

Evidence of strobilurine resistant isolates of *A. solani* and *A. alternata* in Germany

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- introduction
- material and methods
- results

Alternaria solani

Alternaria alternata

- summary and discussion



- EB control by strobilurines widely used due to important benefits and excellent disease control
- mode of action is highly specific (single site mode of action)
 - high risk of fungicide resistance
- first evidence of the F129L mutation in *A. solani* by Gudmestad (2000) and G143A in *A. alternata* by Ma *et al.* (2003)



Alternaria solani:

F129L mutation

→ resulting in a „partial“ resistance (Gisi, Sierotzki, 2008)

reduced fungicide sensitivity, reduced disease control

(see also in *Pyrenophora teres*)

Alternaria alternata:

G143A mutation

→ resulting in a „high level“ of resistance (Gisi, Sierotzki, 2008)

complete loss of disease control by use of QoI fungicides

(see also in *Mycosphaerella graminicola*)

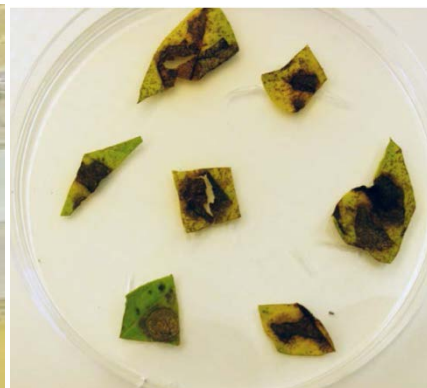
examination of leaf samples for their occurrence of *Alternaria* species
(*A. solani*, *A. alternata*)

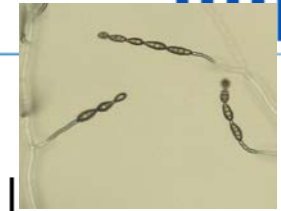
German wide monitoring

period: 2005 - 2014



year	2006	2007	2008	2009	2010	2011	2012	2013	2014
investigated leaf samples	49	28	29	36	33	100	138	264	144



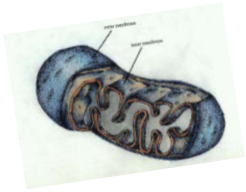


location	sampling date		
Neustadt	20. Mai		✓
Donaueschingen	23. Mai		✓
Bad Kreuznach	28. Mai		✓
Heilbronn	30. Mai		✓
Neuhofen	03. Jun		✓
Frankenthal	03. Jun		✓
Oberding	04. Jun		✓
Weihenstephan	13. Jun		✓
Mintraching	14. Jun		✓
Rain	18. Jun		✓
Jelmstorf	23. Jun		✓
Weihenstephan	25. Jun	✓	✓
Weihenstephan	02. Jul	✓	✓
Buir	14. Jul		✓
Uelzen	15. Jul		✓
Donaueschingen	22. Jul	✓	✓
Ruppin	29. Jul		✓
Teltow	31. Jul		✓
Havelland	05. Aug		✓
Watterdingen	06. Aug	✓	✓
Kirchheim	12. Aug	✓	✓
Neufang	20. Sep	✓	✓
Geltolfing	21. Sep	✓	✓
Atting	21. Sep	✓	✓
Ehetal	21. Sep	✓	✓
Aschheim	23. Sep	✓	✓
Leiblfing	25. Sep	✓	✓



location	sampling date		
Jelmstorf	15. Jun		✓
Schrobenhausen	16. Jun		✓
Aiterhofen	20. Jun	✓	✓
Bredenbeck	27. Jun		✓
Donaueschingen	12. Jul		✓
Freising	14. Jul	✓	✓
Jelmstorf	15. Jul		✓
Donaueschingen	27. Jul		✓
Buir	27. Jul		✓
Borken	27. Jul		✓
Hamerstorf	02. Aug		✓
Leipzig	05. Aug		✓
Jelmstorf	16. Aug	✓	✓
Kirchheim	18. Aug	✓	✓
Rupenest	19. Aug	✓	✓
Neusustrum	19. Aug	✓	✓
Holxen	24. Aug		✓
Buir	25. Aug	✓	✓
Hamerstorf	26. Aug	✓	✓
Obersunzing	29. Aug	✓	✓
Gersten	02. Sep	✓	✓
Buir	02. Sep	✓	✓
Kirchheim	12. Sep	✓	✓
Aschheim	12. Sep	✓	✓
Ismaning	12. Sep	✓	✓
Feldkirchen	12. Sep	✓	✓
Freising	12. Sep	✓	✓
Jelmstorf	12. Sep	✓	✓

