



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

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

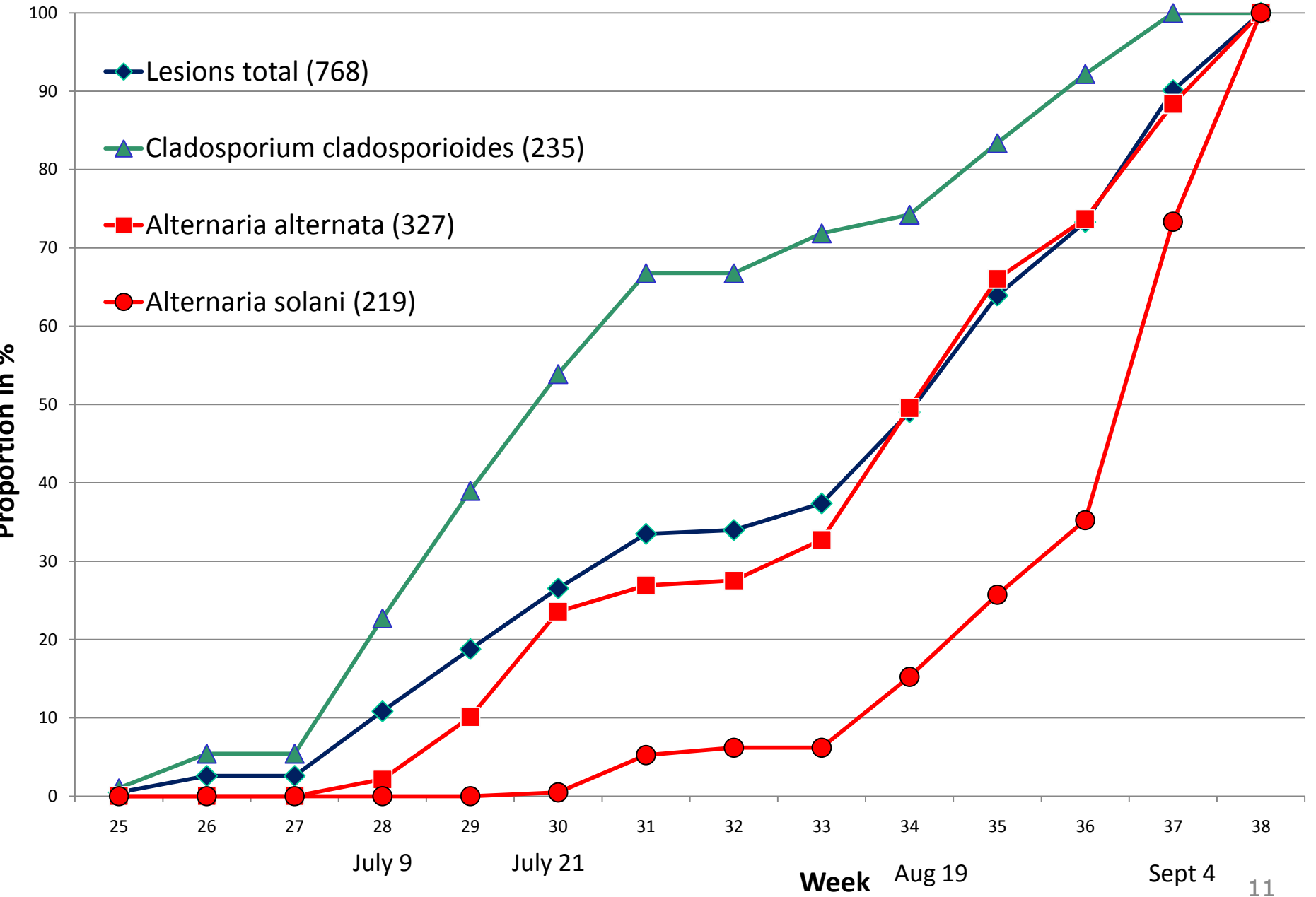
C. cladosporioides - strictly saprophytic (as far as known)

