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Pathogenicity of Alternaria-species on potatoes and tomatoes

GERD STAMMLER, FRANZISKA BÖHME, JASMIN PHILIPPI, SIMONE MIESSNER & VANESSA TEGGE

BASF SE, Agricultural Center, Speyerer Strasse 2, 67117 Limburgerhof, Germany

SUMMARY

Various experiments were performed to analyze the virulence of *Alternaria solani* and *Alternaria alternata* on tomatoes and potatoes. Isolates were made from potatoes from different regions worldwide and a number of isolates were used as pure cultures for inoculum on tomatoes in the greenhouse and potatoes in the greenhouse and in the field. Conditions and host cultivars were varied in order to achieve infections. However, in all trials *A. solani* isolates were highly virulent while *A. alternata* isolates showed low or no symptoms after inoculation. Therefore doubts are justified if *A. alternata* is a causal agent of Early Blight or if it is rather a secondary invader which lives saprophytic on lesions and is therefore often isolated from leaf spots.

KEYWORDS

Alternaria solani, Alternaria alternata, Early Blight, Pathogenicity

INTRODUCTION

Early blight is in several potato growing regions a serious disease. The disease can be controlled by well-timed fungicide applications. In Western Europe two different *Alternaria* species are mainly discussed as causal pathogens, *Alternaria solani* and *Alternaria alternata* (Philippi 2011, Boehme 2013). Studies from Russia postulate also that the species *A. tomatophila, A. tenuissima, A. infectoria* and *A. arborescens* are involved in the Early Blight disease (Orina *et al.,* 2012). In Brasil *A. grandis* has been published as causal agent of Early Blight (Rodrigues *et al.,* 2010). *A. alternata, A. tenuissima, A. arborescens* and *A. infectoria* produce small spores, *A. solani, A. tomatophila* and *A. grandis* produce larger spores. The importance of the Alternaria species in Early Blight is seen differentially. Especially the impact of the small spored species, mainly *A. alternata* is controversially discussed. While some researchers see both species as causal agents or discuss a pathogen complex of *A. solani* and *A. alternata* (Leiminger and Hausladen 2012, 2013), others are convinced that only *A. solani* is pathogenic (Turkensteen *et al.,* 2010). In the last case *A. alternata* would be a saprophyte, which colonizes leaf lesions wherever this lesion came from (e.g. ozone damage, variation specific, caused by *A. solani* etc.) and is therefore a secondary invader. Between the different opinions consensus is found that *A.*

solani is pathogenic. Different experiments were performed with the objective to contribute to the elucidation of the importance of *A. alternata* in the Early Blight disease.

MATERIALS AND METHODS

Isolates

Isolates from 2010, 2011 and 2012 were made from leaf samples from potatoes. Leaf samples were sent dried to BASF, Limburgerhof and leaves with typical Early blight symptoms were sterilized, lesions were cut out and placed on 2 % malt agar (at 16°C or 22°C). Outgrowing mycelium was transferred on new petri dishes. Pure cultures were made by additional transfers. Isolated species were determined by their spore size and shape. Different isolates from different regions were used in the following experiments.

Greenhouse trials

Pathogenicity trials with single isolates on tomatoes in the greenhouse

Three weeks old tomato plants were inoculated with spore suspensions from the different isolates in order to determine the pathogenicity of each isolate. 10 ml spore suspension of each *A. alternata* and *A. solani* isolate was made with deionized water and 2 % malt solution, respectively. This experiment was conducted twice. The concentration of the spore suspensions were around $\sim 10^6$ spores per milliliter for *A. alternata* and $\sim 10^5$ spores per milliliter for *A. solani*. Spores were suspend in deionized water, 0.2 % malt extract or 2 % malt extract. Inoculated plants were incubated in a moist chamber with 20 °C and 95 % relative humidity. During the first 24 hours a lid covered the plants in order to prevent the spore suspension from washing off the leaves. The plants were kept for one week and rated regularly.

Additional trials were performed under same conditions but with mixtures of different isolates of *A. alternata* and *A. solani.* The idea behind was if a complex of both species leads to stronger infections.

