Identification of *Alternaria solani* GI in combination with analysis of the F129L substitution.

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The advantage with this method is that it is species specific for *A. solani* GI (tested against *A. alternata* and sequences on GeneBank) and that the PCR product can be sequenced in search for the amino acid at position 129 in the gene encoding cytochrome *b*, associated with loss of sensitivity to strobilurins. The PCR-conditions have been slightly revised since the publication.

DNA extraction

- Cut out a leaf disc containing one single lesion (*ca.* 5 mm ø), which include both green and necrotic tissue.
- Washed it twice in sterile distilled water and dried with paper towels.
- Homogenised using five glass beads (3mm) in a 2 ml micro centrifuge tube and shake at 5500 repeats per minute during 30 s (*e.g.* Precellys® 24 Bead Mill Homogenizer, Bertin Technologies).
- Add the extraction buffer (800 mL of 3% CTAB-EDTA, pH 8 or the amount recommended if using a kit). Shake again as above.
- Follow the CTAB-EDTA protocol or the kit protocol onwards.
- Re-suspend the DNA pellet in 30 µL distilled water if CTAB-protocol or as written in the kit-protocol.
- Dilute the samples to approx. 1 ng DNA μl^{-1}.

PCR protocol

Forward primer: Asol 129(319) 5´- ATG CGG GTG AAT ACG GTT AA 3´ (Edin, 2012)
Reverse primer: 143 Reverse primer 5´- CTC ACT TTG TTT ATG TTA TTT AAC CAA GAA TG 3´ (Rosenzweig *et al.*, 2008)

PCR solution conditions are: 2.75 mM $MgCl_2$ (final concentration), 0.2 mM dNTP, 0.02 µM of each primer, 0.05 U μl^{-1} of DreamTaq® DNA Polymerase (Fermentas International Ink, Canada) and 10X DreamTaq™ Green Buffer. The amount of DNA is approximately 5 ng to 15 ng dependent on the reaction volume wanted. The amplification product is 417 bp.

Master mix 5µl + 5µl DNA:	H_2O	2.25 µl
	Buffer (10x)	1 µl
	dNTP (2 mM)	1 µl
	MgCl (25 mM)	0.3 µl
	Primer F (10 nM)	0.2 µl
	Primer R (10 nM)	0.2 µl
	Taq (5 U μl^{-1})	0.05 µl

Master mix 35µl + 15µl DNA:	H_2O	21.25 µl
For sequencing	Buffer (10x)	5 µl
	dNTP (2 mM)	5 µl
	MgCl (25 mM)	1.5 µl
	Primer F (10 nM)	1 µl
	Primer R (10 nM)	1 µl
	Taq (5 U μl^{-1})	0.25 µl

PCR condition: 95ºC for 2 min, 40 cycles of 30 s at 95ºC, 30 s at 60ºC and 30 s at 72ºC, followed by a 5 min extension. Separate the products with electrophoresis on 1% agaorose gel stained with suitable dye and visually analyse as under UV-light.

Purify the PCR-products from the 50 µl reaction using *e.g.* Agencourt AMPure XP (Beckman Coulter, MA, USA) according to the manufacturer's manual and send for sequencing.

References

Edin, E. (2012). Species specific primers for identification of *Alternaria solani*, in combination with analysis of the F129L substitution associated with loss of sensitivity toward strobilurins. *Crop Protection* 38, 72-73.

Rosenzweig, N., Atallah, Z.K., Olaya, G. & Stevenson, W.R. (2008). Evaluation of Q_oI fungicide application strategies for managing fungicide resistance and potato early blight epidemics in Wisconsin. *Plant Disease* 92, 561-568