Evolution of the population of Phytophthora infestans in France

Epidemiologic and phenotypic markers

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The evolution of *Phytophthora infestans* in France
(Mating type, Metalaxyl resistance)

Every year, the laboratory of Loos en Gohelle realises phenotypic characterizations of the strains of *Phytophthora infestans*

Since 1993, more than 2000 samples have been analysed to determine the mating type and fungicide resistance, particularly mefenoxam (metalaxyl).
Method of sampling

-Bio vigilance” survey is programmed : with protocols for sampling and analyses :
The samples : the technician takes lot of necroses in the fields or dumps (more than 15 necroses by field).
TEST RESISTANCE TO MEFENOXAM

- Method described by the FRAC (Fungicide Resistance Action Comitee) using the floating leaf disc method
  - 3 doses: 0.1-10 et 100 ppm
  - 2 replications for each concentration
  - Each disc is inoculated with a droplet of the tested isolate inoculum
  - Incubation at 16°C with photoperiod 16h/8h during 7 days
  - Graphically determination of the Ec₅₀

   \[ Ec_{50} \leq 0.1 \text{ ppm} \quad \rightarrow \quad \text{sensitive isolate} \]
   \[ 0.1 < Ec_{50} \leq 10 \text{ ppm} \quad \rightarrow \quad \text{intermediate isolate} \]
   \[ Ec_{50} > 10 \text{ ppm} \quad \rightarrow \quad \text{resistant isolate} \]
DETERMINATION OF MATING TYPE

Mycelium plugs from the strain to be tested are confronted with reference stains A1 and A2 on agar medium.

Incubation at 16°C during 10 days in darkness.

Observation under microscope at the level of the confrontation line to detect the possible presence of oospores.

The oospores formation requires the two opposite mating types.
## Monitoring Before 2003 in North of France

(1) A2 Monitoring in 1997

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of samples</th>
<th>A1</th>
<th>A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>86</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>1994</td>
<td>35</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>62</td>
<td>60</td>
<td>2 (1 on potato and 1 on tomato in garden)</td>
</tr>
<tr>
<td>1997</td>
<td>93</td>
<td>82</td>
<td>11 (1)</td>
</tr>
<tr>
<td>1998</td>
<td>165</td>
<td>164</td>
<td>1 (on tomato in garden)</td>
</tr>
<tr>
<td>1999</td>
<td>151</td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>78</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>52</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of observations</th>
<th>Samples</th>
<th>Mating type</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/06/1997</td>
<td>Potato / field</td>
<td>A2</td>
</tr>
<tr>
<td>6/07/1997</td>
<td>Tomato / garden</td>
<td>A2</td>
</tr>
<tr>
<td>7/07/1997</td>
<td>Potato / garden</td>
<td>A2</td>
</tr>
<tr>
<td>7/07/1997</td>
<td>Potato / garden</td>
<td>A2</td>
</tr>
<tr>
<td>6/07/1997</td>
<td>Tomato / garden</td>
<td>A2</td>
</tr>
<tr>
<td>10/07/1997</td>
<td>Potato / garden</td>
<td>A2</td>
</tr>
<tr>
<td>15/07/1997</td>
<td>Tomato / field</td>
<td>A2</td>
</tr>
<tr>
<td>16/07/1997</td>
<td>Potato / garden</td>
<td>A2</td>
</tr>
<tr>
<td>16/07/1997</td>
<td>Volunteers</td>
<td>A2</td>
</tr>
<tr>
<td>21/07/1997</td>
<td>Tomato / garden</td>
<td>A2</td>
</tr>
<tr>
<td>24/07/1997</td>
<td>Tomato / garden</td>
<td>A2</td>
</tr>
</tbody>
</table>
### « North » of France 2006

