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Comparing pathogenicity of *Alternaria solani* and *Alternaria alternata* in potato

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SUMMARY

Large-scale surveys of leaf lesions on potato in the period of 2009-2012 showed that many symptoms were not caused by *Alternaria solani*, but were of physiological origin. As a large percentage of these lesions contained the fungus *Alternaria alternata*, the question arose whether or not *A. alternata* is capable of causing necrosis. In this study we show and confirm that *A. alternata* appears to be unable to infect potato leaflets and cause lesions, suggesting there is no need to specifically treat against this saprophytic fungus.

KEYWORDS

Koch, lesions, Alternaria, ozone

INTRODUCTION

Early blight receives more attention in recent years as problematic pathogen on potatoes. Due to climate changes and more specific treatments for late blight, an increase in the amount of early blight was observed. However, as the symptoms strongly resemble symptoms caused mostly by deficiencies and ozone damage, people in the field often have difficulty distinguishing early blight symptoms from others. As a result in practice there have been numerous cases of people applying fungicides to a non-biologic cause of symptoms, with little to no result as consequence. In a few instances, they went as far as saying they were dealing with fungicide-resistant early blight.

On top of this problem with diagnostics is the discussion whether or not *A. alternata* is capable of causing lesions like *A. solani* does, therefore warranting a fungicide treatment. As *A. alternata* is present in high concentrations in the air, it is often found inside lesions. Lacking other obvious biological causes, such as *A. solani*, it is often assumed that *A. alternata* is the cause of lesions.

In this study we test and compare the pathogenicity of *A. solani* and *A. alternata* on various cultivars of potato. The study is performed both in a detached leaf assay and in a field trial. Additionally, the survey database allows for statistical analysis of co-occurrence, suggesting either competitive or non-competitive interactions between both species.

MATERIALS AND METHODS

Isolates of A. solani and A. alternata

For both species, three different isolates were used. Two *A. solani* isolates were obtained from practice fields in 2011. The third isolate was obtained from the Fungal Biodiversity Centre (CBS, CBS107.61). Likewise, two *A. alternata* isolates were obtained from practice fields, while the third isolate, A906NL11.2 was included as a commonly used isolate in studies.

Field trial

Plants of seven cultivars (Innovator, Markies, Miranda, Seresta, Festien, Aveka, Valiant) were planted in sandy soil (Wijster, the Netherlands). Three treatments were included: inoculation with *A. solani*, inoculation with *A. alternata* and an untreated control. Each treatment contained three replicates of at least 8 plants per cultivar per replicate. Inoculation was performed using a cocktail of the three aforementioned isolates at the flowering stage of potato growth. 10.000 spores/ml were applied during calm wind conditions. Lesions were counted and analysed in the laboratory for presence for either *Alternaria* species.

Detached leaf assay

Leaflets were obtained from the same plants used in the field trial (Wijster, The Netherlands, sandy soil). These plants had not received any treatments with fungicides with efficacy against early blight. Leaflets were placed on water agar (15g/l), abaxial side up.

In order to give both fungi maximum chances to infect, small wounds were made on the abaxial side of half the leaflets using a sterile scalpel. The other leaflets remained unwounded. Per leaflet 10 drops of 10µl each, containing spore suspensions (10000 spores/ml) were placed. Suspensions contained spores of one of three *A. solani* isolates, one of three *A. alternata* isolates or no spores (untreated control). Petri dishes were sealed and placed into a climate chamber for 7 days for disease development (20°C, 16h light). Lesion development and size were assessed at this point.

RESULTS

Detached leaf assay

Lesions only appeared on leaflets inoculated with *Alternaria solani* (Figure 1). *Alternaria alternata* was unable to create lesions under any condition. Wounding of the leaves increased the size of lesions caused by *A. solani* (Figure 2), most likely due to the easier access the fungus has to the target cells, starting the disease earlier. Isolate 3 of *A. solani* contained a spore solution with less than 10.000 spores/ml, but high amounts of mycelium. It is shown that when wounded, this does not affect the ability of the fungus to cause lesions, but in unwounded leaves, lesions are smaller, pointing to the importance of spores in spread of the disease.