In another experiment, tomato plants were wounded slightly with a tooth brush or stronger with a needle with the intention to enhance the infection by *A. alternata*. Infection was carried out with 2 % malt and the same conditions as described above.

Additional experiments were performed with older tomato plants in order to vary the age of the plants at inoculation time point since there are hints in literature that older leaves are more susceptible than younger ones (Rotem 1994).

Virulence trial with isolates in mixture on potatoes in the greenhouse

Host plants were three weeks old potatoes of the variety Aveka and Kuras which were inoculated with 50 ml of each single suspension as described above. An air sprayer with a nozzle size of 0.8 mm and 0.5 bar was used to coat the leaves with the suspensions. Two plants of each cultivar were sprayed with 2 % malt solution to serve as control plants. In between two suspensions the sprayer was washed with water in order to prevent contamination. Inoculated plants were incubated in a moist chamber with 20 °C and 95 % relative humidity. During the first 24 hours a lid covered the plants in order to prevent the spore suspensions from washing off the leaves. The plants were kept for two weeks and were visually rated regularly.

Field trial

Two potato varieties, Aveka and Kuras, were planted into a field. The field consists of two separate fields each with cultivar Aveka and Kuras, respectively. Every subfield contains 100 completely randomized plots, implying there are four replications per treatment. One plot of the size of 3 m x 1.5 m comprises two rows of potatoes. The field was fertilized once with 350 kg*ha-1 ENTEC® perfect, which is equivalent for 25.5 kg N*ha-1. To avoid infections with *Phytophthora infestans* (Late blight) one application with Ranman® (0.2 kg*ha-1) and one application with Revus® (0.6 kg*ha-1) was carried out. There were additional treatments against weeds and *Leptinotarsa decemlineata* (Colorado potato beetle). Inoculation date was five weeks after emergence of potato sprouts. The preparation of inoculum started a few weeks prior to the field inoculation.

For each *A. solani* strain one two weeks old fungal petri dish was pureed (Ultra Turrax) with 100 ml deionized water. Then 15 flasks with 50 ml of V8 medium were inoculated with 500 µl of the fungal-agar-puree. The flasks were placed in an agitating chamber (150 rpm, 22 °C). After one week the resulting mycelium was pureed for one minute and 1 ml mycelium puree was pipetted on 2 % malt agar plates and incubated for another two weeks at 22 °C and 12 h photoperiod. With this method it was possible to inoculate more than 400 petri dishes for each strain in a relatively short period of time.

As *A. alternata* produces enough spores only 60 plates per strain were needed for the inoculum preparation. Thus fungal material was transferred in the usual five-point manner on 2 % malt agar containing petri dishes two weeks previous to the inoculation date.

The spore suspensions were prepared with 2 % malt solution and adjusted to a spore density of $1.78*10^4$ spores per milliliter. Afterwards the spore suspensions were mixed according to the treatments and 1.4 liters of each treatment were taken into the field. In turns every suspension was poured into a backpack sprayer and sprayed with 2 bar onto the plots. After each spore suspension the backpack sprayer was cleaned with water in order to prevent contamination. To achieve better conditions for infection the field was irrigated before and after inoculation. In frequent time intervals the plots were rated visually. The trial plan is shown in Table 1.

Table 1. *Trial plan for the field trial 2012 in potatoes (var. Aveka and Kuras). As 121, As 185, Aa 204 and Aa 257 represent isolates of A. solani and A. alternata, respectively*

Treatment number	Isolates	Ratio A. solani (As) : A. alternata (Aa)
1	Not inoculated control	-
2	As 121	100
3	Aa 204	100
4	As 121 : Aa 204	10 : 90
5	As 121 : Aa 204	50 : 50
6	As 121 : Aa 204	90 : 10
7	As 185	100
8	Aa 257	100
9	As 185 : Aa 257	10 : 90
10	As 185 : Aa 257	50 : 50
11	As 185 : Aa 257	90 : 10
12	As 121 : Aa 257	50 : 50
13	As 185 : Aa 204	50 : 50