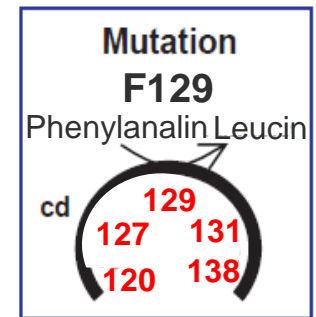




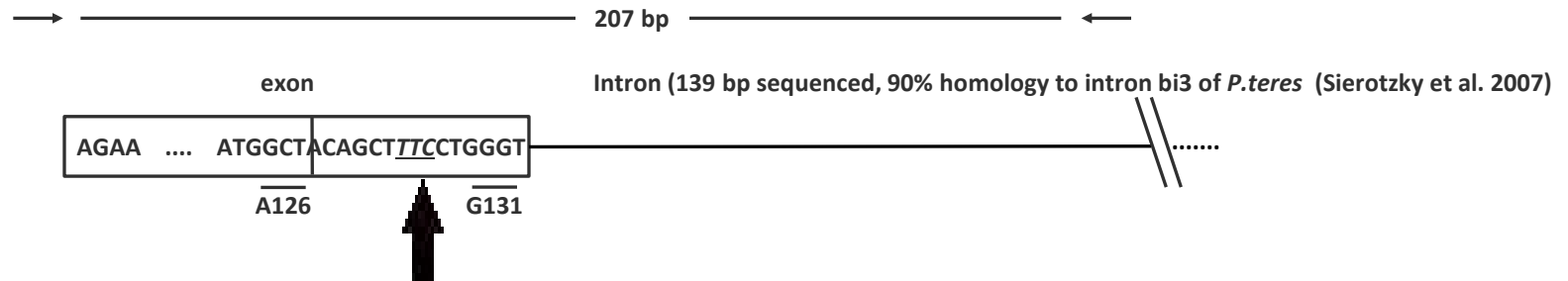
- site mutation at position 129 from TTC to TTA, CTC, TTG
- substitution from phenylalanin to leucin (Phe→ Leu)



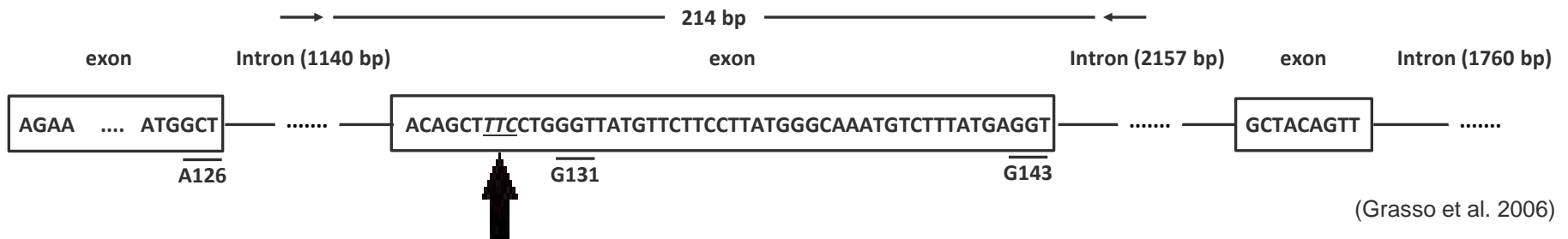
F 129 L



Alt. sol. type II - USA



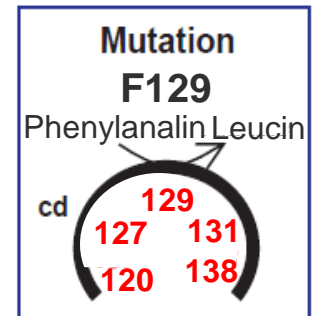
Alt. sol. type I - Europe



- site mutation at position 129 from TTC to TTA, CTC, TTG
- substitution from phenylalanine to leucine (Phe→Leu)
- sequencing of a 207 and 214 bp fragment of the *cyt b* complex in *A. solani*

