

Isolation of *phytophthora infestans* from field collected leaves

- Collect leaves with a singles lesion, place the leaves individually in plastic bags or Ø 9 cm Petri plates containing 1,5 % water agar, and keep the leaves cool.
- Incubate the leaves until sporulation occurs at 15°C at a light intensity of 12 Wm⁻² 16 hours a day.
- Surface sterilizes a potato tuber with tap water and ethanol.
- Cut the tuber in slices of ± 0,5 cm, and place one or two slices in a Petri plate.
- Place a small piece of the edge of a growing lesion (ca. 0,5 cm²) under a tuber slice. For one lesion it's advised to place 4 pieces under tuber slices.
- After 5 days of incubation at 15°C the mycelium has grown through the tuber slice.
- Transfer a little plug of mycelium with a needle and put it on Rye agar (normal and selective) or V8 agar. Try not to touch the tuber slice. Incubate the Petri plates at 20°C.
- It's also possible to transfer the mycelium to potato leaves. Put the leaves abaxial site up in a Ø 90-mm Petri plate containing 1,5% water agar (WA). Place little drops (10 µl) of tap water on the leaf. Place a little mycelium plug in each droplet. Incubate at 15°C at a light intensity of 12 Wm⁻² 16 hours light a day.