RESULTS AND DISCUSSION

Isolates from diseased leaf samples

Both species, *A. alternata* and *A.solani* were isolated from diseased leaves. Interestingly, the temperature during the incubation of the leaves in the agar plates played an important role in the success of isolation of *A. solani* or *A. alternata*. Lower temperatures favored isolation of *A. solani* and higher ones the isolation of *A. alternata* (Figure 1). This is a very important finding because it shows that the isolation success depends at least on the temperature. However the fact that both species could be isolated from typical lesions does not necessarily show that both species are virulent. To show this, the Koch's postulates needed to be fulfilled. Therefore we made inoculation trials under various conditions which are described in the next chapters.

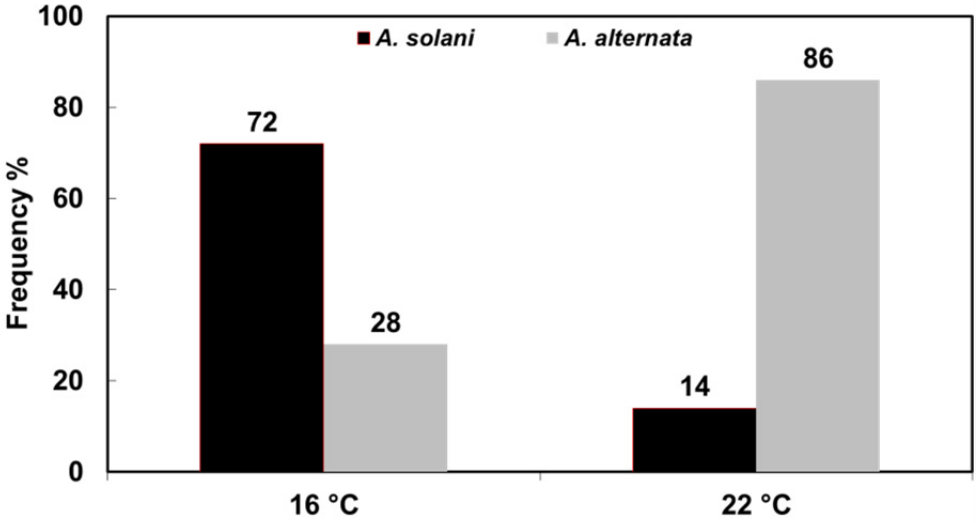

Figure 1. *Influence of temperature during isolation process on the success of isolation of A. solani or A. alternata. Data represent 161 isolates which were made from 10 samples*

Greenhouse trials with tomatoes

In all greenhouse trials no typical infections were achieved with various isolates of *A. alternata* but easily with all isolates of *A. solani* (Figure 2, 3). Mixtures did not show higher infection rates, which indicates that a complex of two species did not enhance virulence. When spores were suspended in 2 % malt extract, the infection was much higher. The very weak symptoms caused by *A. alternata* in the 2 % malt extract experiments were untypical black spots on the leaf surface which could be physically removed.

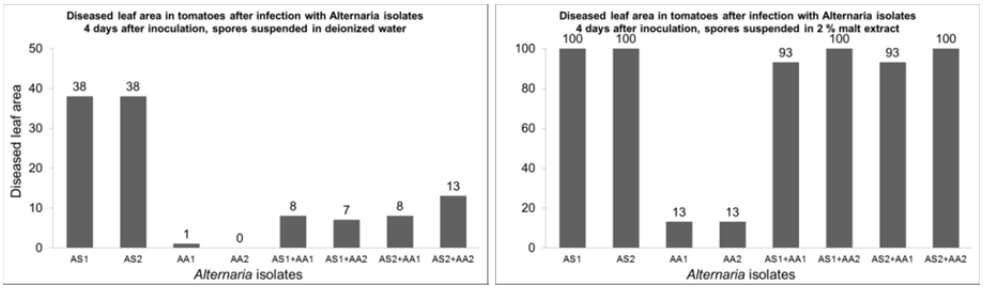

Figure 2. *Infection of tomatoes with strains of A. solani (AS1, AS2), A. alternata (AA1, AA2) or mixtures of the isolates (AS1+AA1, AS1+AA2, AS2+AA1, AS2+AA2). Spores were suspended in deionized water (left) or 2 % malt extract (right)*