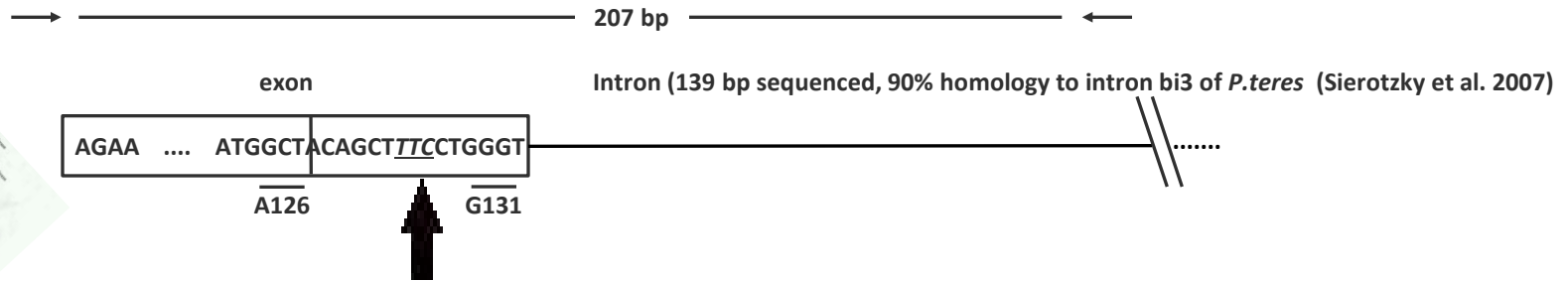


F 129 L



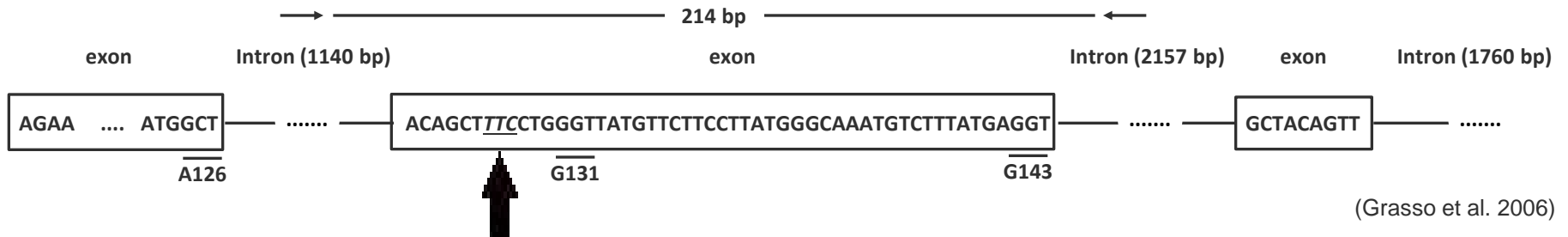
year	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
isolates	5	24	20	5	39	13	66	88	183	62
nr of locations	3	22	13	3	22	9	12	14	25	27
Strobilurin applied	0	0	6	3	18	9	12	12		
Locations with F129L	0	0	0	0	1	2	7	6	20	18
Nr mutants	0	0	0	0	2	2	39	15	69	20
Frequency % F129L					5,1	15,3	56,5	17,0	37,7	32,2

Alt. sol. type II - USA



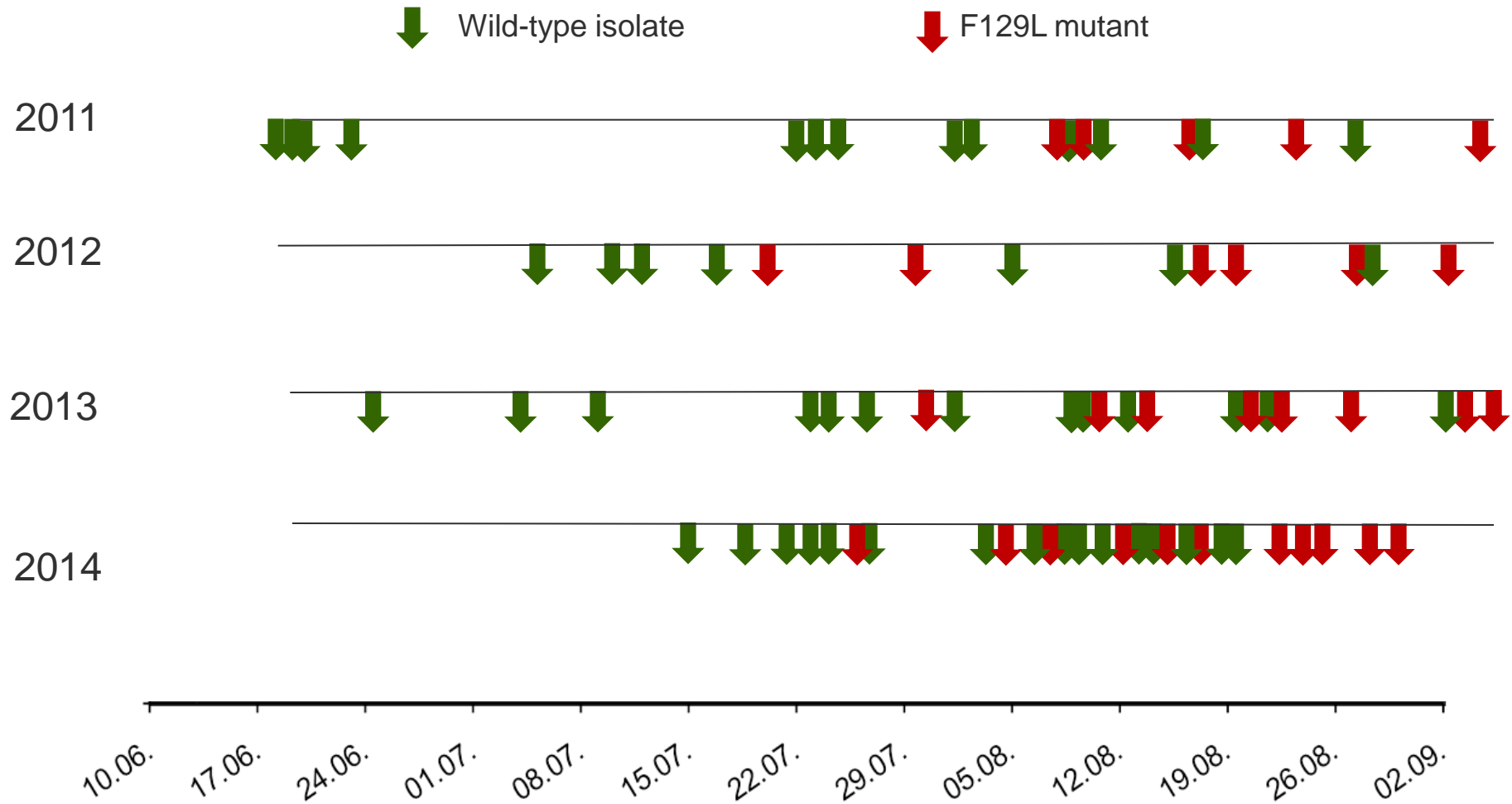
- 144 out of 147 F129L mutants were genotype II, carrying TTA, TTG mutation

