Fungicide Resistance in Bavarian *Alternaria solani* and *Alternaria alternata* Field Isolates

Nottensteiner Mathias, Absmeier Carolin, Zellner Michael

Bavarian State Research Center for Agriculture (LfL), Institute for Plant Protection, Lange Point 10, 85354 Freising, Germany

Abstract

Alternaria leaf spots are a foliar disease of potatoes. Target-site mutations in *Alternaria solani* and *A. alternata* field isolates against Quinone outside inhibitors (QoI) fungicides (strobilurines) have been present in Germany since a decade. QoI-insensitive *A. alternata* isolates carry a G143A amino acid exchange caused by a single nucleotide polymorphism (SNP) in the cytochrome *b* gene. *A. solani* evolved a similar F129L mutation. A shift from the predominant *A. solani* genotype I to genotype II, which was exclusively associated with the F129L mutation, was reported from Germany after QoI approval for Alternaria control. Here, we found QoI mutations to be highly abundant in *A. solani* and *A. alternata* field isolates collected in 2016 in Bavaria, in southeastern Germany. The frequency of the F129L mutation, but not of *A. solani* genotype II, was about 10 % higher than in the last published data. Since 42 % of our screened *A. solani* genotype I field isolates were F129L mutated, this indicates a progression of QoI resistance mutation through the previously unaffected genotype. QoI mutations were present in all examined areas. An analysis of SNP diversity pointed to at least one independent evolution of the F129L mutation in each of both *A. solani* genotypes in Bavaria. Besides QoI mutation spread, reduced sensitivity of *Alternaria* spp. towards succinate dehydrogenase inhibitors (SDHI) fungicides (e.g. boscalid) is an emerging topic in Alternaria leaf disease control. SDHI target-site mutations were present in our *A. solani* and *A. alternata* field isolates collection at a rate of around 40 %. Remarkably, they only co-appeared in combination with a QoI mutation, suggesting a further adaption of *Alternaria* spp. populations to applied fungicide strategies.

Objectives

This study aimed to:

I) Record QoI mutation rates in *A. solani* and *A. alternata* field isolates from major potato growing regions in Bavaria
II) Determine the distribution of genotype I and genotype II within the collected *A. solani* field isolates
III) Explore whether QoI mutations can also be found in *A. solani* genotype I
IV) Investigate whether QoI mutations evolved multiple times by analyzing SNP diversity
V) Check the presence of SDHI fungicide target-site mutations in Bavaria in a subset of randomly selected *A. solani* and *A. alternata* field isolates

Symptoms


Alternaria leaf disease of potato


Early Blight
Alternaria solani


Brown Spot
Alternaria alternata

Methods

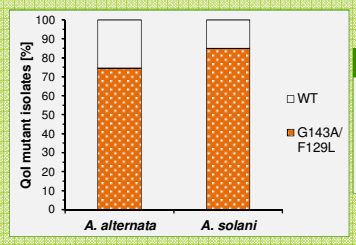
Field isolates: Alternaria diseased potato leaves were collected in August and September 2016 from 26 commercial and experimental potato fields , of which 47 *A. solani* and 55 *A. alternata* isolates were generated.

Target-site mutation identification: DNA was extracted from single-spore cultures. Gene segments encoding for target-site mutations against QoI (*cyt b*) and SDHI (*sdhb*, *sdhc*, *sdhd*) fungicides were amplified by PCR, gel-excised and sequenced.

Conclusions

- **85.1 % of *A. solani* field isolates and 74.5 % of *A. alternata* showed QoI target-site mutations, rendering QoI solo applications questionable**
- **The shift from *A. solani* genotype I to F129L-mutated genotype II has not progressed, but the overall presence of F129L has increased by about 10 %**
- **The F129L QoI mutation was present in *A. solani* genotype I field isolates at a rate of 41.7 %, possibly explaining the observed overall increase in the QoI mutation**
- **The presence of each two distinct SNPs causing F129L in *A. solani* genotype I and II indicates at least one independent evolution of QoI target site mutations in both genotypes**
- **QoI mutations were accompanied by SDHI target site mutations in 42.1 % of *A. solani* and 43.5 % of *A. alternata* field isolates, indicating a potential upcoming problem of dual fungicide efficacy loss in the control of *Alternaria* spp. in potatoes in Bavaria**