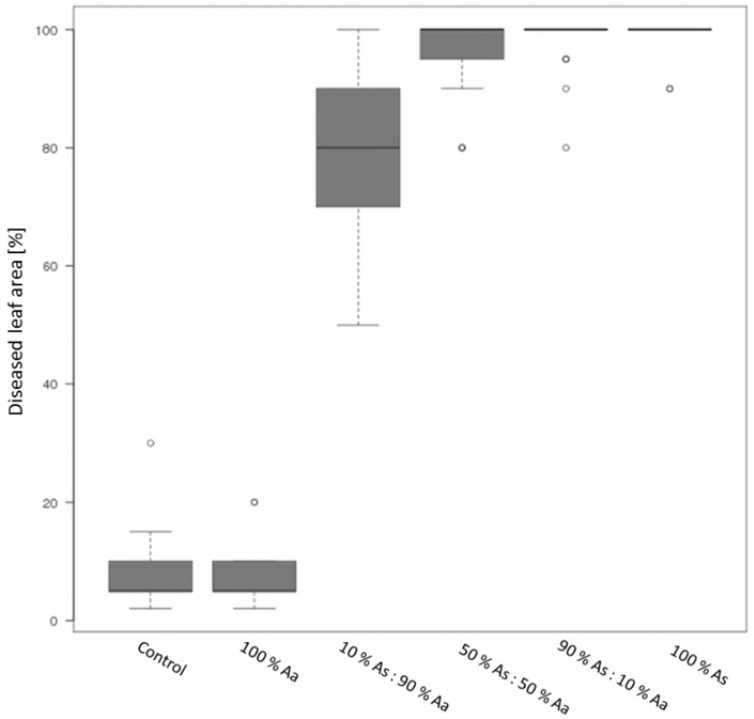

Figure 3. *Box and Whisker plot of a greenhouse trial with tomatoes. Plants were infected with isolates of A. solani (As), A. alternata (Aa) or mixtures of the isolates in different ratios (90:10, 50:50, 10:90). Spores were suspended in 2 % malt extract*

A trial on two months old tomato plants was done according to the previous experiments in the greenhouse. Ten leaves of two months old tomato plants were inoculated in order to divide the whole plant into sections with differently old leaves. The idea was to see whether each section shows different susceptibility against Early Blight. There were already Early Blight symptoms visible after three days post inoculation. Plants that were inoculated with *A. solani* showed lesions and the leaves started to hang (Figure 4). On the contrary the treatment with 100 % spore suspension of *A. alternata* looked as good as the control plants with no inoculation.

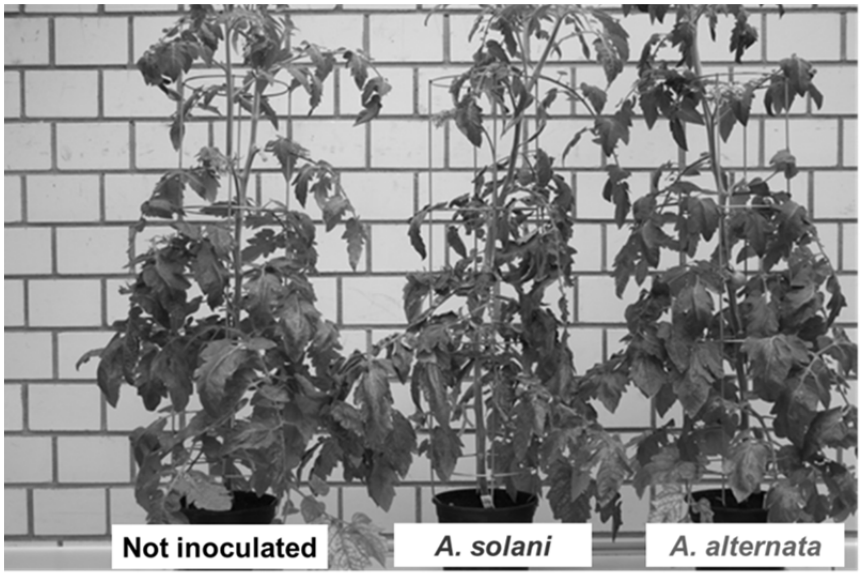

Figure 4. *Two months old tomato plants inoculated with spore suspensions made with 2 % malt solution. From left to right: Control, plant inoculated with a spore suspension of 100 % A. solani (As) and one with 100 % A. alternata (Aa), 3 days after inoculation*