Alt. sol. type I - Europe



- only 3 out of 147 F129L mutants, were genotype I, carrying CTC mutation
- first observed in 2013 (2 isolates) and 2014 (one isolate)
- all wildtype isolates were genotype I

appearance of isolates with F129L mutation



- investigation of various isolates out of each sampled location

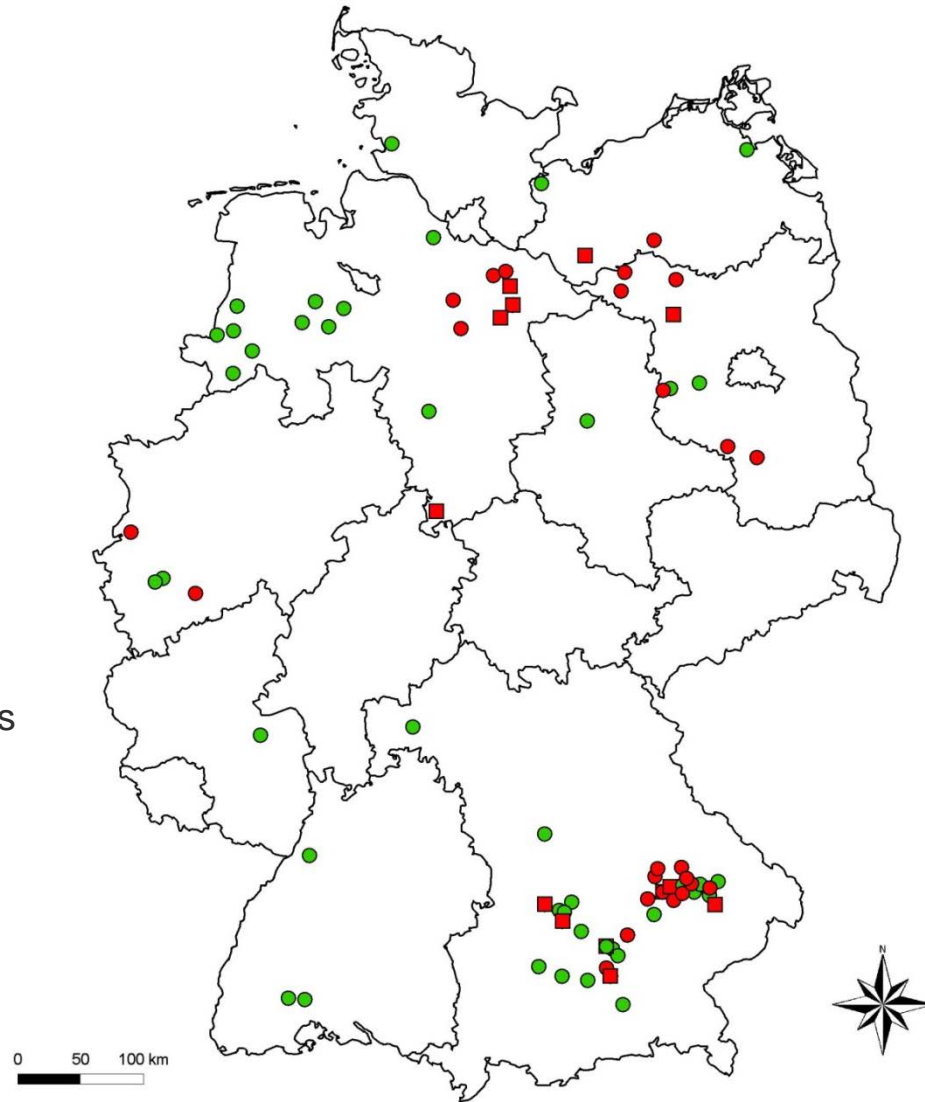
year	2011	2011	2011	2011	2012	2013	2013
sampling date	aug 16 th	aug 16 th	aug 16 th	sep 11 th	aug 28 th	sep 18 th	sep 18 th
location	Aschheim	Aiterhofen	Kirchheim	Laberweinting	Freising	Freising	Freising
treatment	AZ,	AZ,	AZ,	AZ,	unknown	untreated	AZ,
number of isolates	10	10	9	10	14	10	10
proportion of wild-type isolates	10	0	0	0	14	8	9
proportion of F129L isolates	0	10	9	10	0	2	1

