

Methods for inoculation of tomatoes and potatoes with *Alternaria solani* in the greenhouse and field

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Gerd Stammler, Simone Miessner, Franziska Böhme
BASF SE, Agricultural Center, Speyerer Strasse 2, 67117 Limburgerhof, Germany

Isolation of strains of *A. solani*

Dried leaf samples with typical Early blight symptoms are sterilized, lesions were cut out and placed on 2 % malt agar (at 16°C or 22°C). Outgrowing mycelium is transferred on new Petri dishes. Pure cultures are made by additional transfers. Isolated species are determined by their spore size and shape.

Inoculation of tomatoes with single isolates in the greenhouse

Three weeks old tomato plants (cv. Goldene Königin) are inoculated with spore suspensions from different isolates. 10 ml spore suspensions of each *A. solani* isolate are made with deionized water or 0.2 % or 2 % malt solution, respectively. The concentration of the spore suspensions should be around $\sim 10^5$ spores per milliliter. Inoculated plants are incubated in a moist chamber at 20°C and 95 % relative humidity. During the first 24 hours a lid covers the plants in order to prevent the spore suspension from washing off the wet leaves. The plants are kept for one week and rated regularly to measure the disease progress. Disease progress is highest if spores are suspended in 2 % malt, followed by 0.2 % malt and water (Figure 1). Experiments can also be done in the same way with older plants (Figure 2)

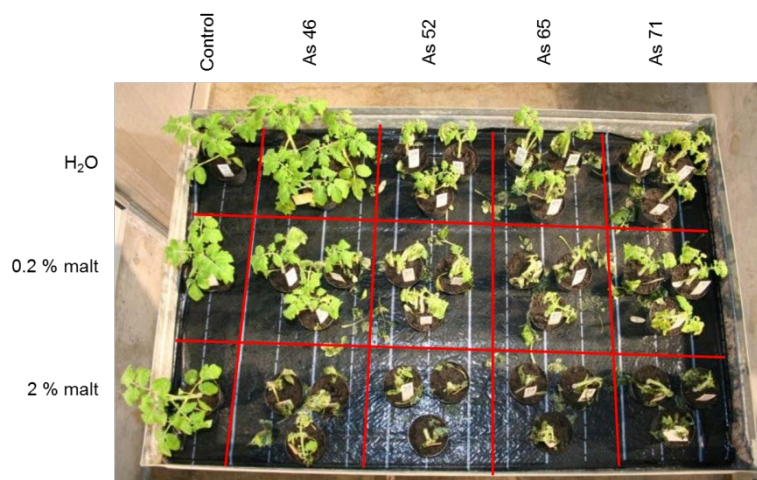


Figure 1. Tomato seedlings inoculated with different isolates of *Alternaria solani*. Spores were suspended in water, 0.2 % malt and 2 % malt, respectively. All strains caused disease; figure shows

disease progress 6 days after inoculation. Plants treated with medium without spores are still healthy (left).



Figure 2. Two months old tomato plants inoculated with spore suspensions made with 2 % malt solution. Left: not inoculated control plant. Right: inoculated with a spore suspension of *A. solani*. Photos were made 7 days after inoculation.

Inoculation of potatoes with single isolates in the greenhouse

Host plants are three weeks old potatoes of the variety Aveka and Kuras which are 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 is used to coat the leaves with the suspensions. Some plants of each cultivar are sprayed with 2 % malt solution to serve as control plants. In between suspensions of different isolates the sprayer is washed twice with water in order to prevent contamination. Inoculated plants are incubated in a moist chamber with 20 °C and 95 % relative humidity. During the first 24 hours a lid covers the plants in order to prevent the spore suspensions from washing off the wet leaves. The plants are kept for around two weeks and are visually rated regularly. Often infected leaves are dropped after first symptoms; this should be regarded in the evaluation! Figure 3 show typical disease symptoms in the greenhouse, 7 days after inoculation.



Figure 3. Infection of potato plants in the greenhouse with *A. solani*. Lower leaves were present at inoculation time point. Plants infected with *A. solani* showed yellowing of leaves, the typical brown target spots and dropping of leaves. Photo was made 7 days after inoculation.

Inoculation of potatoes with single isolates in the field

Susceptible potato varieties, such as Aveka and Kuras, should be selected for field trials and are planted into a field as usual done. Four replications per inoculation (i.e. spore suspension) and non-inoculated control is recommended. Plots should have a size of 3 m x 1.5 m, which comprises two rows of potatoes and plots must be randomized distributed. Fertilization with e.g. 350 kg*ha⁻¹ ENTEC® perfect, which is equivalent for 25.5 kg N*ha⁻¹ is recommended, but depends on location. To avoid infections with *Phytophthora infestans* (Late blight) applications with registered rates of Ranman® or Revus® are recommended since these products have no side effect on Early blight. Additional treatments against weeds and *Leptinotarsa decemlineata* (Colorado potato beetle) might be necessary. Inoculation date can be e.g. five weeks after emergence of potato sprouts.

For field trials a higher amount of inoculum is needed. The following paragraph describes a method for preparation of such higher amounts. Be aware that the preparation of inoculum has to start in advance, i.e. a few weeks prior to the field inoculation. For each *A. solani* strain one two weeks old fungal Petri dish is pureed using an Ultra Turrax with 100 ml deionized water. Then 15 flasks with 50 ml of V8 medium are inoculated with 500 µl of the fungal-agar-puree. The flasks are placed in an agitating chamber (150 rpm, 22 °C). After one week the resulting mycelium is pureed for one minute and 1 ml mycelium puree is pipetted on 2 % malt agar plates and incubated for another two weeks at 22 °C and 12 h photoperiod. With this method it is possible to inoculate more than 400 Petri dishes for each strain in a relatively short period of time.

The spore suspensions are prepared with 2 % malt solution and adjusted to a spore density of 1.78*10⁴ spores per milliliter. For each suspension 1.4 liters are prepared, placed on ice and taken into the field. Suspensions are poured into a backpack sprayer and sprayed with 2 bar onto the plots. After each spore suspension the backpack sprayer is cleaned twice with water in order to prevent contamination. To achieve better conditions for infection, the field should be irrigated before and after inoculation. In 1-3 days time intervals the plots should be evaluated. First symptoms might occur 4 days after inoculation and typical symptoms develop in the following weeks (Figure 4).



Figure 4. 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.