<table>
<thead>
<tr>
<th></th>
<th>A1</th>
<th>A2</th>
<th>A1/A2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Field</strong></td>
<td>48 (32 fields, 5 with A1 and A2)</td>
<td>151 (109 fields, 5 with A1 and A2)</td>
<td>1 (1 field)</td>
</tr>
<tr>
<td><strong>Garden tomato</strong></td>
<td>1</td>
<td>5 (1 garden)</td>
<td></td>
</tr>
<tr>
<td><strong>Garden potatoes</strong></td>
<td></td>
<td>6 (2 gardens)</td>
<td></td>
</tr>
<tr>
<td><strong>Dumps</strong></td>
<td>14 (12 dumps, 6 with A1 and A2)</td>
<td>24 (12 dumps, 6 with A1 and A2)</td>
<td>2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>63</td>
<td>186</td>
<td>3</td>
</tr>
</tbody>
</table>

➤ 252 samples, analysed for the part « North » of France

74 % strains are A2 (79% fields with A2)
### « North » of France

#### 2007

<table>
<thead>
<tr>
<th></th>
<th>A1</th>
<th>A2</th>
<th>A1/A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field</td>
<td>30 (17%)</td>
<td>81 (73%)</td>
<td>0</td>
</tr>
<tr>
<td>Garden</td>
<td></td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Dumps</td>
<td>5 (18%)</td>
<td>23 (82%)</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>35 (24%)</strong></td>
<td><strong>110 (76%)</strong></td>
<td><strong>1</strong></td>
</tr>
</tbody>
</table>

- 146 samples analysed for the part « North » of France
- 76 % strains are A2 (73% fields with A2)
MATING TYPE 2003-2007

(Fields: crops and volunteers, dumps, gardens)

North Pas de Calais, Picardy, Champagne Ardenne, Normandy
MATING TYPE

OTHERS REGIONS IN FRANCE

2005 - 2007

N : Number of analysed samples

Centre ➞ 2007 : 100% A2 ( N=10)
Fig. 2. % A2 by region in 2006 ( ), 2007 ___

1. Nord-Pas-de-Calais
2. Haute-Normandie
3. Basse-Normandie
4. Haute-Normandie
5. Picardie
6. Champagne-Ardenne
7. Lorraine
8. Alsace
9. Bretagne
10. Pouilou-Limousin-Charentes
11. Poitou-Charentes
12. Auvergne
13. Bourgogne
14. Franche-Comté
15. Languedoc-Roussillon
16. Midi-Pyrénées
17. Provence-Alpes-Côte d'Azur
18. Corse

(74) 76
(58) 79
(25) 70
Metalaxyl resistance : 2006

Situation in North of France (North Pas de Calais, Champagne Ardenne, Picardy, Normandy)

<table>
<thead>
<tr>
<th></th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Résistant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fields</td>
<td>33 (20 fields)</td>
<td>14 (12 fields)</td>
<td>137 (97 fields)</td>
<td>184 (129 fields)</td>
</tr>
<tr>
<td>Dumps</td>
<td>19 (12 dumps)</td>
<td>2</td>
<td>17 (8 dumps)</td>
<td>38 (22 dumps)</td>
</tr>
<tr>
<td>Garden potatoes</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Garden tomato</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>17</td>
<td>156</td>
<td>231</td>
</tr>
</tbody>
</table>

➢ Total ≈ 67.5 % resistant strains

On dumps : 45 % resistant strains

Fields : 74 % fields with resistant strains
Metalaxyl resistance : 2007

Situation in North of France (North Pas de Calais, Champagne Ardenne, Picardy, Normandy)

<table>
<thead>
<tr>
<th></th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>field</td>
<td>32 (25)</td>
<td>5 (4)</td>
<td>76 (60)</td>
<td>113 (89)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dumps</td>
<td>25 (17)</td>
<td>2</td>
<td>14 (11)</td>
<td>41 (29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>volunteers</td>
<td></td>
<td></td>
<td>4 (3)</td>
<td>4</td>
</tr>
<tr>
<td>Garden tomato</td>
<td>1</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>7</td>
<td>95</td>
<td>160</td>
</tr>
</tbody>
</table>

