Whole genome sequencing elucidates the genomic background of fungicide resistant and -susceptible *Alternaria solani* strains

#ECFG15

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*Alternaria solani* (causes early blight) is of increasing relevance in Germany.
Alternaria solani (causes early blight) is getting more relevant for the farmers in Germany (Leiminger, J. H.; Hausladen, H.; 2011) -> yield losses of more than 50% are possible

High development of resistance against strobilurines (QoI´s) and carboxamides (SDHI´s) (Leiminger, Hausladen, Adolf, 2014; Gudmestad et al., 2012; Landschoot et al., 2016)

Incedence of SDHI resistant strains is increasing in Germany

Development of resistance against strobilurines (QoI’s) and carboxamides (SDHI’s)
Incedence of SDHI resistant strains is increasing in Germany
SDH mutants in Germany in 2014

- Locations w/ mutants
- Locations w/ only wildtype
SDHI resistance caused by point mutations in Sdh complex

- Affect the mitochondria of *A. solani* conidia
  - inhibit electron transport in mitochondrial respiration at complex II (Sdh)
- Binding site is formed by subunits B-C-D

**Known mutations in *A. solani*:**
- B: H278R/Y
- C: H134R
- D: D123E, H133R

Marcin Sarewicz, Artur Osyczka 2015 Physiol. Rev

Nicole Metz
Are there phenotypic differences between the mutants?
Do SDH mutations arise once and then spread?

Resequencing study:

What?
Two regions
Strains with and without mutations

How?
SNP calling to reference genome (NL03003)

Then what?
Looking for genetic clustering
>What is the genetic variation in SDHI mutants?
20 isolates, 16 from Germany
Half from Bavaria, Half from Lower Saxony

“Dutch” isolates kindly donated by Vivianne Vleeshouwers & Jaap Wolters, Wageningen University
Half with SDH mutation, Half without

- **Wild type**
- **B: H278R/Y**
- **C: H134R**
- **unknown**
SNPs found throughout the genome
nearly 600,000 SNPs, one every 213 bases!
We recover the SDH mutations

SDHB

SDHC
Our samples form three groups
Some samples are very similar
Geographical clustering?

- Bavaria
- Lower Saxony
- Netherlands
SDH mutations might seem to originate in different genetic backgrounds

- Wild type
- B: H278R/Y
- C: H134R
- unknown
SDH mutations might seem to originate in different genetic backgrounds

- Wild type
- B: H278R/Y
- C: H134R
- unknown
Establish the number of distinguishable genotypes: 4 (or 5)
Genotypes, partly geographical?

- Bavaria
- Lower Saxony
- Netherlands
- USA
Genotypes, partly defined by SDH mutations

- Wild type
- B: H278R/Y
- C: H134R
- Unknown
- Bavaria
- Lower Saxony
- Netherlands
- USA
Fitness, linked to SDH mutation?

- SpDensity3wk vs. Sdhregion
- GermRate3wk vs. Sdhregion

Geography:
- Bavaria
- Lower Saxony

Tamara Susanto
Fitness, linked to „genotype“
Conclusions:

SDH mutants belong to different genotypes
  So, potato trade did NOT spread the resistance!

H134R mutation arose at least twice (or three times?)

No trade off between mutation & virulence on this level
  yet „Genotypes“ might define fitness of the strains
Open Questions:

Besides SDH, which other genes are affected?
How do they define fitness? Can a aggressive SDH mutant arise easily?

These are ~2015 strains, what happens now?
Changes over time? One dominant SDH mutant strain?
Do we see development of new fitness effects?

This is a German / USA sample set. What are the backgrounds elsewhere in Sweden? Belgium? Poland?

H134R mutation in many genetic background, suggest it arose more than once. What are the implications?

Mutations against other fungicides also arose before / after What is their effect on the population genetics?
Next steps

Looking for collaborators!

> Expand sample sets

> Develop high throughput phenotyping

Link genetic diversity to life traits

Dive deeper into these low / high variation regions on the genomes

Follow-up study: are the fitter mutants spreading faster? Sample 2020!
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Poster B2-07
Genetic diversity of Alternaria in natural populations