***Figure 1**. Detached Leaf Assay. Lesions caused by A. solani appear on all cultivars, whereas A. alternata is unable to create lesions on leaves. Shown here is cultivar "Seresta", although results apply to all cultivars. Wounding did not allow A. alternata to create any lesions*

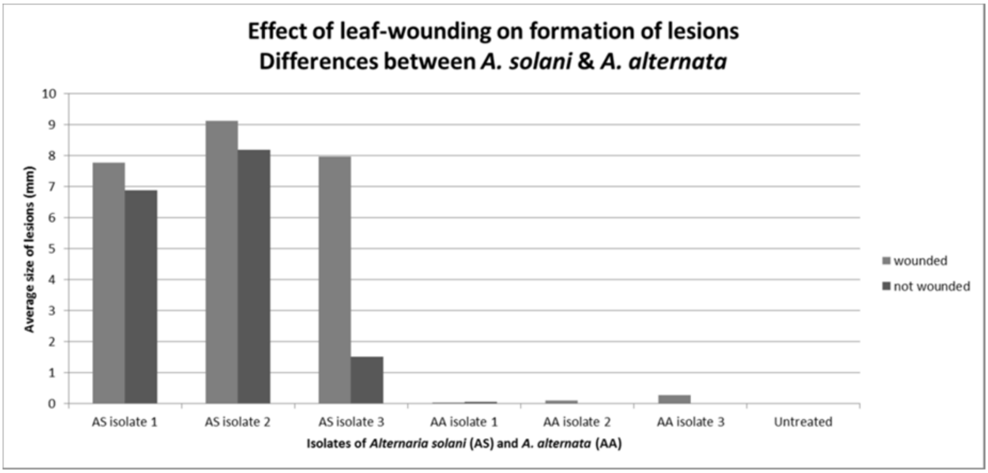

***Figure 2**. Effect of wounding of leaves on the lesion size. Seven cultivars are included in the trial. A. solani (AS isolate 1-3) causes lesions on both wounded and unwounded leaves, showing its role as a pathogen. A. alternata (AA isolate 1-3) shows no significant lesions compared to the untreated control*

While *A. alternata* does not create lesions on its own, the discussion arose whether or not it can make lesions created by other causes, like *A. solani*, bigger. This would still warrant treatment of *A. alternata*. On top of lesions caused by *A. solani*, we applied spores of *A. alternata.* Measuring the lesions after one week showed that *A. alternata* did not enhance lesion growth (Figure 3).

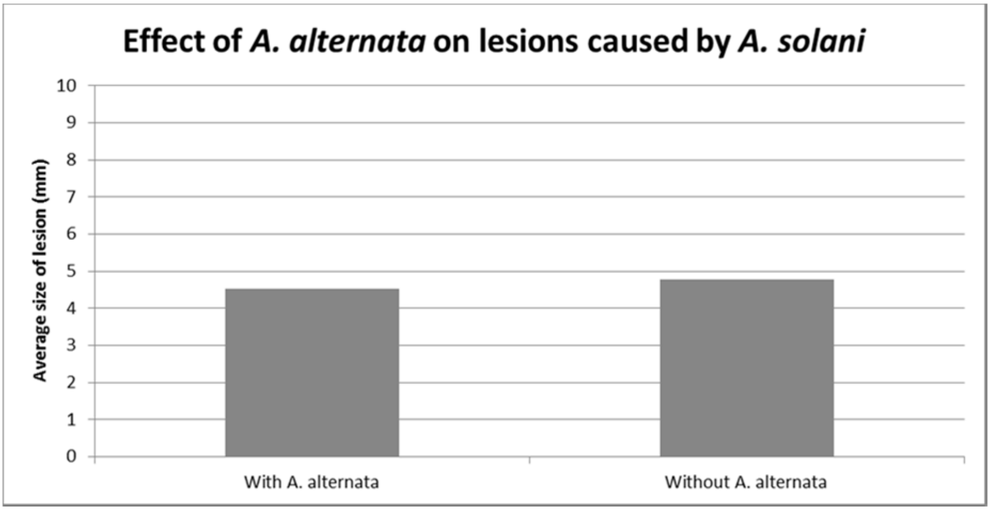

***Figure 3**. A. alternata on top of A. solani. Based on this small experiment, where A. alternata was placed on top of lesions caused by A. solani, there is no enhancing effect of A. alternata on lesion growth*

Field trial

Similar to the detached leaf assay, presence of *A. alternata* did not lead to more lesions in the field. Problematic with field trials concerning *A. alternata*, is the naturally high concentration of the fungus in the air. Spores of the fungus are a known allergen for hay fever-like reactions. Due to this high concentration, *A. alternata* is often found in lesions caused by pathogens or physiological damage. Inoculation of plants in the field with *A. alternata* therefore did not lead to more or bigger lesions (Figure 4). All fields contained a certain amount of lesions before inoculation, most likely caused by ozone. The fields inoculated with *A. solani* contained more lesions, caused by this fungus.