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



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

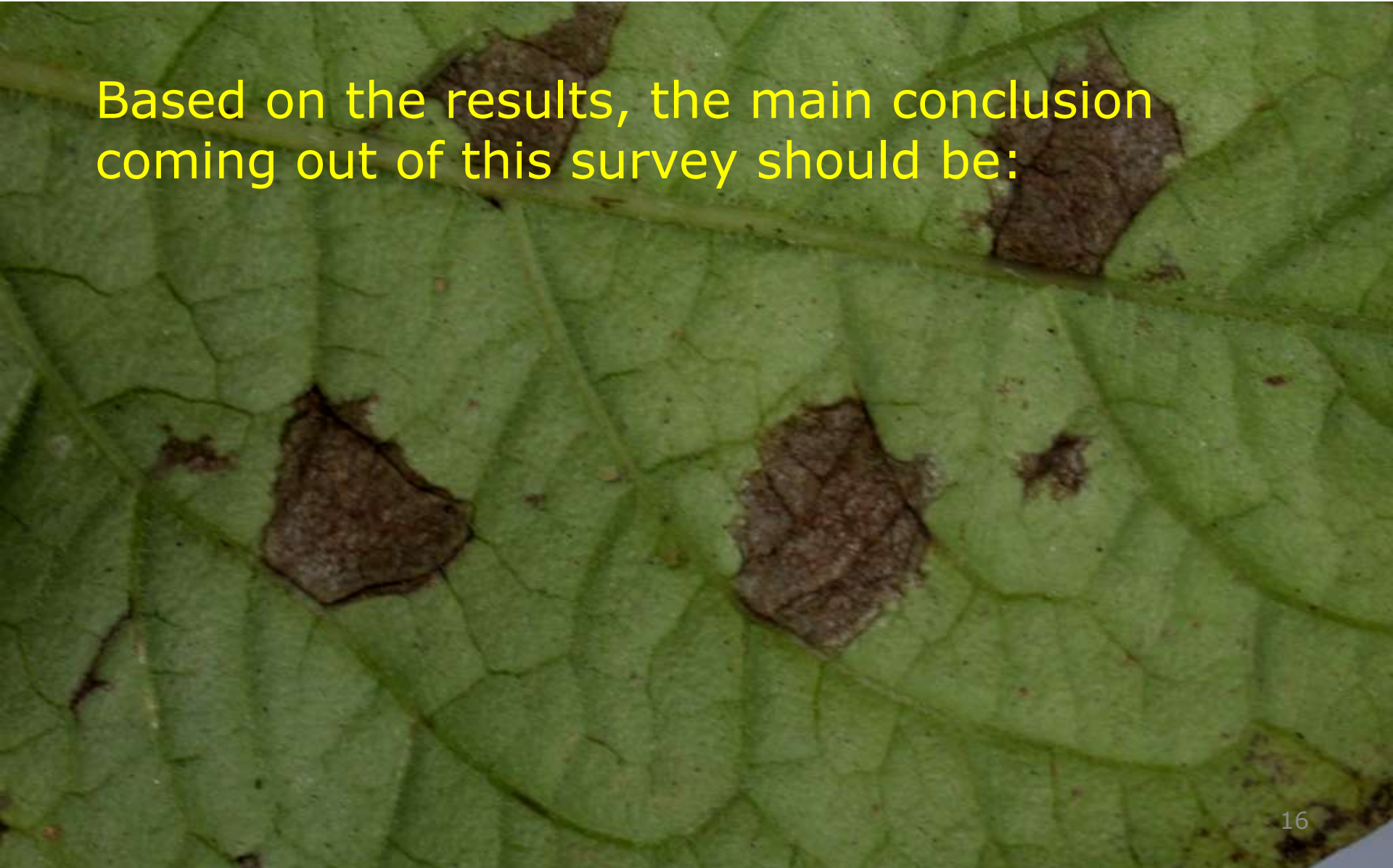
99 lesions yielded *A. solani* + *A. alternata*

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

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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

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

Alternaria

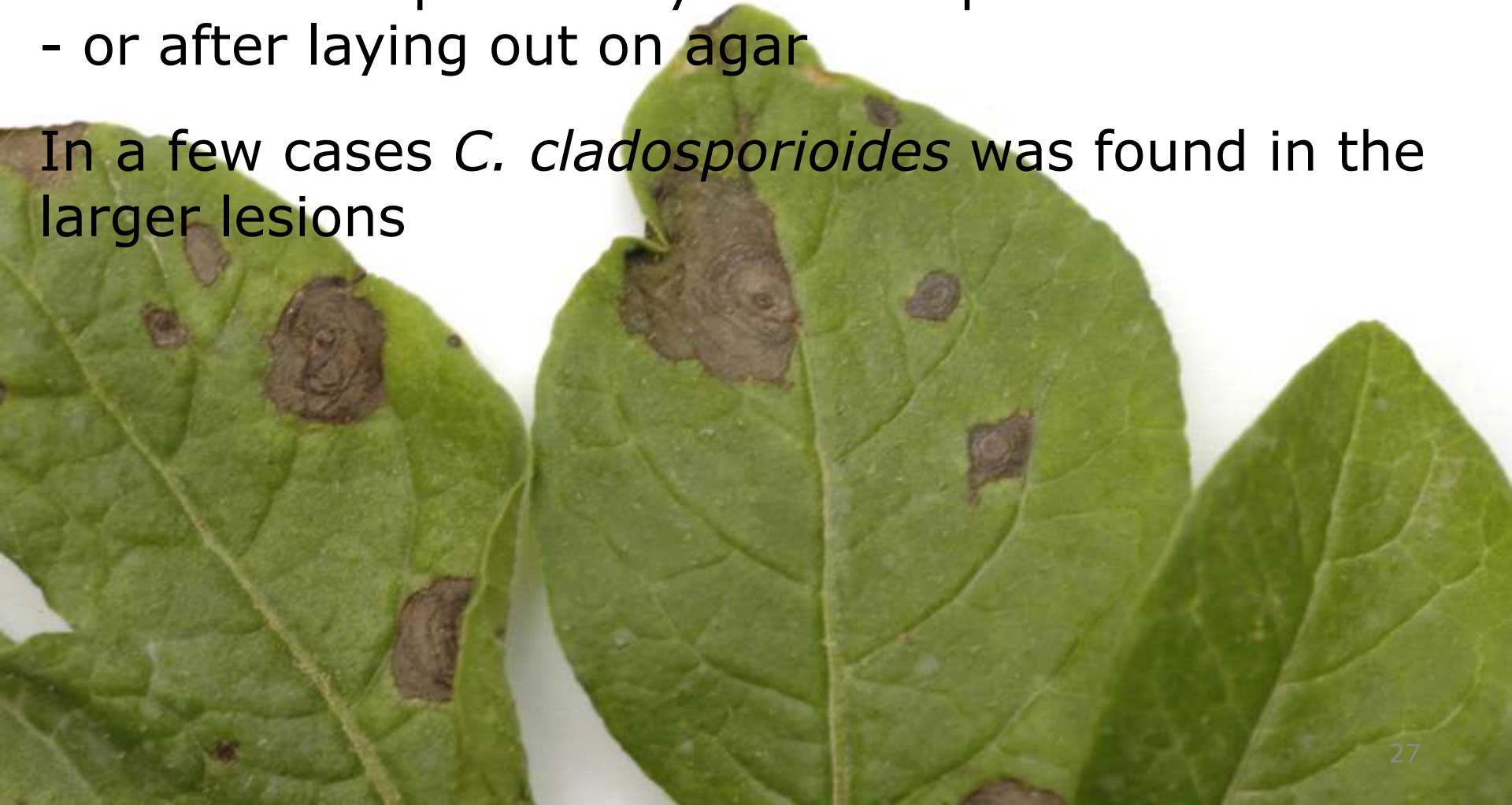
On June 23, 2006 cultivar Mimi was found severely affected by "Alternaria"; at that time nowhere in The Netherlands Alternaria was found



