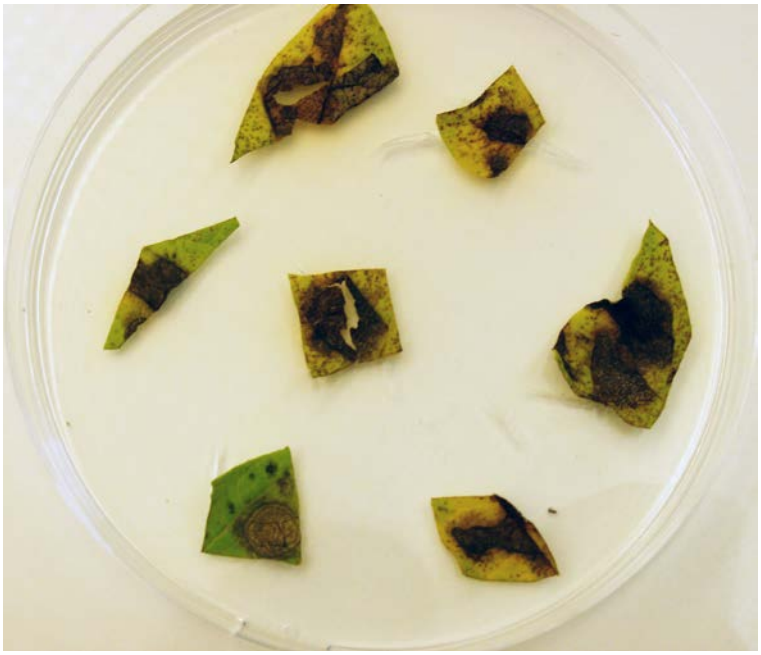
Isolation of *A. solani*

June 2014

- sample early blight (EB) infected leaflets at random during early blight disease epidemic within July and September
- cut out EB infected leaf pieces bearing a single lesion (0.5 to 1.0 cm in diameter) from infected leaves
- surface-sterilize leaf cuts in 5% NaOCl for 1 min and then wash in sterile, distilled water

  transfer necrotic leaf cuts to petri dishes containing synthetic low nutrient (SN) media (1 g KH_2PO_4; 1 g KNO_3; 0.5 g $MgSO_4x7H_2O$; 0.5 g KCl; 0.2 g Glucose; 0.2 g Sucrose; 600 µl 1M NaOH; 22 g agar; dissolved in 1 l distilled water). SN medium favours the production of spores, while the ratio of mycelium to spores increases on media with higher concentrations of nutrients.



- incubate agar plates for 3 days at 20°C under near ultraviolet light (Philips LTD 36W/80) with an alternating 12 h photoperiod
- check agar plates for sporulation. Sporulation is favoured on SN medium surrounding necrotic tissue
- Transfer single spores to new SN media. Single-spore isolations are carried out with a fine dissecting needle. Collect only one isolate per diseased plant in order to avoid collecting the same genetic individual.
- Incubate petry dishes at 23°C and UV light for further investigations