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Presentation

# Will the real Alternaria stand up please
# Experiences with Alternaria-like diseases on potatoes during the 2009 growing season in The Netherlands

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## INTRODUCTION

During the period June 25 till September 18, 2009, a survey was made on causes of Alternaria-like lesions present on potato leaves. This survey was part of a project called Development of Digital Detection and Diagnosis Service (short name DDDD-Project) financed by the Provincial Authority of Drenthe and European Regional Development Fund (ERDF). The aim of the project is to link images of disease symptoms in the field to a databank of causal agents and corresponding symptoms and sequently to select a cause. Then to the farmer an advice is to be formulated how to manage the problem concerned. As a first crop to deal with, potato was chosen and as a first target disease Alternaria-like lesions.

## MATERIAL AND METHODS

*Target organisms.*

A first target organism is *Alternaria solani*. However, from prior research (see for example the Mimi-case, this article), it was learned that in Alternaria-like lesions as well as in lesions of *Phytophthora infestans*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*, two organisms are commonly found to be present. It concerns *Alternaria alternata* and *Cladosporium cladosporioides*. Among the three named organisms, *A. solani* is a true pathogen whereas *A. alternata* is a weakly pathogenic organism capable to affect wounded foliar tissues of potato (Pits *et al.,* 2005). *C. cladosporioides* is an extremely common saprophyte.

*Collection.*

Cooperators were requested to collect potato leaflets with symptoms, which to their opinion were typical for *A. solani*. For the collection of leaflets with symptoms, Petri-dishes with water agar amended with 250 mg streptomycin per l were made available to cooperating institutions. After receiving samples by mail or by handing over, both lesions and agar were examined on the presence of spores of the target organisms with the help of a binocular loupe. Then as far as available, for a minimum of three leaflets of each sample three lesions per leaflet were excised and placed on water agar amended with 250 mg/l streptomycin. The three lesions per leaflet were placed in a single petri-dish. After 3 and 9 days, lesions and agar were examined on the presence of the target organisms as well as for other organisms showing up. The three target organisms show a strong tendency to profusely sporulate both on the lesion tissues as well as on mycelium entering the water agar connected to the excised tissues.



## RESULTS

Results are presented in Tables 1, 2 and 3 and Figure 1. In total 112 samples were received, which yielded 768 excised lesions incubated on water agar. The first sample yielding *A. solani* was obtained on July 21. Before that date, already 22 'Alternaria' samples had been received, which yielded 286 excised lesions void of *A. solani*. From that date onwards another 90 samples were received, which yielded 48 samples and corresponding 219 excised lesions with *A. solani* and 42 samples and corresponding 263 excised lesions void of *A. solani* (Table 1).

**Table 1.** *Results on presence of A. solani in Alternaria-like lesions in samples received during the growing season of 2009 from June 25 till September 18.*

| **Sample information** | **June 25 till July 21** | **July 21 till September 4** | **September 4 till 18** | **June 25 till September 18** |
|---|---|---|---|---|
| ***<u>Samples received</u>*** | **22** | **59** | **31** | **112** |
| yielding *A. solani* | 0 | 19 | 29 | 48 |
| without *A. solani* | 22 | 40 | 2 | 64 |
| ***<u>Lesions excised</u>*** | **286** | **301** | **181** | **768** |
| yielding *A. solani* | 0 | 68 | 151 | 219 |
| without *A. solani* | 286 | 233 | 30 | 549 |

For the period of June 25 till July 21, *A. alternata* was found to be present in 33.9% of the excised lesions. In that period there was no *A. solani* found.
A first conclusion is that in 123 or 66% of the lesions, which were very similar to lesions of Alternaria, both *A. alternata* and *A. solani* were absent (Table 2).