Alternaria

In most lesions no fungus was found present
- either at inspection by microscope
- or after laying out on agar

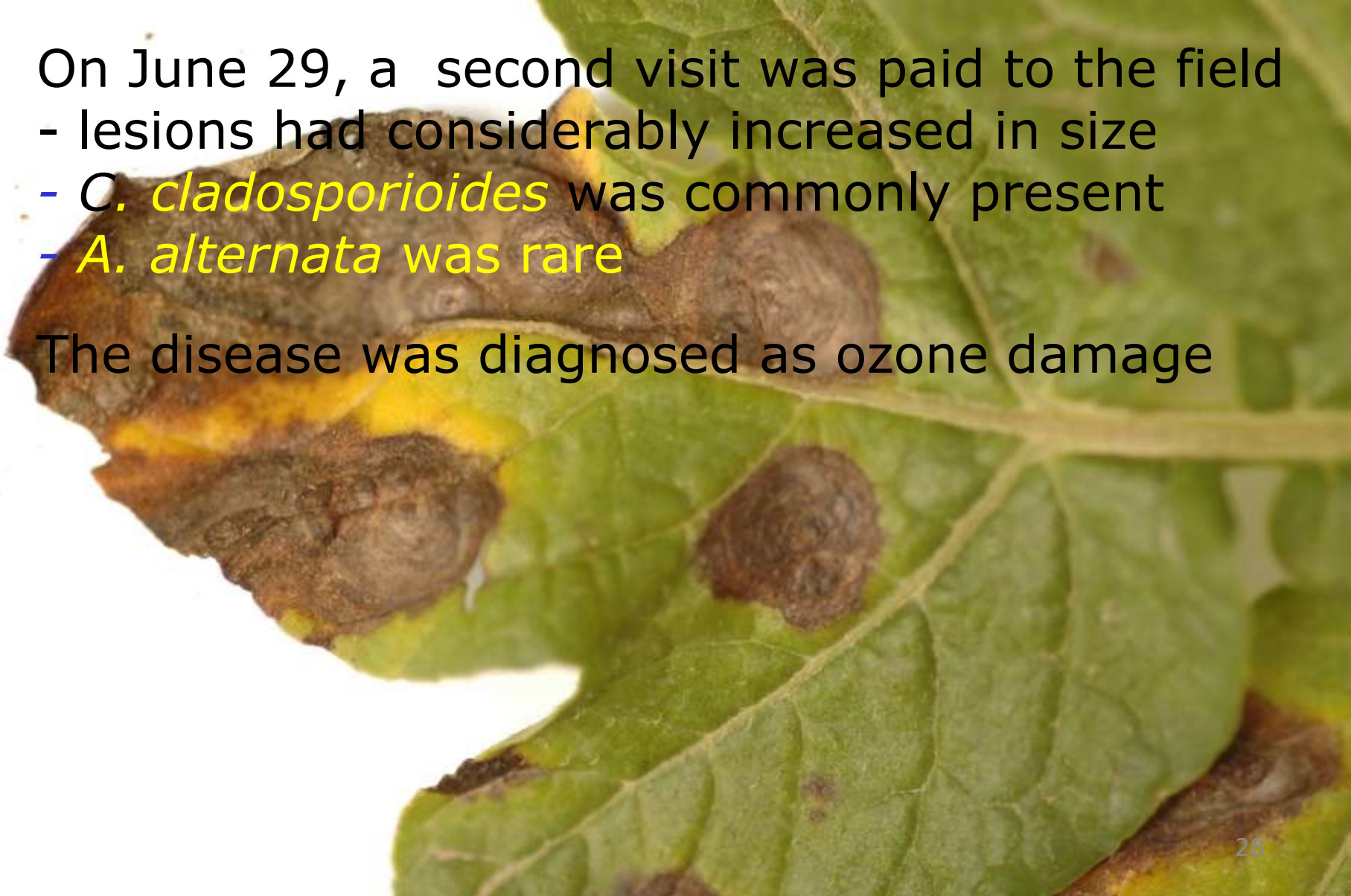
In a few cases *C. cladosporioides* was found in the larger lesions