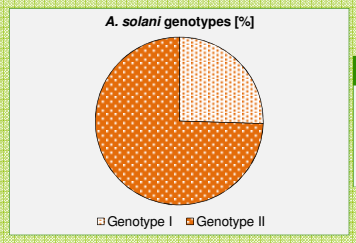
Results



Percent QoI fungicide mutants

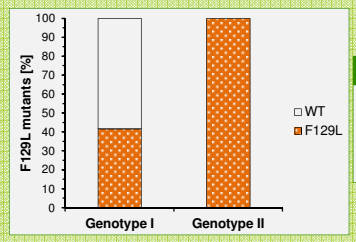
74.5 % of *n* = 55 *A. alternata* field isolates showed the G143A mutation and 85.1 % of *n* = 47 *A. solani* field isolates showed the F129L mutation in Bavaria in 2016.

WT: wildtype. QoI: Quinone outside inhibitor



Frequency of *A. solani* genotypes

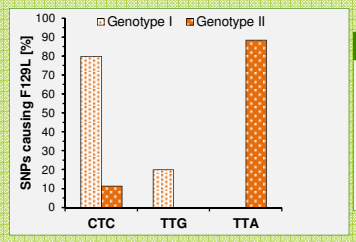
25.5 % of *n* = 47 *A. solani* field isolates were genotype I and 74.5 % were genotype II in 2016 in Bavaria. This resembled the results of Leiminger *et al.* (2014), who found 24.5 % (*n* = 94) *A. solani* genotype I isolates in 2011.



F129L in *A. solani* genotypes

41.7 % of *n* = 12 *A. solani* genotype I and 100 % of *n* = 35 genotype II field isolates showed the F129L amino acid exchange in Bavaria in 2016.

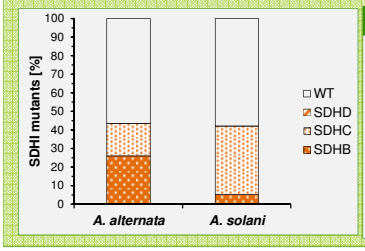
WT: wildtype



SNPs causing F129L in *A. solani*

In genotype II, the distribution of SNPs was 88.6 % TTA, 11.4 % CTC and 0 % TTG in *n* = 35 field isolates.
In genotype I, the ranking of SNPs was 80 % CTC, 20 % TTG and 0 % TTA in n = 5 field isolates.

SNP: Single nucleotide polymorphism
Wildtype codon = TTC



Percent SDHI fungicide mutants

43.5 % of *n* = 23 *A. alternata* field isolates showed a SDHI fungicide mutation. In 26.1 % of cases, the mutation was located on subunit SDHB, in 17.4 % on SDHC, but not on SDHD. In *A. solani*, 42.1 % of *n* = 19 field isolates were SDH mutated. The mutation was located on subunit SDHC in 36.8 % of isolates and in 5.3 % on SDHB, but not on SDHD.

SDH: Succinate dehydrogenase. SDHB, SDHC, SDHD: Subunits of succinate dehydrogenase enzyme complex. WT: wildtype. SDHI: Succinate dehydrogenase inhibitor

References & Acknowledgments

Leiminger, J. H., Adolf, B., & Hausladen, H. (2014) Occurrence of the F129L mutation in *Alternaria solani* populations in Germany in response to QoI application, and its effect on sensitivity. *Plant Pathology*, 63 (3), 640-650.

Landschoot, S., Carrette, J., Vandecasteele, M., De Baets, B., Höfte, M., Audenaert, K., & Haesaert, G. (2017) Boscalid-resistance in *Alternaria alternata* and *Alternaria solani* populations: An emerging problem in Europe. *Crop Protection*, 92, 49-59.

The authors thank the colleagues from the Bavarian State agricultural offices for collecting Alternaria diseased potato leaves.

LfL Pflanzenschutz

Bavarian State Research Center for Agriculture,
Institute for Plant Protection
Lange Point 10, 85354 Freising
https://www.lfl.bayern.de/ips/blattfruechte/index.php,

Contact:
Prof. Dr. Michael Zellner
Michael.Zellner@LfL.Bayern.de