- Total ≈ 60% resistant strains
- on dumps :34 % resistant strains
- on field :67% fields with resistant strains
MEFENOXAM RESISTANCE
(Fields: crops and volunteers, dumps, gardens)
(North Pas-de-Calais, Picardy, Champagne Ardenne, Normandy)
2004 à 2007

N = 70
65% fields with resistance

N = 185
37% fields with resistance

N = 231
74% fields with resistance

N = 160
67% fields with resistance

S: sensitive
I: intermediate
R: resistant

% samples

2004 2005 2006 2007
0 10 20 30 40 50 60 70 80 90 100

N: Number of analysed samples
MEFENOXAM RESISTANCE ON DUMPS
(North Pas-de-Calais, Picardy, Champagne Ardenne, Normandy)
2004 à 2007

% samples

- Sensitive
- Intermediate
- Resistant

POTATO
RESULTS MONITORING MEFENOXAM
(fields)

(Others regions)
2005 - 2007

POTATO

BRETAGNE
N = 26

2005 84 53 15
2006 N = 43
2007 N = 40

AQUITAINE
N = 10

2005 50 15
2006 N = 55
2007 N = 8

ALSACE
N = 29

2005 24 29
2006 N = 22
2007 N = 12

% samples

sensible
intermédiaire
résistant

N = 26
N = 10
N = 15
N = 40
N = 8
N = 22
N = 29

2005 2006 2007
2005 2006 2007
2005 2006 2007
Others tested fungicides

<table>
<thead>
<tr>
<th></th>
<th>Cymoxanil</th>
<th>Diméthomorphe</th>
<th>Propamocarbe</th>
<th>Zoxamide</th>
<th>Fenamidone</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>66 samples</td>
<td>38 samples</td>
<td>40 samples</td>
<td>35 samples</td>
<td>-</td>
</tr>
<tr>
<td>2006</td>
<td>106 samples</td>
<td>64 samples</td>
<td>72 samples</td>
<td>25 samples</td>
<td>-</td>
</tr>
<tr>
<td>2007</td>
<td>69 samples</td>
<td>43 samples</td>
<td>48 samples</td>
<td>45 samples</td>
<td>37 samples</td>
</tr>
</tbody>
</table>

It was observed no evolution
### The epidemics in 2004, 2005, 2006, 2007 in North of France

<table>
<thead>
<tr>
<th>Years</th>
<th>Severity</th>
<th>First symptoms</th>
<th>Epidemic Threshold on susceptible cultivars</th>
<th>Periods risks</th>
<th>Control</th>
<th>Model and DSS forecast</th>
<th>Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Moderate</td>
<td>End of May on dumps and volunteers</td>
<td>(May 20) June 17</td>
<td>some risks mid of July</td>
<td>easy and good success</td>
<td>Correct</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>&quot;usual&quot; in France very severe in North of France</td>
<td>May on dumps</td>
<td>early 8 of May</td>
<td>5 of July - July</td>
<td>Very difficult in July high risks difficulties of sprays rainfastness growth of crop cultivar resistance &quot;broken&quot; many symptoms in fields</td>
<td>Correct</td>
<td>Climatics conditions aggressivess? metalaxyl resistance not proved</td>
</tr>
<tr>
<td>2006</td>
<td>Relatively severe</td>
<td>Beginning of May on dumps</td>
<td>25 of May</td>
<td>End of May August</td>
<td>Some syptoms on isolate plant during difficult emergence en of May August : difficult (rain) some symptoms on tubers</td>
<td>Correct</td>
<td>Contamination in may → seed origine? (oosporer? Probably not</td>
</tr>
<tr>
<td>2007</td>
<td>The most severe since 10 years in all regions of France</td>
<td>25 May on dumps</td>
<td>10 of May</td>
<td>Since June very severe in July</td>
<td>Very difficult in July with lot of symptoms mong sprays risks every days and rain every days</td>
<td>Correct</td>
<td>Many sources of contamination climat: no cold in winter, April very soft aggressiveness?</td>
</tr>
</tbody>
</table>
The Epidemic