Alternaria

- 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



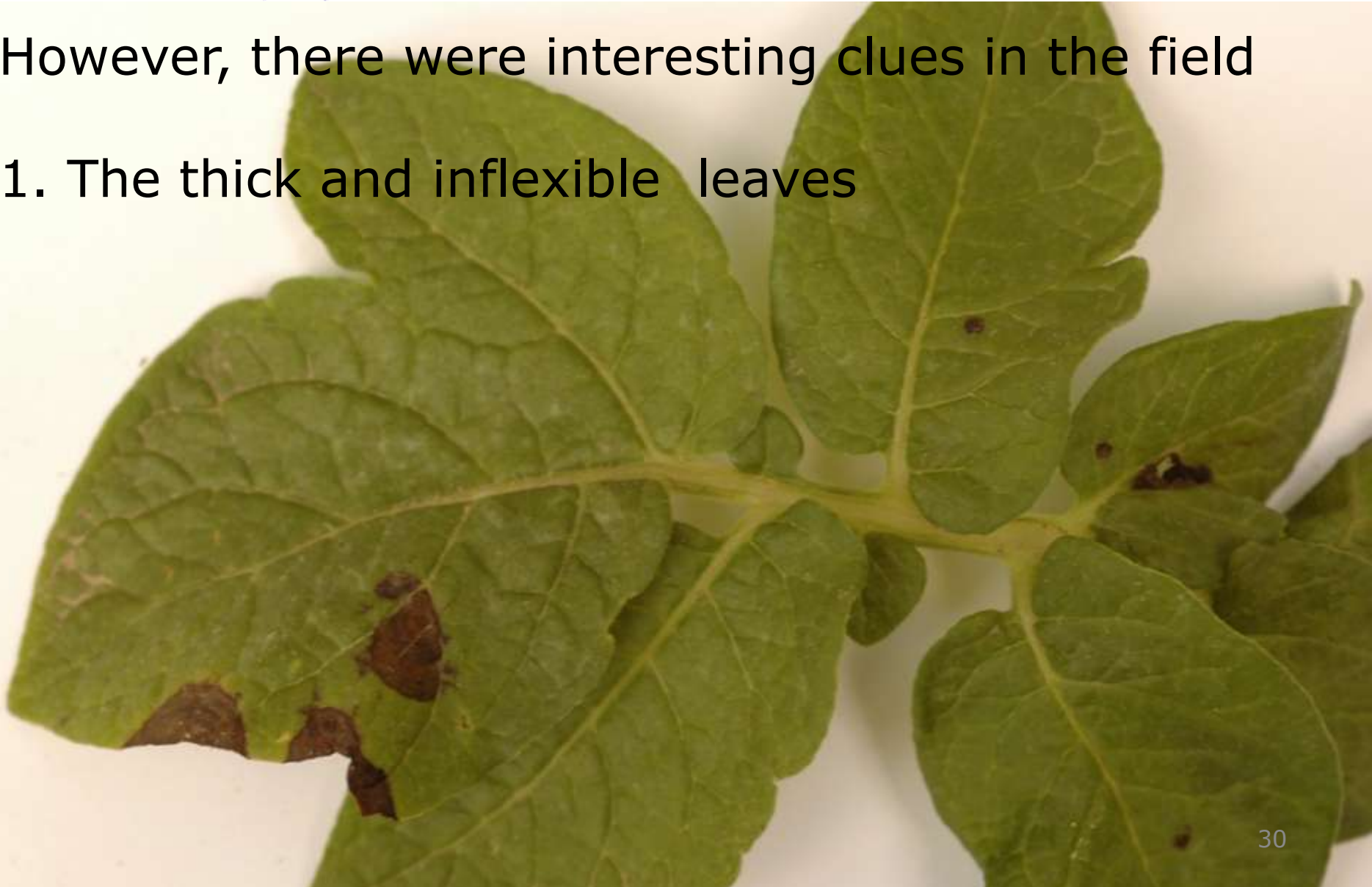
Alternaria

However, there were interesting clues in the field



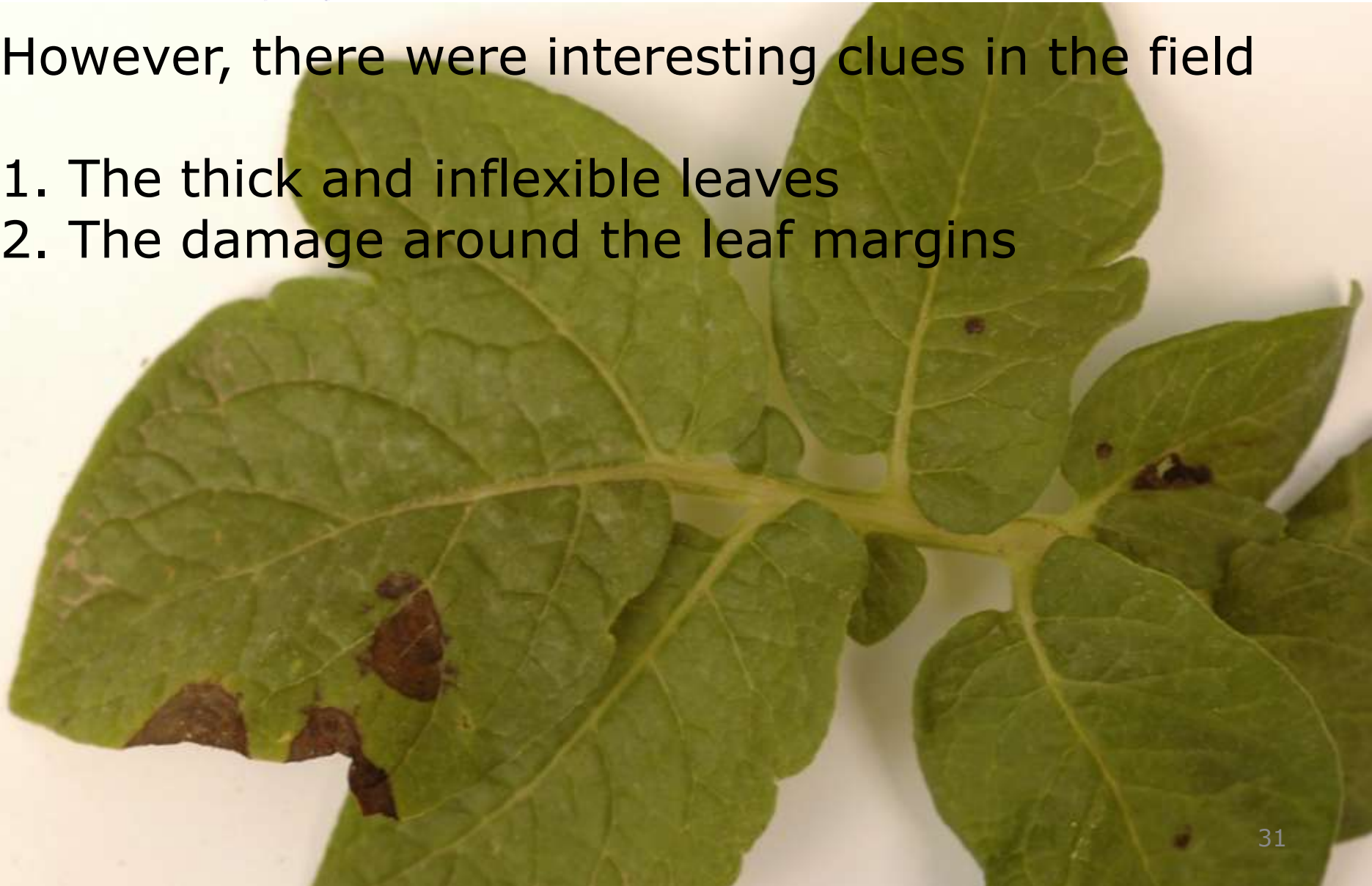