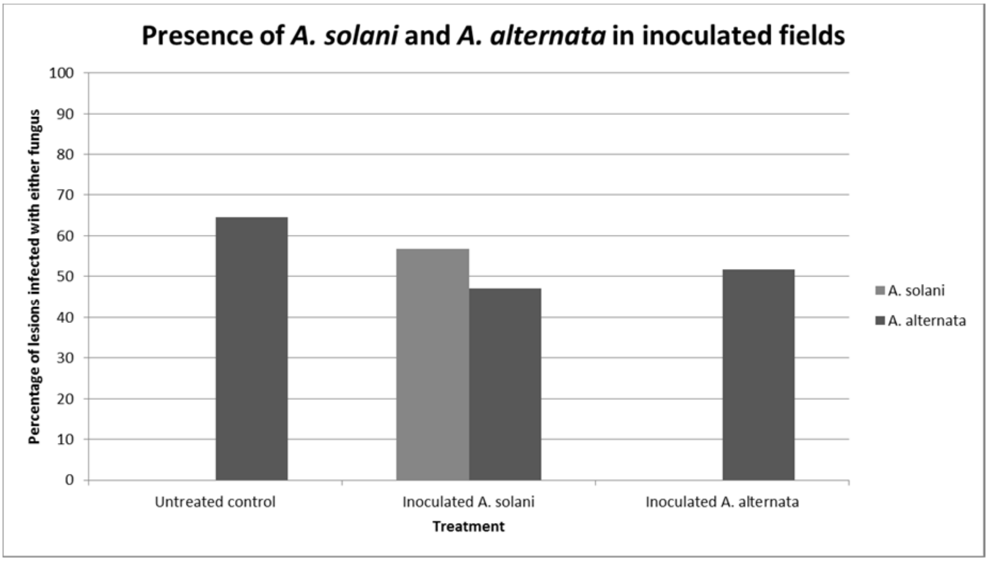

***Figure 4**. Inoculation with Alternaria alternata does not lead to development of lesions. As a saprophytic fungus on potato, it does find its way into lesions already present. A. solani was only found in the trial fields inoculated with this fungus, confirming its role as a pathogen and showing there was no cross-contamination to other fields*

Statistical analysis of co-occurrence

Based on the hypothesis that two different species that are both pathogens on the same host plant are competitors of each other, we analysed our data on co-occurrence of both *Alternaria* species. If both *A. solani* and *A. alternata* are pathogens, presence of one fungus would depend on the presence or absence of the other. In the treatment where both fungi are present, we gathered 265 lesions and determined the presence of either fungus. Table 1 shows the statistical analysis of the results. *A. alternata* appears independent of *A. solani*. This is in line with our expectations. *A. alternata* lives on dead tissue, regardless of the cause. It appears in 51,7% of the lesions, regardless of whether or not these are infected with *A. solani*.

Table 1*.* *Analysis of co-occurrence of A. alternata and A. solani. Based on the chi-square test, both fungi appear independent of each other*

2012 trials		
Total # of lesions	265	
Total # of lesions infected with A. alternata	137	
% of total lesions infected with A. alternata	51,7	
Total # of lesions infected with A. solani	197	
	With A. alternata	Without A. alternata
Observed # of lesions of A. solani	101	96
Expected # of lesions of A. solani (51,7% of 197)	101,85	95,15
Chi-square test	p = 0,9041	

DISCUSSION

Based on our large-scale studies into lesions on foliar tissue of potato in recent years, we started to have our doubts about the pathogenic capabilities of *Alternaria alternata*. If the fungus is a true pathogen and present in such high quantities that it warrants hay fever alerts, there should hardly be any healthy plant left standing in the field. Combined with the fact that damage caused by ozone and deficiencies is an underestimated problem this led us to investigate the role of the fungus compared to its big brother *A. solani*.

In the study we presented here, we applied the postulates of Koch. Surprisingly, in literature we were unable to find a study where this was already done for *A. alternata*. In the field, *A. alternata* is already present in high concentrations, which is why it is often found in lesions and named as the cause of it, especially if the lesion lacks other fungi due to being caused by physiological damage. When plants were inoculated with high concentrations of *A. alternata*, this did not lead to the appearance of more lesions (figure 4). *A. solani*, as expected, did create more lesions.

In the lab, we provided both fungi with ideal circumstances: applied directly on the leaf; wounded to ease access and development under controlled conditions. *Alternaria alternata* once again failed to create lesions on any of the cultivars.

While our experiments were relatively simple, they are important. In Dutch agricultural practice, growers sometimes apply fungicides specifically for *A. alternata*. Our results indicate that this is not necessary. *Alternaria solani* is the true pathogen in the family and is the one that should be treated with fungicides. These results are confirmed elsewhere in these Proceedings by Stammler *et al.* Other causes of leaf spots, such as damage caused by ozone or nutrient deficiencies, are the bigger danger to potato yield.