- constant site-specific composition of species

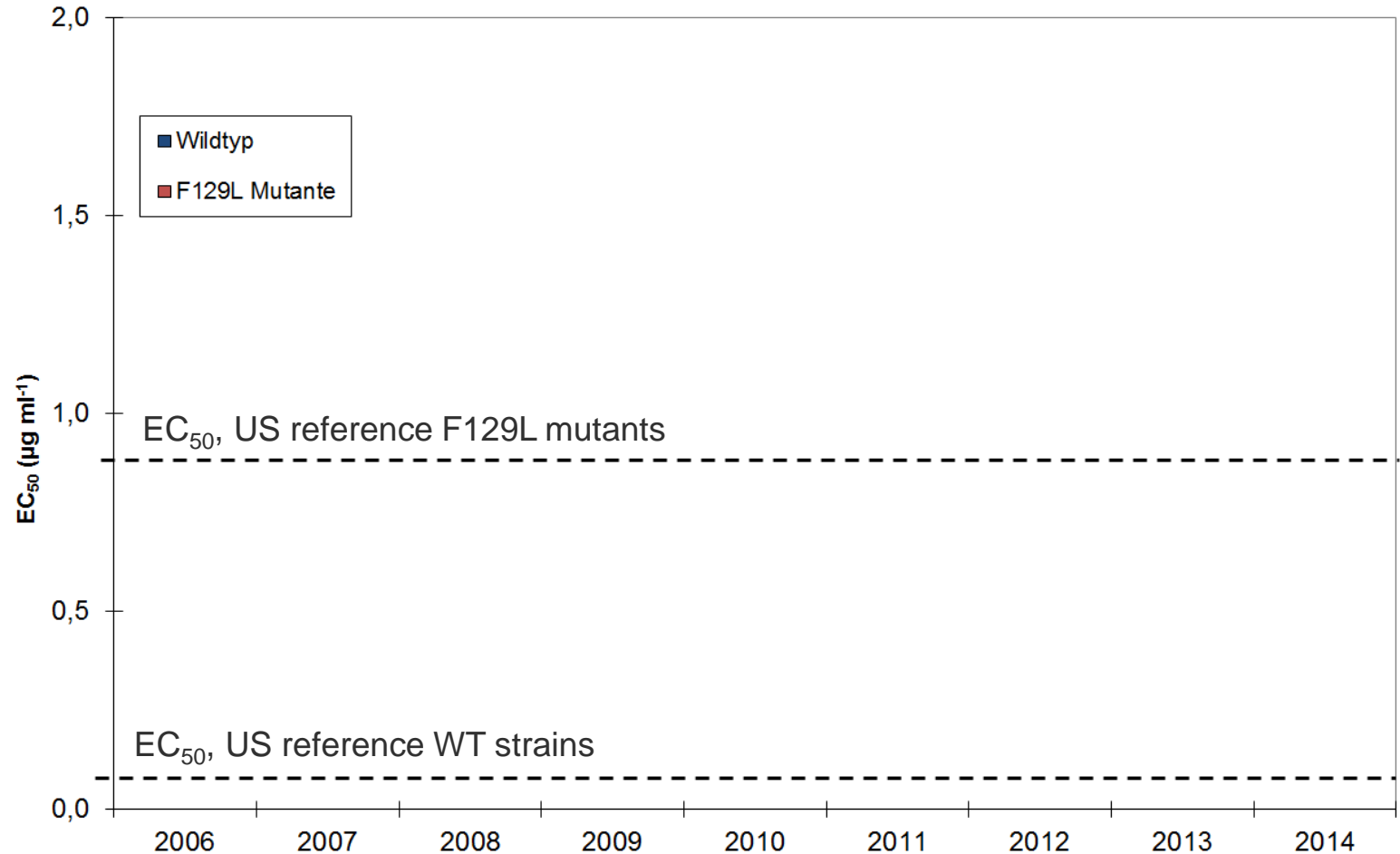
A. solani
2005-2014

location with

- F129L mutant
- wild-type
- wild-type and F129L mutants

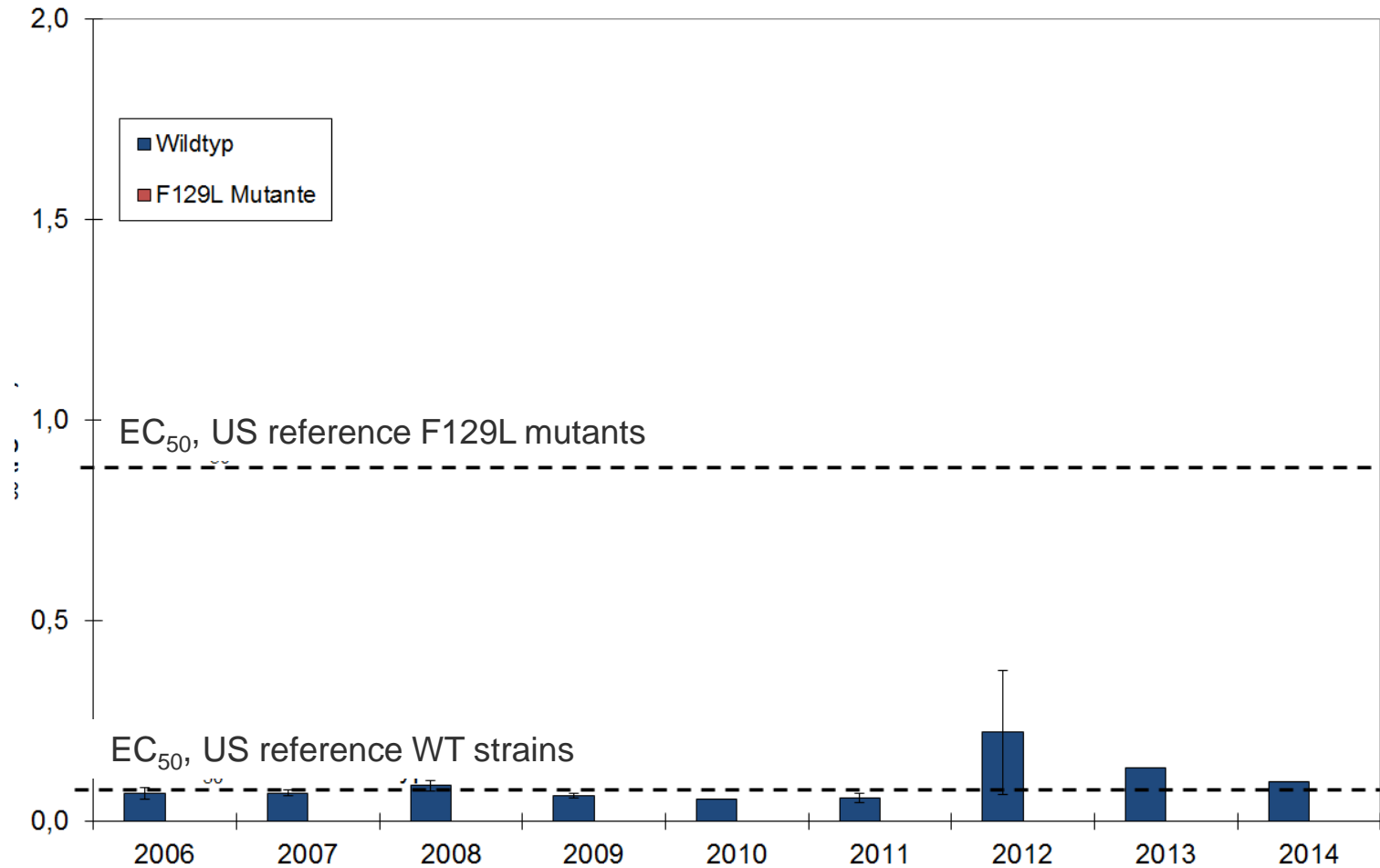


evaluation of fungicide sensitivity of *A. solani* wild-type and F129L mutants