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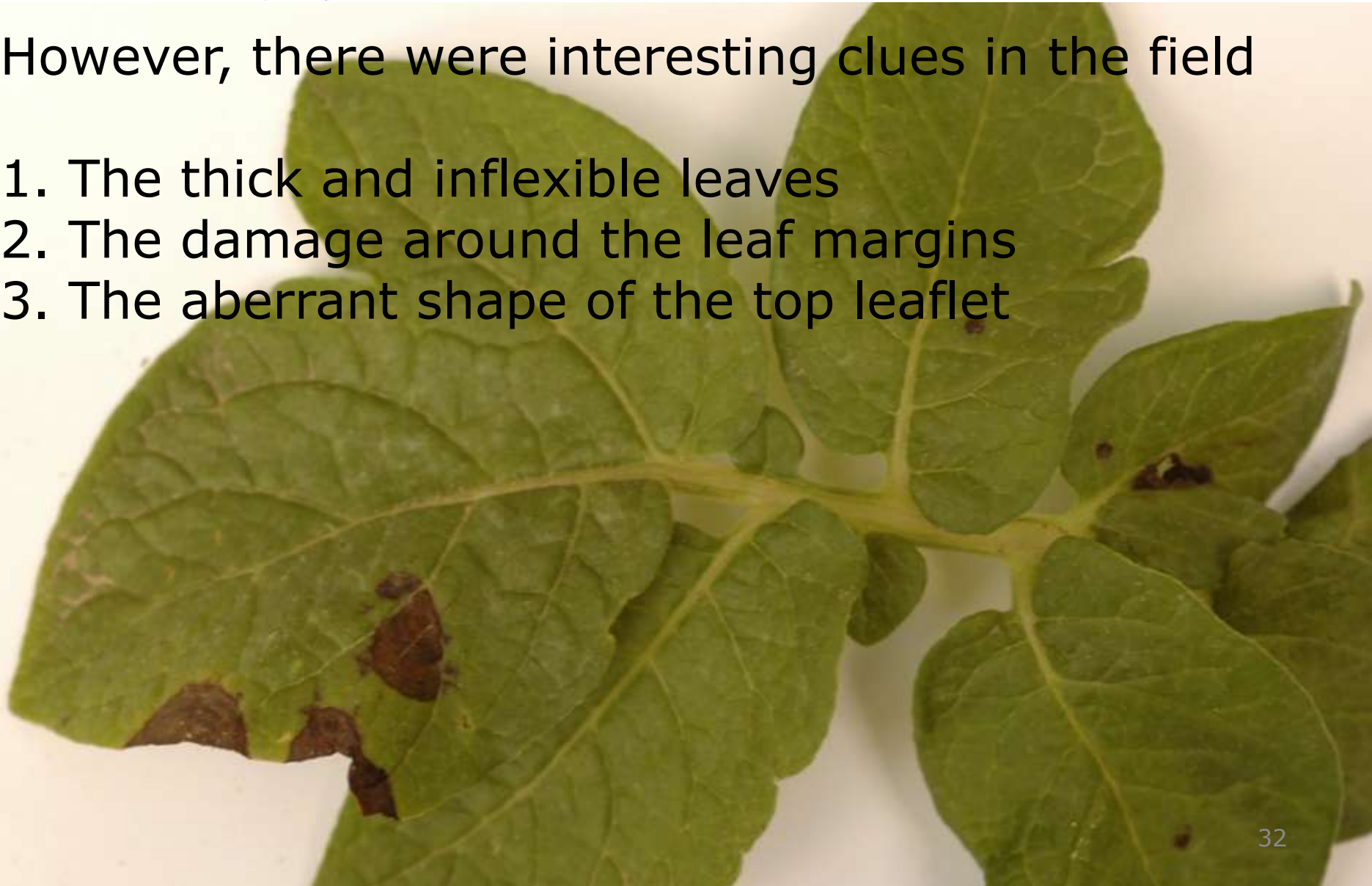
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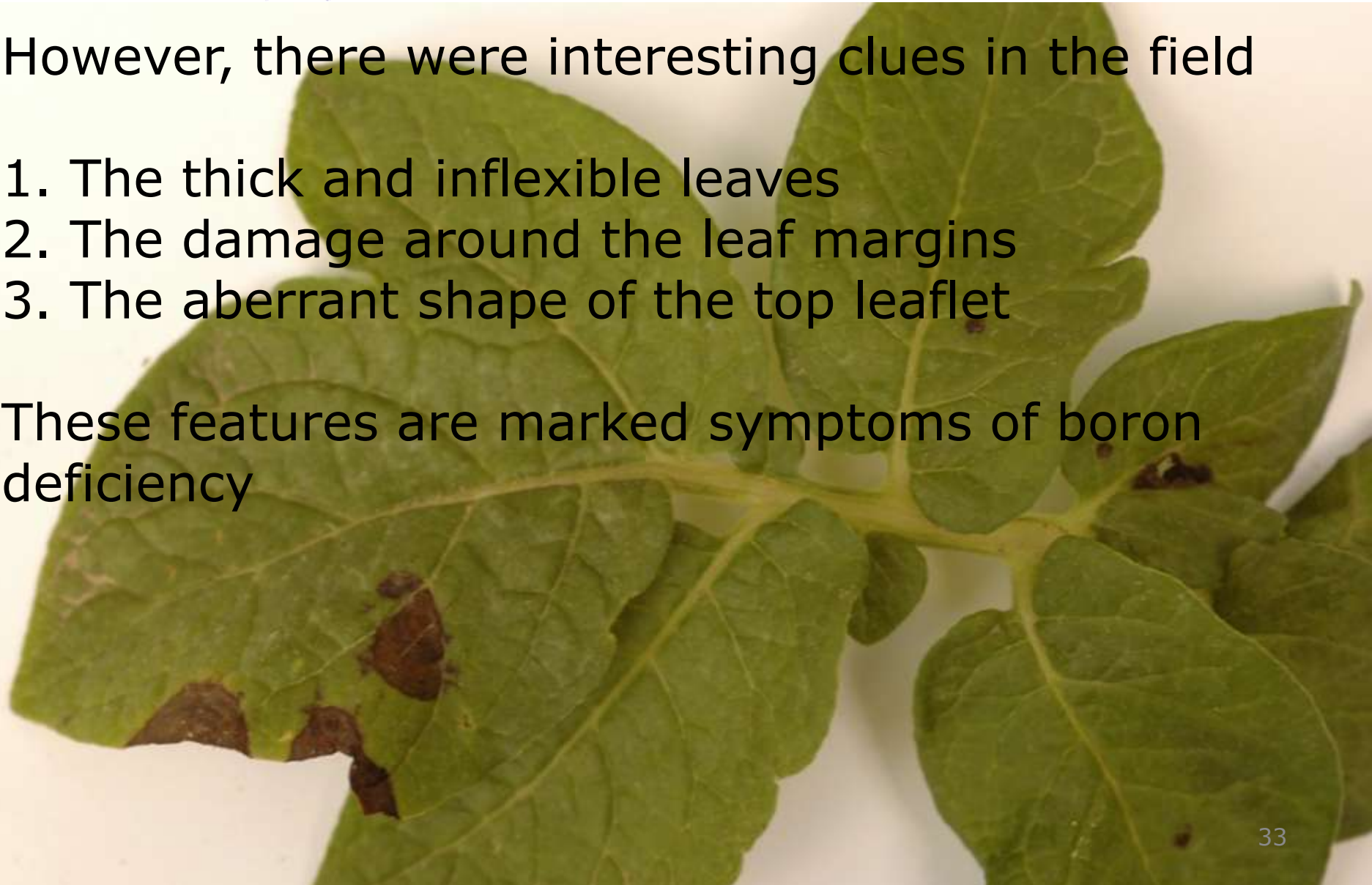