**Table 2.** *Occurrence of A. solani and A. alternata in Alternaria-like lesions sampled for incubation during the growing season of 2009.*

| **Period** | ***A.solani*** | ***A.alternata*** | ***A. solani* and *A. alternata*** | **Lesions void of *Alternaria* spp.** | **All lesions** |
|---|---|---|---|---|---|
| June 25 till July 21 | 0 | 67 | 0 | 123 | 186 |
| July 21 till Sept. 4 | 50 | 215 | 18 | 88 | 401 |
| Sept. 4 till Sept. 18 | 70 | 19 | 81 | 24 | 181 |
| Total period | 120 | 301 | 99 | 261 | 768 |

For the growing season of 2009, the presence of *A. alternata* in lesions occurred according to an average infection rate of 43.8% (Table 3). To be able to do so and if the average size of lesions is supposedly 1 $cm^2$ then there must be at least one active propagule per 1 $cm^2/0.44= 2.28$ $cm^2$ to achieve the encountered percentage of invaded lesions. If *A. alternata* was acting as a pathogen and infecting independently of *A. solani* to form lesions, there should be at least one lesion with *A. alternata* at any 2.28 $cm^2$ leaf surface of any leaflet. This was not the case. In fact, *A. alternata* is not an organism that is strongly pathogenic to potato foliage, but is much more an extremely successful invader of necrotic lesions due to any agent to cause those.

**Table 3.** *Incidence of lesions with A. alternata with or without A. solani and C. cladosporioides and the corresponding Chi square test.*

| **Presence of *A. alternata in lesions* with *A. solani* and with *C. cladosporioides*** | **Frequency** | |
|---|---|---|
| (Pairs of *A. alternata* and *A. solani*) / (all lesions with *A. solani*) | 0.4393 | |
| (Pairs of *A. alternata* and *C. cladosporioides*) / (*all lesions with C. cladosporioides*) | 0.4345 | |
| (All *A. alternata* isolates) / (All isolates) | 0.4394 | |
| **Average frequency of *A. alternata*** | **0.4377** | |
| | | |
| **Chi square test based on average frequency of *A. alternata*** | **Expected** | **Encountered** |
| **Group 1**: *A. alternata* and *A. solani* together | 93.67 | 94 |
| **Group 2**: *A. alternata* and *C. cladosporioides* together | 129.93 | 126 |
| **Group 3**: *A. alternata* versus all lesions | 393.50 | 395 |
| **Chi square test for groups 1, 2 and 3: P = 0.9931** | | |
| **Chi square test for groups 1 and 2: P = 0.9285** | | |

For the period of July 21 till September 4, *A. alternata* was present in 59.1% and *A. solani* was present in 17% of the lesions excised. For that period still 114 or 28.4% of Alternaria-like lesions were found to be void of both *A. solani* and *A. alternata*.
For the period of September 4 till September 18, *A. solani* was commonly found in the field. From 181 lesions collected during that period, 151 lesions yielded *A. solani* and 100 lesions yielded *A. alternata*. In 91 of these lesions, *A. solani* and *A. alternata* occurred together. From 181 lesions collected during this period only 24 lesions or 13.3% were found to be void from both Alternaria species.
In samples obtained from the field *A. solani* gradually increased from 0% in the first period to 17.0% in the second one and to 83.4% in the third period (Table 2, column *A. solani*).
In general, it can be said that in the Netherlands, *A. solani* came late during 2009. At the end of the growing season, most of the Alternaria-like lesions contained *A. solani*, but also to a high percentage *A. alternata*. It should however be mentioned here that those crops, which yielded so many lesions void of *A. solani* and *A. alternata*, had already acutely died off in early August; this without any further increase in the number of lesions.