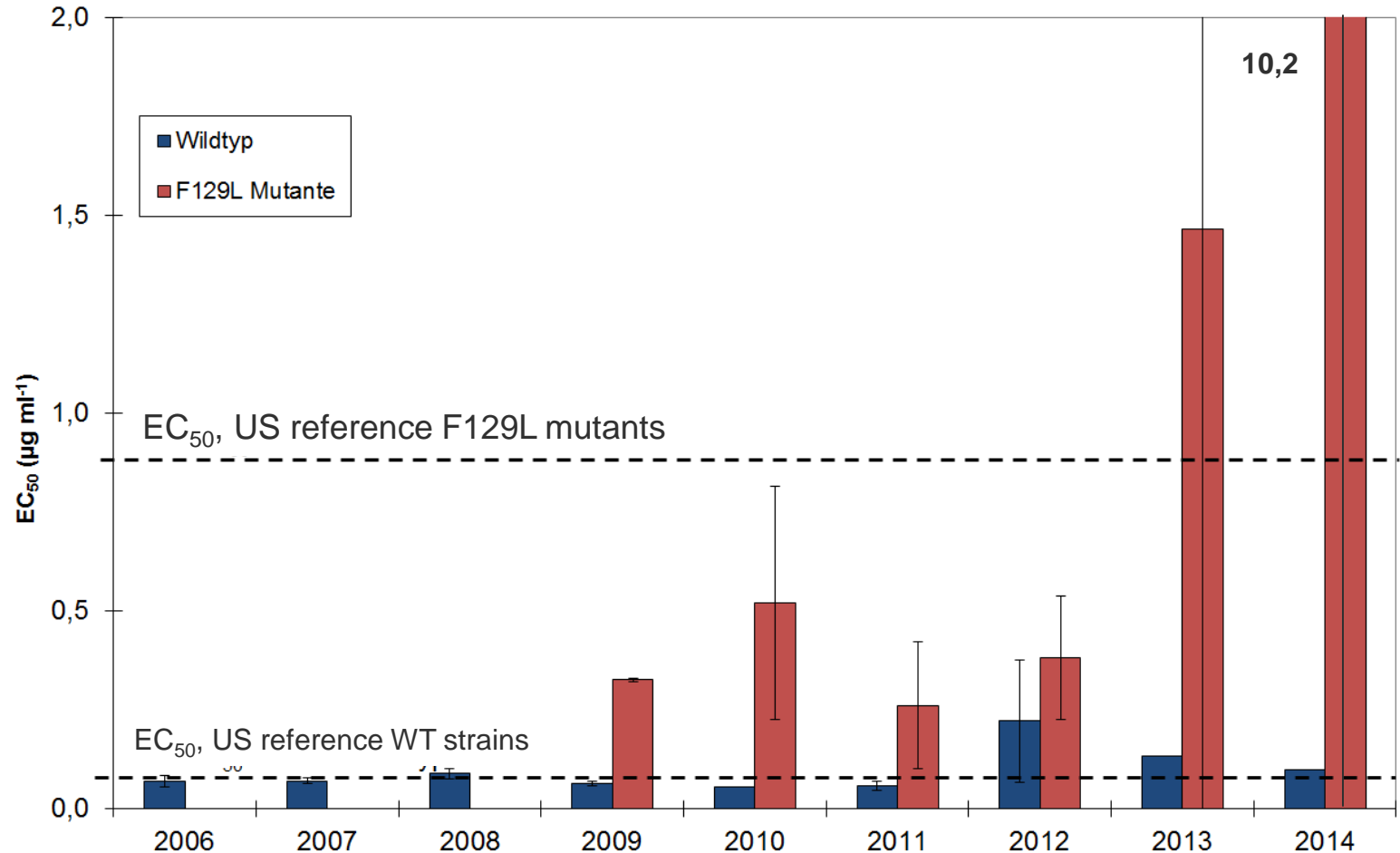


EC_{50} : concentration, at which 50 % of the spores die or do not germinate

evaluation of fungicide sensitivity of *A. solani* wild-type and F129L mutants



evaluation of fungicide sensitivity of *A. solani* wild-type and F129L mutants





detection of the G143A mutation in *A. alternata*

Point mutation within the *cyt b* complex , mutation site 143 GGT -> GCT
(Gly → Ala)

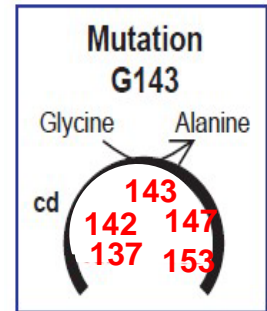
analysis of 150 single spore isolates (2005-2014)
and 58 EB infected leaf samples