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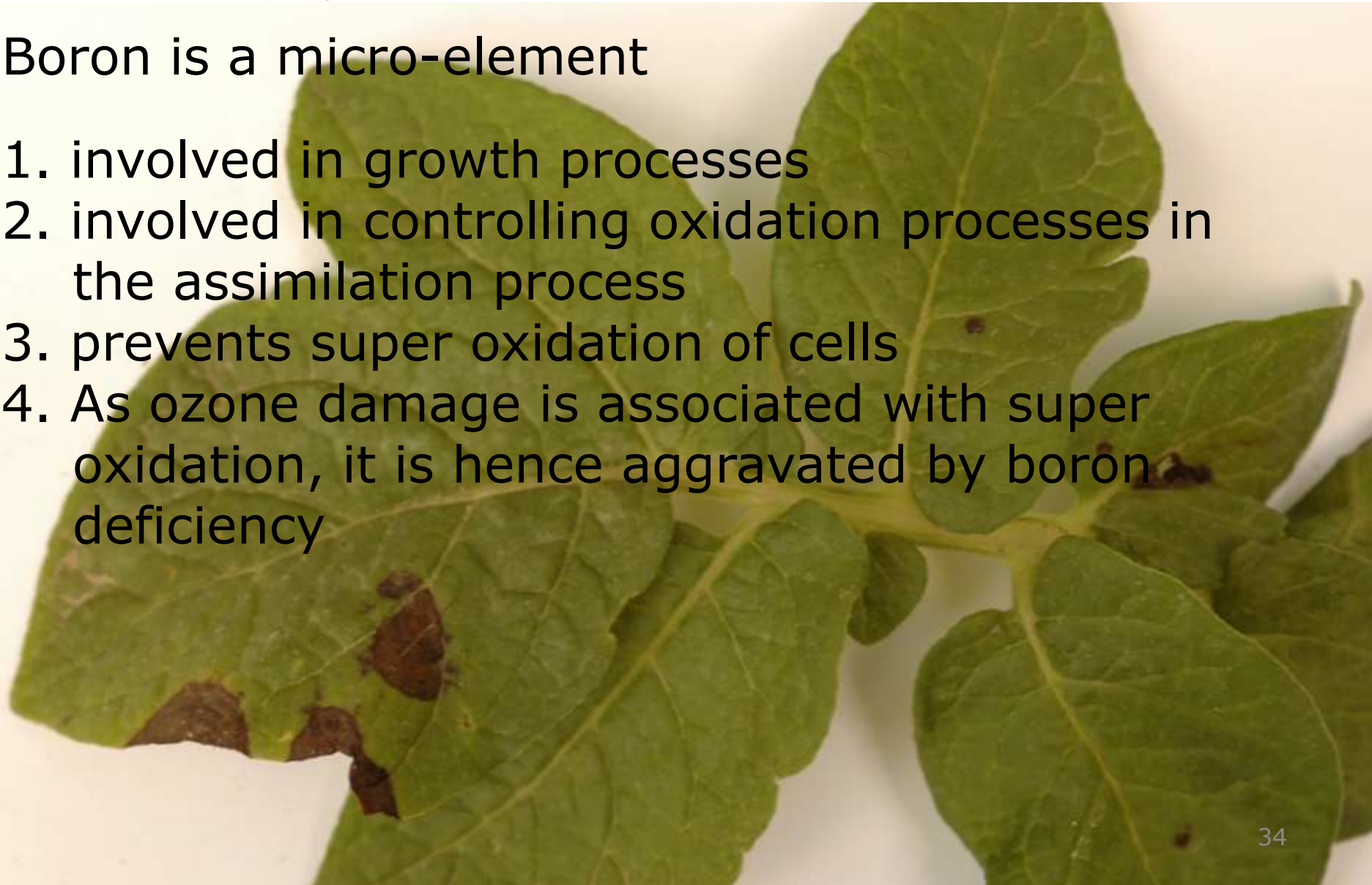
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These features are marked symptoms of boron deficiency