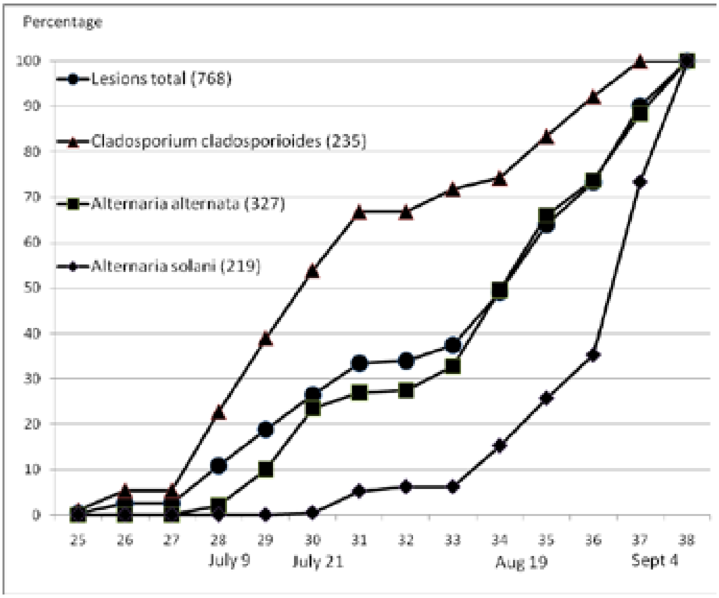


**Figure 1.** *Proportional increase in percentages of the three target organisms and number of lesions laid out on water agar during the period of June 25 till September 18, 2009. On the x-axis numbers of weeks are presented as well as specific dates mentioned in the text. The 50% line presents when 50% of the lesions concerned were collected.*

Regarding the whole testing period there were considerable numbers of Alternaria-like lesions void of both *A. solani* and *A. alternata* (Photo 2). It means there is symptom development in farmers' fields leading to Alternaria-like lesions for which the presence of either *A. solani* or *A. alternata* is not needed. The key question is what the origin of this symptom development may be. An interesting candidate is ozone stress associated with boron deficiency (See The Mimi case).

**The Mimi case**

In 2007 two rows of the very early Scottish potato variety Mimi were grown in the Noordoost Polder in the neighbourhood of Marknesse. On the 23rd of June, the first and third author were called in as the cultivar was battered by early blight. Indeed, the crop showed relatively large Alternaria-like lesions with diameters ranking between 4 and 15 mm with the typical Alternaria-like concentric rings (Photo 1). However, at that period nowhere in the Netherlands *A. solani* had been reported. Leaflets with lesions were collected in Petri-dishes with water agar. Lesions concerned were internally and externally studied microscopically. However, no fungal hyphae were to be seen either on or inside the lesions. Thirty lesions were laid out on water agar. Of those, a single lesion yielded an isolate of *C. cladosporioides*; all other lesions did not show any development of fungi. On the 29th of June a second visit was paid to this field. Lesion's size had increased considerably with diameters increases of more than

**Photo 1.** *Alternaria-like lesions on leaflets of cv. Mimi void of Alternaria spp.*

100% and many lesions had coalesced to form large areas of affected necrotic tissues. Again leaflets were collected as described before. This time, all 30 lesions yielded *C. cladosporioides*. *A. alternata* was found to be present in a single lesion only. Due to foregoing weather conditions and based on experiences in the laboratory, the disease was claimed by the two authors to be due to ozone stress and not because of attack by any pathogen.

However, in addition to the development of Alternaria-like lesions, cultivar Mimi showed typical symptoms of boron deficiency such as: thickened and inflexible leaflets, coalescence of the top leaflet with one or two of the two closest lateral leaflets, typical damage of the leaflet borders due to failing of meristematic activity, and shortening of the petioles and stems. The cultivar is reputed for its shallow and weak root system, which curbs its possibilities to obtain boron from the soil, as this element is in a very low concentration directly available to the plant. This is even the case for soils, which in general are not boron deficient.



**Relation between ozone stress and boron**

What could be the relationship between boron deficiency and development of ozone stress associated with foliar lesions imitating early blight? Well, apart from being involved with plant growth and especially with the growing points and other meristematic tissues, the micro-element boron is involved in the assimilation process of plants and especially so in preventing super oxidation through formation of radicals as a consequence of the most critical process of transforming $H_2O$ and $CO_2$ with the help of sun light into sugars. If boron is short in supply, super oxidation leads to a rapid disintegration of affected foliar tissues at sunny days (Mulder and Turkensteen, 2008). Ozone, which is also a super oxidant, is produced out of $NO_2$ and alkanes under the influence of sunlight. Both compounds originate from industrial processes as well as traffic. It enters leaflets through stomata and affects cells of inside tissues. In case of insufficient availability of boron, this reaction is very difficult to stop and especially so on sunny days. There is quite a difference between cultivars. Mainly cultivars with weak root systems are affected as for such varieties, it is most critical to obtain sufficient boron to prevent super oxidation.