After seven days post inoculation plants inoculated with high percentages of *A. solani* in the spore suspension lost already most of their leaves and showed high rates of necrosis as to compare in Figure 5. On the other hand high percentages of *A. alternata* in the mixture led only to weak symptoms and plants looked almost like the untreated control. The plants were divided into lower, middle and upper leaf level. As there was no visible differentiation in disease severity between the middle and the lower section during the rating process, they were combined to lower leaf level. So there were in total 2 sections, upper and lower leaf level, to rate and evaluate.

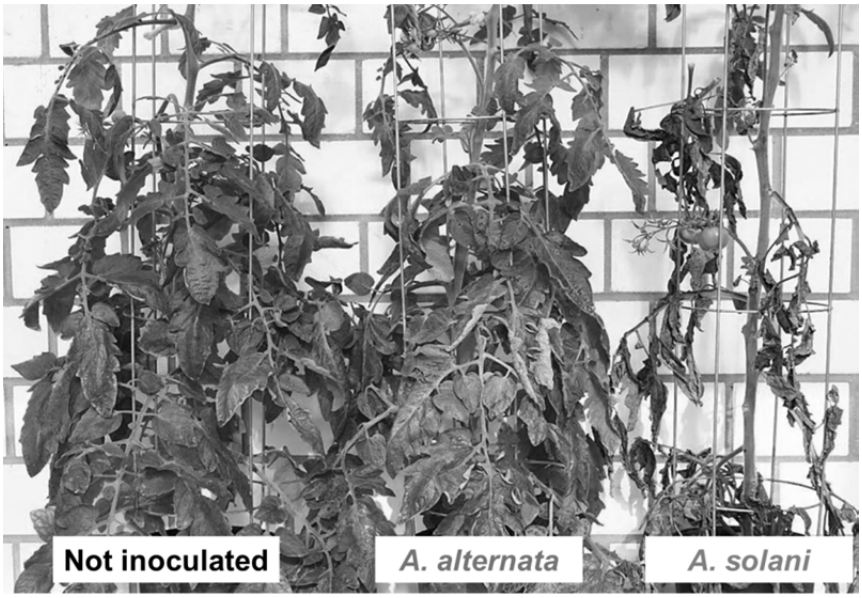

Figure 5. *Two months old tomato plants inoculated with spore suspensions made with 2 % malt solution. From left to right: Control, plant inoculated with a spore suspension of 100 % A. alternata and one with 100 % A. solani, 7 days after inoculation*

At the end of the period of observation the rating results were used to calculate the area under disease progress curve (AUDPC) to determine the intensity of disease. Figure 6 shows the results concerning the differences between the treatments. The tendency is the same as in previous experiments. The higher the content of *A. solani* in the mixture the more severe is the infection. Infections caused by a 100 % spore suspension of *A. alternata* cause only weak infections. Thus AUDPC is small and significant smaller than in the other treatments but not significantly different from the untreated control.