• Two tools are use
  – Warning system
    • Qualitative model: Guntz Divoux. 1960, some modifications by Conce in 1987
    • Quantitative model: milsol
    • Scouting in fields and around (dumps, volunteers) by technicians
  – DSS MILPV
    • Models Guntz Divoux and Milsol
    • Observations by the growers (field, dumps, volunteers)

• + questions by phone of growers and technician

=> Informations on the epidemic, condition of control, and success of control
Example of risks in 2004 and 2005
Exemple of risk give for the DSS MILPV

No risk

2006

2 periods of risks/day

2007
First symptoms in the field

We have appearance of isolated symptoms in the plot of land. Some are destroyed in 2007
Exemple of destruction in the field early july 2007
discussion

• Mating type:
  – before 2003, the french territory was « free » of A2 strains (only exceptional cases in fields in 1997 and more in garden)
  – Since 2003 the A2 strains increase very quickly in north of France (now around 80% of samples)
  – The situation is very different according to the regions:
    • high in North and Alsace
    • In increasing in Brittany but a lower level
    • Fast increasing in south ouest and center (information INRA 100% A2 in 2007)

• Metalaxyl resistance
  – The resistance is in the France since the begining of using the fungicide
  – It is variable according to the year and region (high level in Brittany: usual use on covered crop)
  – It seems to increase particulary on dumps in North of France

• First conclusion: the late blight is in evolution
  – It is confirm bien genotypic studies (INRA Didier ANDRIVON; SYNGENTA Dr GISI)
Why the evolution

- Some results of research (INRA Rennes)

Isolates A1 et A2 collected in 2004 and 2005 in the Nord Pas de Calais

Agressiveness evaluated on Bintje

Isolats A1 a little more aggressive than isolat A2 (but not significant: p=0.016)

indice: log: (spore production x lesion size)/ latence période

At present:
- study to compare A and A2 collected in 2006
- study to compare aggressiveness of isolates collected 90 years and 2000 years
Why the evolution

• Some results of research : Dr GISI (SYNGENTA)
  – isolates from north of France
    – 1997 : 16 samples A2: 0 metalaxyl resistance : 62%
    – 2006 : 69 samples A2: 80% metalaxyl resistance : 72%
    – 2007 : 21 samples A2: 76% metalaxyl resistance : no result for the moment
  – Aggressiveness : measured by the increasing of lesion size between day 4 and 5 and increasing of sporulation day 8 – day 5
    • On 2006 : no difference of aggressiveness between A1 and A2
    • Isolates of 1997 a little more aggressive than the isolates of 2006

• Questions
  – A1A2 no difference of aggressiveness
  – Importation of seeds? North region of France import more seed from The Netherlands?
  – Proximity of region : North near The Netherlands, Alsace near Germany
  – Evolution of weather conditions : higher temperature
  – Types of crops (resistance cultivar, earlier growth, …)
Evolution of the epidemy

- The new strains, A2 strains seem to be not more aggressive.
- The models (with “old” data of biology) are always correct except in some case of early risk.
- The most important cause seems to be a softer climate with consequence particularly in:
  - Winter: if there is no frost, the initial inoculum is more important
  - April: there are earlier plantations, and faster growth
  - May: the production of spores on dumps is probably higher
  - June and July: the epidemic is faster because there are more numerous periods with risks (T° 12 °C to 20°C)
Evolution of our advices to the growers

• on the method:
  – No change in our models and DSS
  – But beginning of use in January (until now it was first of April because of frost very often before, it was not the case in 2007 and to measure the global epidemiological risk)
  – More intensive survey of durability of cultivar resistance

• Advise:
  – Using fungicide no change, except metalaxyl:

    To be very careful early in the season, because the crops in North of France come back on fields with A2 strains in 2003-2004-2005, so with risks of oospores?

    Very good prophylaxis
    Early sprays with adapted fungicides according to model and DSS advises
Thank you very much for your attention!