**State of crops with (Pseudo-)Alternaria-like lesions**

Crops concerned showed some typical symptoms pointing at acute deficiency of boron as cause, as there is the sudden halt of stem growth, growth and development of new leaves and formation of micro-rosettes in the highest axils. Furthermore, the highest leaves showed symptoms of acute manganese deficiency associated with the typical olive green colour, loss of luster and the presence of tiny speckles along major veins. In addition on these same leaves symptoms of ozone stress developed, leading to lesions increasing in size due to the formation of new concentric zones (Mulder and Turkensteen, 2008; Photo 2).

**Photo 2.** *Close-up of Alternaria-like lesions on a leaflet due to ozone toxicity*

**DISCUSSION AND CONCLUSIONS**

Concerning the date 21st of July, *A. solani* came about relatively late during the growing season. Thirty percent of the samples yielding *A. solani* were obtained during August, the other 70% between September 4 till 18. The question is whether this was the common moment for *A. solani* to occur or that this late occurrence was specific for the season of 2009. It should be considered that a relative great part of the increase of *A. solani* is due to the fact that the crops with symptoms due to ozone stress had already died off.

During most of the season *A. alternata* was found to be present. The first isolate of *A. alternata* was obtained from leaflets collected on the 9th of July. However, the first Alternaria like lesions came in on June 24, most of which yielded *C. cladosporioides*. Averaged over all isolates, it was found to colonize 43% of all lesions independently whether it concerned lesions with or without *A. solani* and with or without lesions due to ozone stress (Table 3). *A. alternata* showed towards the end of the season a relatively strong affinity to *A. solani*, as in the period of September 4 till 18, it was found within 51.9 % of the lesions of *A. solani*, whereas in the period July 21 till September 4, it was found within 26.5% of the lesions only. The higher incidence later in the season is most likely due to the much higher frequency and hence much shorter distance between lesions of *A. solani* and the invader *A. alternata*.



*C. cladosporioides* was found to have invaded 38.4% of the lesions studied. It was present from the beginning, but became less frequently seen at the end of the season, which is probably due to the fact that lesions associated with *A. solani*, which were the most common ones observed at the end of the season, did apparently not present a favourable substrate to support invasion by *C. cladosporioides*. It was found with 40.5% of lesions with *A. alternata* but only with 10,5% of the lesions associated with *A. solani*.

From the 768 lesions laid out on water agar, 71.8% was void of *A. solani*, 48.0% was void of *A. alternata* and 34.0% was void of both *A. alternata* and *A. solani*. Nevertheless, all these lesions looked very similar to those of early blight. Considering the large group of lesions void of Alternaria spp., there must be another cause for the development of such lesions than *A. solani* and *A. alternata*. Taking into consideration the particular state of the affected crops in 2009, boron deficiency may be part of it. In addition, ozone stress may be a second player. The combination of boron deficiency and ozone stress may be the cause for the development of early blight like lesions. (See Mimi case, this article).

*A. alternata* is not as successful as *A. solani* to form lesions in the open. Only in 6% of the lesions with *A. alternata*, it was found as the single organism present. If we consider *A. alternata* not as an invader but instead to infect healthy foliar tissues to form lesions, and lesions of *A. solani* and *A. alternata* to be formed at random, than there should not be more than 6% of the lesions co-infected by the two organisms at the maximum. Considering that at the time of sampling about 10% of the foliar surface was covered by lesions, it could be in fact only 0.6%. This value is far from the found value of 94%. Like earlier in the season lesions yielding *A. alternata* only may be caused by other causes as well.

## FINAL CONCLUSIONS

During the growing season of 2009 and especially so in June and July, many lesions were formed, which looked very similar to lesions caused by *A. solani*, but were void of *A. solani* and *A. alternata* (Photo 2).

The most probable cause for the early blight like lesions in June and July is ozone stress in combination with shortage of boron, and especially so with varieties respectively crops with shallow roots or weekly developed root systems.

It must be concluded that *A. alternata* is a very successful invader of necrotic lesions, but has a very poor capability if any to infect green foliage in the field (Spits *et al.*, 2005).

## LITERATURE

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