Will the real Alternaria please stand up

In response to the 2009 Alternaria case in The Netherlands

Euroblight Workshop
Arras, France
3-6 May 2010

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At the Hilbrands Laboratory for Soil-borne Diseases, a project named 4D - Digital Detection en Diagnosis Service (Dienst) has been started in July 2009.

subsidized by:
• the Provincial Authority of Drenthe
• the European Community
The project is meant to develop tools to help farmers with their complex task to take the right decisions concerning which disease to treat in which way at which time.
As first test items were selected:
- the potato crop
- the Alternaria disease

Potato as being an important crop and Alternaria as a well-known disease requiring proper treatment at the proper time
The most simple approach is to
- photograph a symptom concerned
- have the result digitalized
- broadcast it to a central server
- analyze the picture
- compare symptoms with a database
- based on the outcome select a treatment procedure
Alternaria

It sounds simple
It looks simple

Everybody knows the potato crop
Everybody “knows” Alternaria

What could ever go wrong?
Procedures were devised to

1. receive leaflet samples with leaf spots
2. observe leaf spots by binocular for spores
3. lay out excised lesions on water agar: up to 3-4 lesions per leaflet, up to 3 leaflets per sample
4. inspect excised lesions by binocular after 2 to 3 days and after 10 days for the presence of spores
Which were the spores (hence fungi) to look for:

- *Alternaria solani*
- *Alternaria alternata*
- *Cladosporium cladosporioides*

Results of prior experiments learned that *A. alternata* and *C. cladosporioides* are commonly present in any type of lesion on potato leaves (late blight, Botrytis, Sclerotinia)
Classification of the three fungi involved

A. solani - a genuine pathogen
A. alternata - saprophytic to weakly pathogenic
C. cladosporioides - strictly saprophytic (as far as known)

All three fungi sporulate readily and are identifiable through shape and size of spores or sporophores
Alternaria
Proportional increase of the three test fungi in 2009

Lesions total (768)
Cladosporium cladosporioides (235)
Alternaria alternata (327)
Alternaria solani (219)
Some facts on * Alternaria solani * in 2009

1. The first isolate of * A. solani * was obtained on July 21
2. Early blight came (comes?) relatively late
3. Nevertheless, many people had already seen early blight in the field and sent 62 samples accordingly
4. After July 21, still 66% of the samples did not contain * A. solani *
5. From September 14 till 18, 50% of the lesions with * A. solani * were received, which concerned 151 out of 181 lesions laid out (83.3%)
Some remarks on *Alternaria alternata*

Up to July 21,
179 lesions were laid out of which:
41 yielded *C. cladosporioides*
67 yielded *A. alternata* and
0 yielded *A. solani*

It means there were many lesions (112) void of both *A. solani* and *A. alternata* which to many people were convincingly similar to early blight
From the total number of 768 lesions laid out:

- 400 lesions yielded *A. alternata*
- 295 lesions yielded *C. cladosporioides*
- 219 yielded *A. solani*
- 74 did not yield any of these three fungi

Hence:

there were 74 Alternaria-like lesions without any of the three fungi mentioned
If *C. cladosporioides* is left out as being non-pathogenic than from 768 lesions laid out:

120 lesions yielded *A. solani* single
301 lesions yielded *A. alternata* single
99 lesions yielded *A. solani* + *A. alternata*
248 lesions did not yield any of these two fungi

Hence:
In total there were 248 Alternaria-like lesions without none of the two *Alternaria* species involved
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Hence the lecture is not over yet
Alternaria-in-the-field

A principal problem is that merely based on the presence of lesions with concentric rings any leaf spot disease is considered to be Alternaria
Alternaria-in-the-field

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However, concentric rings are a consequence of the influence of environmental conditions on growth and development of any type of lesion
Alternaria

A typical example is ozone damage
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Ozone damage occurs in the Netherlands
- With sunny and quiet weather
- It may occur locally along busy traffic roads or more general
- Concerning symptom development, there are great differences between cultivars
The “Mimi-case”

- Mimi is a very early potato cultivar from Scotland
- It was experimentally grown in 2006 to find out about its aptness as a potential cultivar for the Netherlands
On June 23, 2006 cultivar Mimi was found severely affected by “Alternaria”; at that time nowhere in The Netherlands Alternaria was found.
In most lesions no fungus was found present - either at inspection by microscope - or after laying out on agar.