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

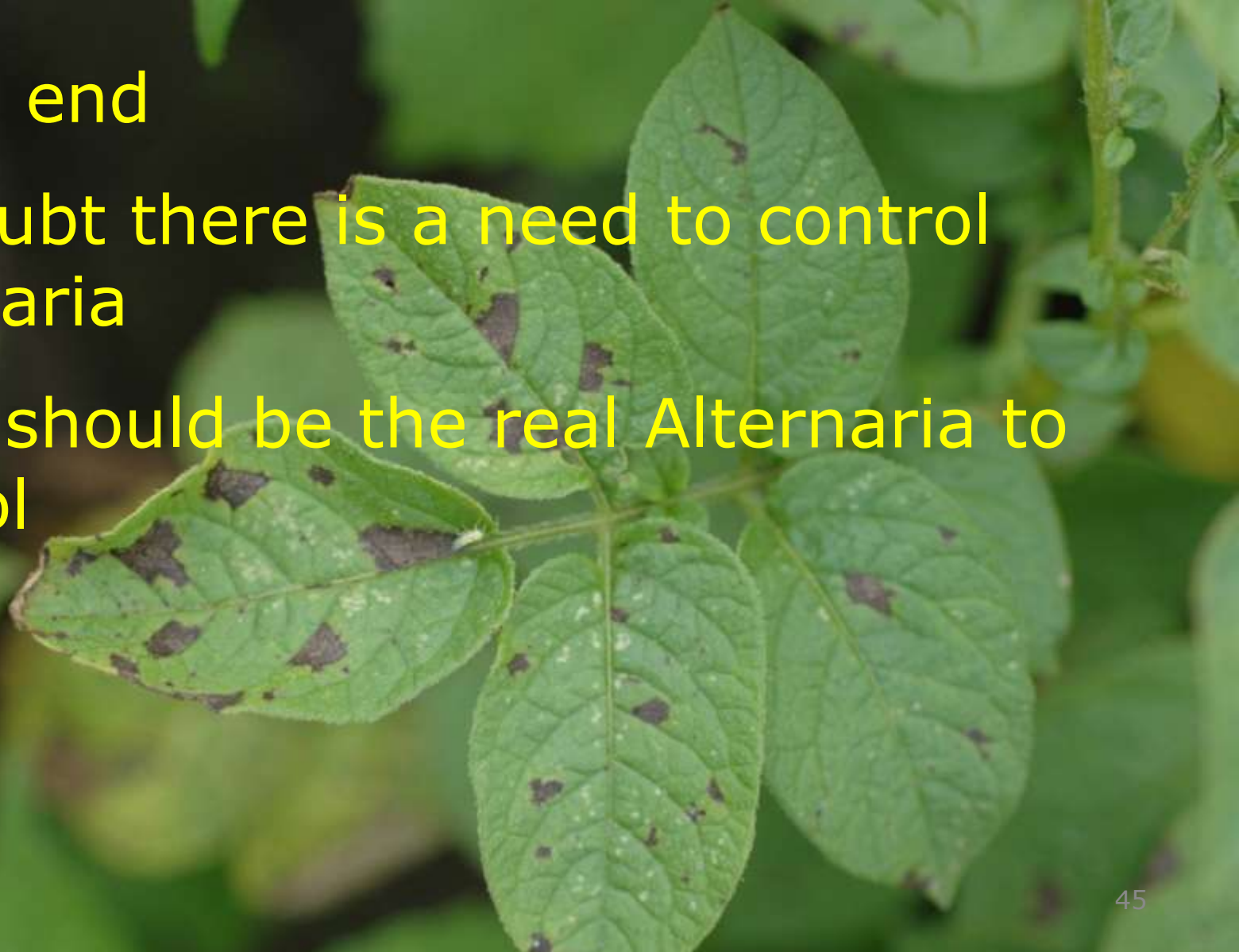
A first action to control “Alternaria-likes” is proper fertilization of the crop

