, 2003)
Results

SENSITIVITY TO AZOXYSTROBIN

$0.004 \leq EC_{50} \leq 0.18 \text{ mg/l}$

Referring values: $EC_{95} \leq 1 \text{ mg/l}$ (from \textit{in vivo} tests)

(Godwin \textit{et. al.}, 1992)
Results

SENSITIVITY TO IPOVALICARB

$0.07 \leq \text{EC}_{50} \leq 0.28 \text{ mg/l}$

Referring values: $0.03 \leq \text{EC}_{50} \leq 0.26 \text{ mg/l}$

(Stenzel et al., 1998)
Results

SENSITIVITY TO ZOXAMIDE

\[0.01 \leq EC_{50} \leq 0.08 \text{ mg/l}\]

Referring values: \[0.025 \leq EC_{50} \leq 0.09 \text{ mg/l}\]

(Cooke et al., 2002)
Results

SENSITIVITY TO CYMOXANIL

$0.03 \leq \text{EC}_{50} \leq 2.48 \text{ mg/l}$

Referring values: $0.06 \leq \text{EC}_{50} \leq 1.48 \text{ mg/l}$

(Ronald and Power, 1998)
Results and Conclusions

SENSITIVITY TO FUNGICIDES

✓ All *P. infestans* populations were sensitive to tested fungicides.

✓ The presence of mating type A2 in North-Eastern and Central Italy did not influence sensitivity of *P. infestans* populations to fungicides commonly used in disease control.

Even if *P. infestans* populations collected from 2002 to 2006 did not show resistance to fungicides used in management control of potato and tomato Late Blight.

Isolates with low sensitivity to metalaxyl were found in Central and Southern Italy in 1995.
Results and Conclusions

SENSITIVITY TO FUNGICIDES

in 1995 and 1996, Cristinzio and Testa sampled 149 populations of *P. infestans* from Marche, Abruzzo, Molise, Campania, Puglia, Basilicata, Sicilia and Sardegna regions. Isolates were characterized by mating type, sensitivity to metalaxyl and dimethomorph

Results showed the presence of 82% of isolates with an Intermediate Metalaxyl resistance and 5% of Metalaxyl Resistant isolates

Our monitoring in North-Eastern and Central Italy can not exclude the presence of resistance to metalaxyl or to other fungicides applied in disease control therefore

particular care both in disease management and laboratory assessments are necessary to avoid or limit the risk of reduced activity of fungicides
Thank you for attention