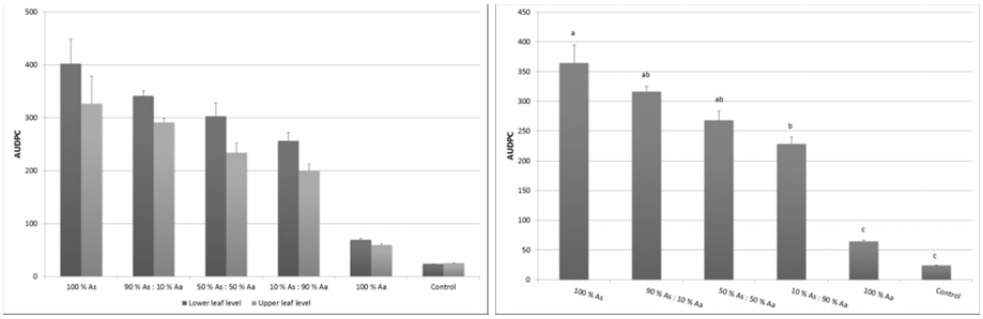

Figure 6. *Left: AUDPC for infection progress in the mixture experiment on two months old tomato plants in the greenhouse for upper and lower leaf level. Rating took place 3 - 7 dpi. Simple means with standard error. Right: Statistical analysis of AUDPC of the mixture experiment on two month old tomato plants in the greenhouse. Means with standard error. Same letter means no significant differences (α=0.05, LSD). 7 dpi*

Even wounding (slight with tooth brush on upper and lower leave side or stronger with needles) did not lead to infections with *A. alternata* (Figure 7). This experiment was not done with *A. solani*, because under this incubation conditions a 100% disease severity would occur even without wounding.

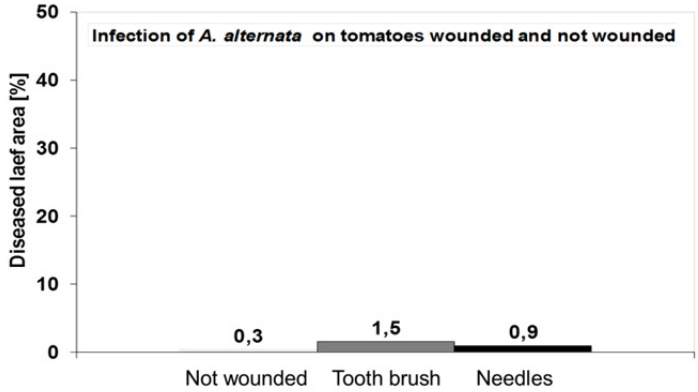

Figure 7. *Mean values of 2 trials with 3 replicates of 8 isolates each on 14 days old tomato plants. Inoculation was done directly after wounding with 2 x 10^6 Spores/ml. Evaluation 9 days after inoculation*

Greenhouse trials with potatoes

Figure 8 shows a typical result of inoculation experiments with potatoes in the greenhouse. Infected leaves are sometimes dropped soon after symptoms appeared. *A. solani* infected potato plants but *A. alternata* did not. No differences in infection between the two potato cultivars Aveka and Kuras were observed.

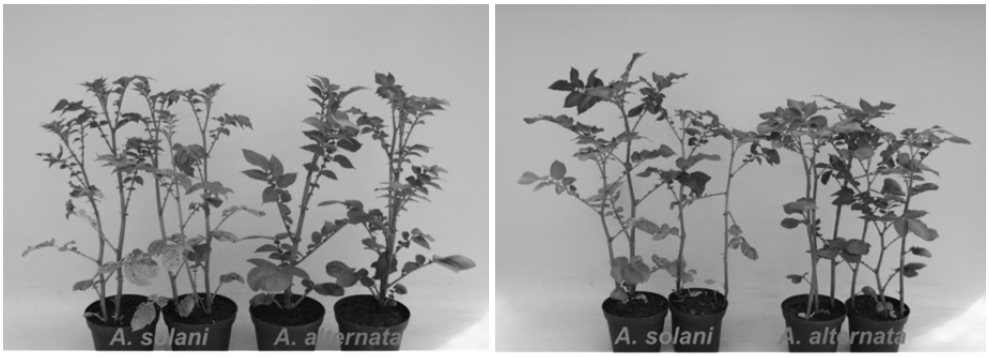

Figure 8. *Infection of potato plants in the greenhouse with A. solani and A. alternata. Lower leaves were inoculated. Plants infected with A. solani showed yellowing of leaves, the typical brown target spots and dropping of leaves. No symptoms occurred on plants infected with A. alternata. Left: cultivar Kuras. Right: cultivar Aveka*