A second action is monitoring development of *A. solani* and than 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

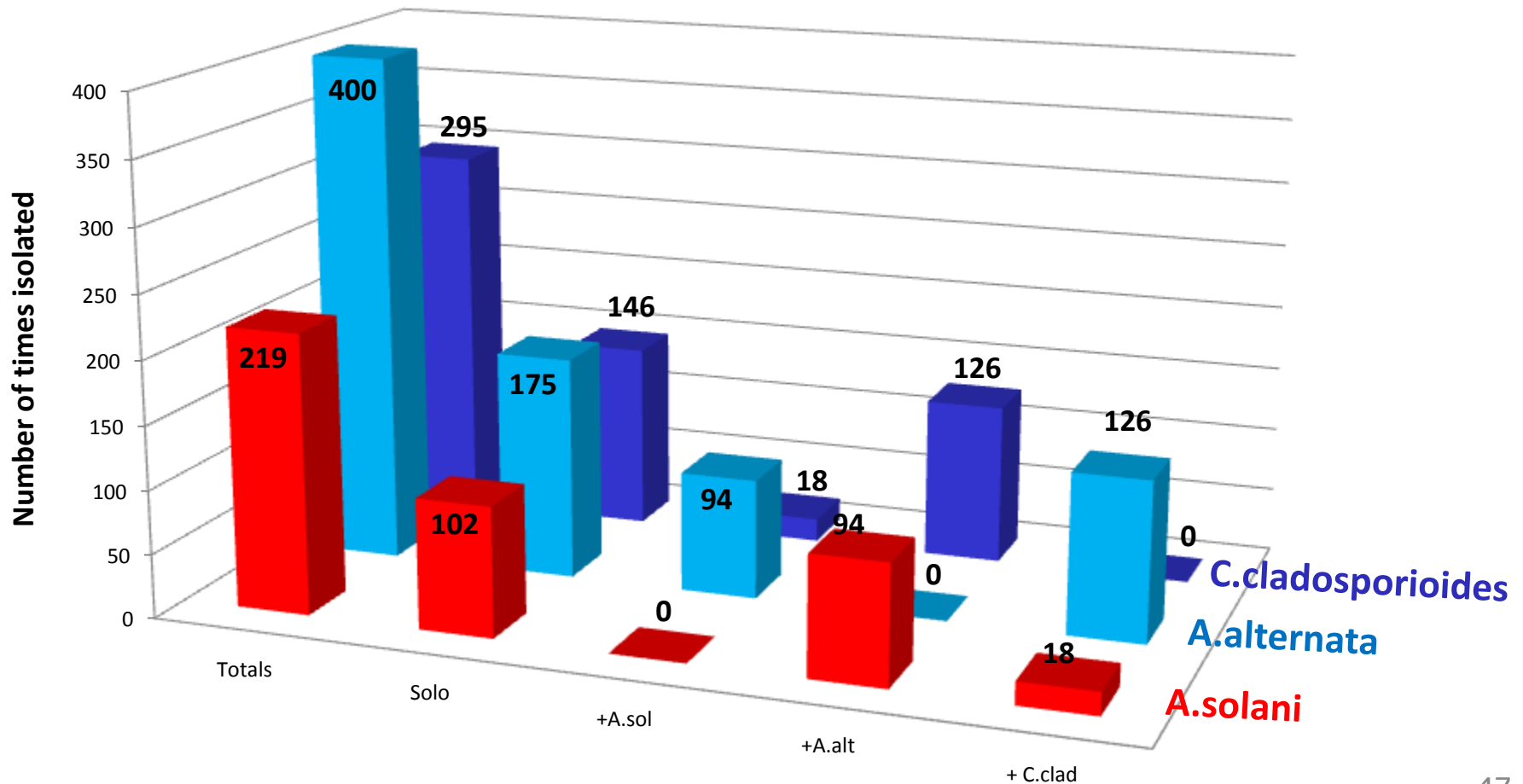




End of presentation

Thank you for your attention

Results Prospection "Alternaria" - 4D-project The Netherlands 2009



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

Commensalism	Frequency	
(Pairs of <i>A. alternata</i> and <i>A. solani</i>) / (All <i>A. solani</i>)	0,43925	
(Pairs of <i>A. alternata</i> and <i>C. cladospor</i>) / (All <i>C. cladospor</i>)	0,43448	
(All <i>A. alternata</i>) / All isolates	0,43937	
Average frequency	<u>0,43770</u>	
Chi square test based on average frequency	Expected	Encountered
1. <i>A. alternata</i> and <i>A. solani</i>	93,6687	94
2 . <i>A. alternata</i> and <i>C. cladosporioides</i>	126,9342	126
3 . <i>A. alternata</i> versus all isolates	393,4960	395
Chi square 1,2,3 is	0,9931	
Chi square 1,2 is	0,9285	

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

The results of this Chi square test indicate that the presence 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 cm², there should be at least 1 lesion caused by *A. alternata* per $1/0.4377 \text{ cm}^2 =$ about any 2.5 cm² 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