Artificial inoculation with *Alternaria solani* & *Alternaria alternata* (detached leaf, greenhouse and field)

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In vitro: detached leaf assays

- Used to test pathogenicity of isolates on various cultivars under controlled conditions
  - Spore concentration used is 10.000/ml.
  - Single leaflets are taken from untreated plants and placed with the bottom side facing upwards on water agar in a Petri dish (15g/L agar).
  - Optional: wounding of the leaflets using a sterile scalpel for easy access of the fungus
  - Spore suspensions are applied in droplets of 10µl (100 spores per droplet). If wounded, the droplets get sucked into the leaf.
  - Depending on size, one or two leaflets fit in a Petri dish
  - Petri dishes are closed and sealed with parafilm
  - Dishes are stored in a climate chamber, 20°C, 16 hours light/8 hours darkness.
  - Measuring of lesion size is done after 7 days of incubation

Greenhouse studies

- We usually do not perform greenhouse studies, as these plants often do not represent the plants in the field from a physiological point of view.

Field studies

- Spore suspensions are concentrated to 10.000 spores/ml
- In case of pathogenicity testing, spores are applied directly on the crop using a backpack sprayer ("worst case scenario" for the plants)
- In case of fungicide or host resistance testing, we work with infection rows in order to simulate more natural conditions whilst still maintaining control over the epidemics. Where possible, infection rows are planted on the west side of the trial to make use of the wind.
- Spores are applied preferably in evenings to avoid heavy sunshine and high temperatures. Wind needs to be minimal.
- Spores are applied when the plants are most susceptible. This is right before and during flowering

In case of host resistance testing, we prefer to separate 'early', 'middle' and 'late' cultivars and inoculate separately in order to strike at the right physiological stage.