Field trials with potatoes

Field trials were performed with the varieties Aveka and Kuras. Two isolates of *A. solani* and 2 isolates of *A. alternata* were used for inoculation alone and in mixtures with different ratios. Only isolates of *A. solani* or mixtures of *A. solani* and *A. alternata* infected the plants. Four days after infection first lesions were visible in the *A. solani* infected plots (Figure 9). In *A. alternata* plots no or very low symptoms occurred. From most of the spots which occurred in *A. alternata* plots, *A. solani* was reisolated (20 isolates were made, 18 were *A. solani*, 2 were *A. alternata*) which indicates a natural "backround" infection. This was also seen in the not inoculated control plots. Figure 10 shows the infection of the different isolates and mixtures during the time period of the trial. The values are mean values over both trials in Aveka and Kuras. Data show that the more *A. solani* is in the inoculation suspension, the higher the disease rating is.

Figure 9. *Infection of potato plants in the field with A. solani. First symptoms occurred four days after inoculation (left) and disease developed further within the next three weeks (right) to very high disease levels and many dropped leaves. Here symptoms on variety Aveka.are shown*

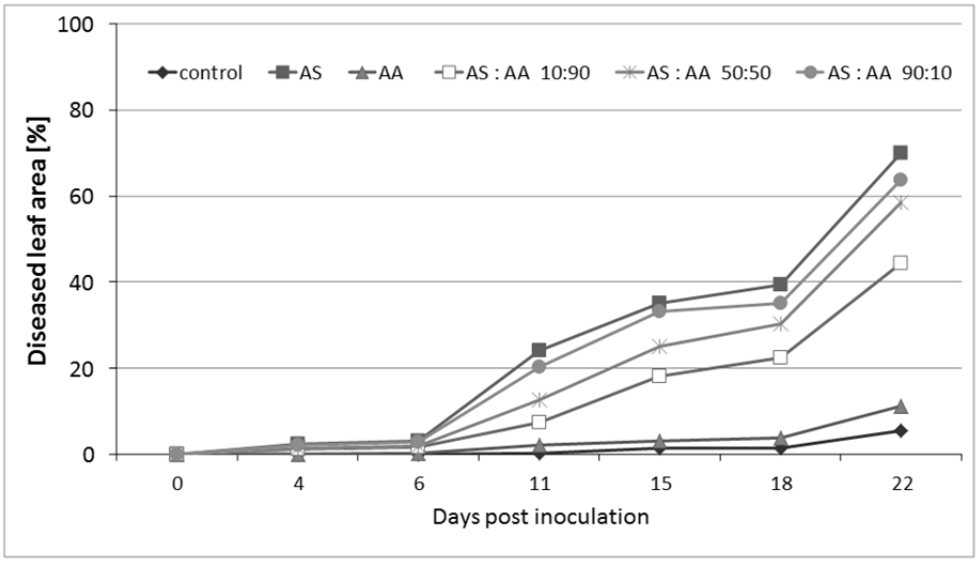

Figure 10. *Infection of potato plants in the field over the time period of evaluation. Data show that the more A. solani was in the spore suspension, the higher the disease level was. AS means A. solani and represents two different isolates, AA means A. alternata and represents two different isolates. The values given are mean values of two isolates and mixtures in two different varieties from 4 replicates, which is then the mean value of 16 plot estimations*

CONCLUSIONS

Different trials were made to evaluate the virulence of *A. solani* and *A. alternata* in potatoes and tomatoes. All trials showed that the species *A. solani* is highly virulent, while *A. alternata* is not or very low virulent under the different conditions we used. Since mixtures of *A. solani* and *A. alternata* did not lead to higher infection levels than *A. solani* alone, the theory of a disease complex is not justified from our view. Therefore our conclusion is that the main pathogen of Early Blight is *A. solani*. If other large spored Alternaria species, such as *A. grandis* and *A. tomatophila* play a role in Early Blight in Europe is currently under investigation. The fact that *A. alternata* can often be isolated from lesions on potato plants is from our view the result that *A. alternata* grows as a saprophyt there and is a secondary invader on such lesions.

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