In a few cases *C. cladosporioides* was found in the larger lesions.
On June 29, a second visit was paid to the field - lesions had considerably increased in size
- *C. cladosporioides* was commonly present
- *A. alternata* was rare

The disease was diagnosed as ozone damage
However, there were interesting clues in the field
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1. The thick and inflexible leaves
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2. The damage around the leaf margins
3. The aberrant shape of the top leaflet
Alternaria

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1. The thick and inflexible leaves
2. The damage around the leaf margins
3. The aberrant shape of the top leaflet

These features are marked symptoms of boron deficiency.
Boron is a micro-element

1. involved in growth processes
2. involved in controlling oxidation processes in the assimilation process
3. prevents super oxidation of cells
4. As ozone damage is associated with super oxidation, it is hence aggravated by boron deficiency
Concerning this type of Alternaria-like lesions

1. A physiological disorder – Ozone toxicity (stress) associated with boron deficiency
2. Lesions with growth rings
3. Initially void of pathogens
4. Rings are the effect of interaction with the day (light) and night regime
5. Such lesions are a prey for fungi like *A. alternata* and *C. cladosporioides*
6. These lesions are strictly not colonized by *A. solani*, which goes for the green stuff
Physiological (non-fungal) leaf spot diseases may be due to

- Ozone toxicity
- Manganese deficiency
- Manganese toxicity
- Magnesium deficiency
- Zinc deficiency
Physiological (non-fungal) leaf spot diseases

These diseases are marked by tiny to small sized lesions, which earlier or later become colonized by A. alternata and/or C. cladosporioides as well as by quite a number of other fungi.
Magnesium deficiency

sent in as Alternaria
Ozone damage sent in as Alternaria
The typical symptoms of early dying Dutch potato crops in 2009 manganese, boron and ozone related
There were many small leaf spots in farmers’ fields.

Most were initiated by physiological disorders.

Nevertheless, because of lack of knowledge on the real cause of the problem, treatment with fungicides to control “Alternaria” was more the rule than the exception.
To determine the cause and treatment of an Alternaria-like disease, the information gathered should be better than “lesions marked by concentric rings” to start treatment with a fungicide to control Alternaria.
Control

A first action to control “Alternaria-likes” is proper fertilization of the crop. A second action is monitoring development of *A. solani* and then to act correspondingly when control of Alternaria becomes a real need.
At the end

No doubt there is a need to control Alternaria

But it should be the real Alternaria to control
Alternaria

End of presentation

Thank you for your attention
Results Prospection "Alternaria" - 4D-project
The Netherlands 2009

Number of times isolated

- Totals: 400
- Solo: 295
- +A.sol: 146
- +A.alt: 94
- +C.clad: 126

C. cladosporioides
A. alternata
A. solani

Alternaria
## Alternaria

### Results Prospection "Alternaria" - 4D-project
**The Netherlands  2009 – Chi square test**

#### Commensalism

<table>
<thead>
<tr>
<th>Commensalism</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Pairs of <em>A. alternata</em> and <em>A. solani</em>) / (All <em>A. solani</em>)</td>
<td>0.43925</td>
</tr>
<tr>
<td>(Pairs of <em>A. alternata</em> and <em>C. cladospor</em>) / (All <em>C. cladospor</em>)</td>
<td>0.43448</td>
</tr>
<tr>
<td>(All <em>A. alternata</em>) / All isolates</td>
<td>0.43937</td>
</tr>
<tr>
<td>Average frequency</td>
<td><strong>0.43770</strong></td>
</tr>
</tbody>
</table>

#### Chi square test based on average frequency

<table>
<thead>
<tr>
<th></th>
<th>Expected</th>
<th>Encountered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>A. alternata</em> and <em>A. solani</em></td>
<td>93.6687</td>
<td>94</td>
</tr>
<tr>
<td>2. <em>A. alternata</em> and <em>C. cladosporioides</em></td>
<td>126.9342</td>
<td>126</td>
</tr>
<tr>
<td>3. <em>A. alternata</em> versus all isolates</td>
<td>393.4960</td>
<td>395</td>
</tr>
</tbody>
</table>

Chi square 1,2,3 is **0.9931**

Chi square 1,2 is **0.9285**
The results of this Chi square test indicate that the presence of of *A. alternata* in the various lesion types is not according to an at random distribution pattern, but is the same for all

So *A. alternata* appears to have invaded the various lesion types with a frequency of about 43.8%

Further, if the combined occurrence of *A. solani* and *A. alternata* in single lesions would be at random and the average “Alternaria” lesion size estimated at 1 cm$^2$, there should be at least 1 lesion caused by *A. alternata* per $1/0.4377$ cm$^2 = \approx$ about any 2.5 cm$^2$ of leaf area. This was not the case.
Results Prospection "Alternaria" - 4D-project
The Netherlands 2009 – Chi square test

These results are not in agreement with the hypothesis that that A. *alternata* acts like A. *solani* as an independent pathogen
Nevertheless, it is for sure that A. *alternata* has a tremendous invasive power and as such to strike at least at average any 2.5 cm² of leaf area throughout the testing period
Manganese deficiency related disorder
A few conclusions:

A. *alternata* mixes well with both *A. solani* and *C. cladosporioides*

However, *A. solani* appears not to like *C. cladosporioides* too much