Sequencing of an 228 bp fragment *cyt b* incl. G143A mutation

Primer: **AF** (5-ACA CTG CTT CAG CAT TTT TCT TCA TAG-3)

AR (5-TTG TCC AAT TCA TGG TAT AGC ACT CA-3) (Ma et al. 2003)

G 143 A






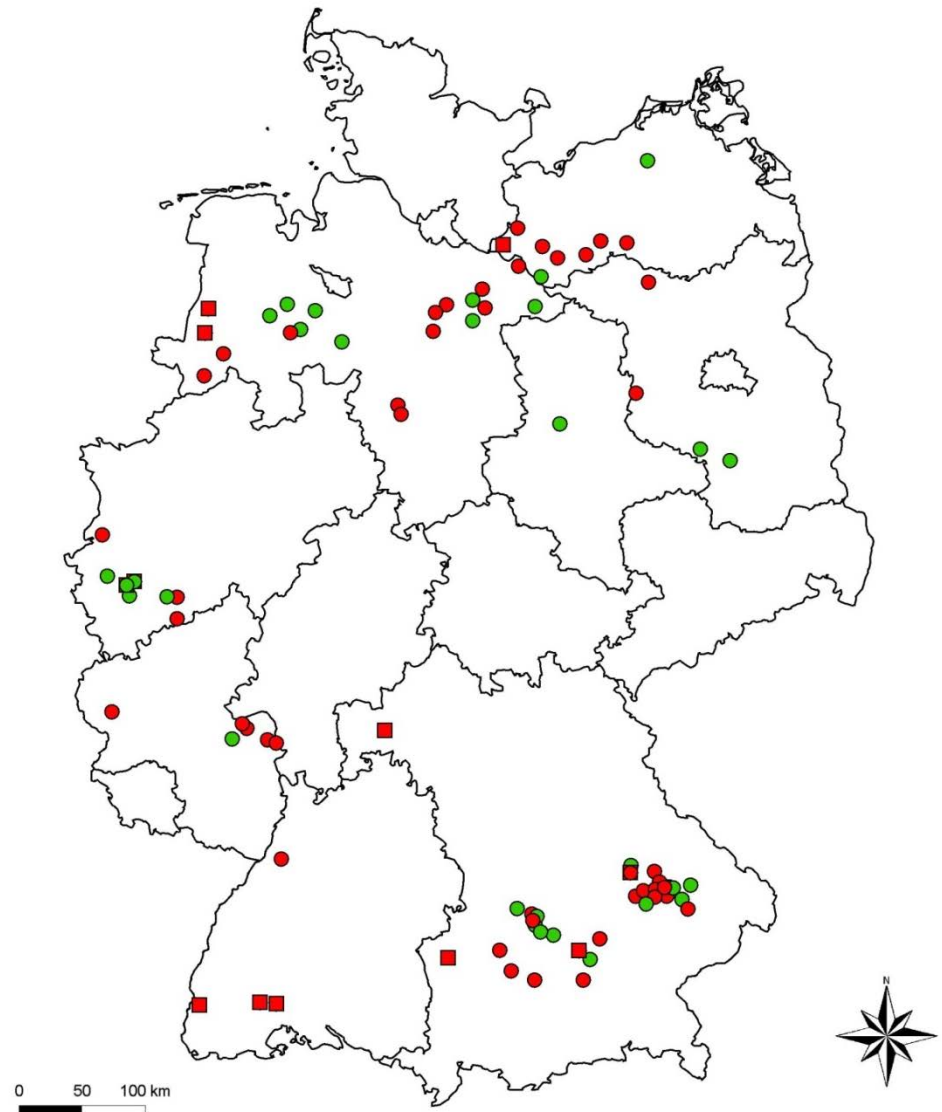
		143	
		↓	
A.alt 100	-	GATACGTCTTGCCATACGGGCAAATGTCATTATGAGCTGCAACAGTT	M
A.alt 101	-	GATACGTCTTGCCATACGGGCAAATGTCATTATGAGCTGCAACAGTT	M
A.alt 11	-	GATACGTCTTGCCATACGGGCAAATGTCATTATGAGGTGCAACAGTT	WT
A.alt 12	-	GATACGTCTTGCCATACGGGCAAATGTCATTATGAGGTGCAACAGTT	WT

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	in total
isolates evaluated	7	43	/	/	14	12	30	34	17	51	208
wildtyp isolates	7	34	/	/	5	0	7	20	7	22	102
G143A mutants	0	9	/	/	9	12	23	14	10	29	106
investigated locations	1	29	/	/	10	1	11	10	4	35	101
locations with G143A	0	9	/	/	5	1	8	5	0	18	46

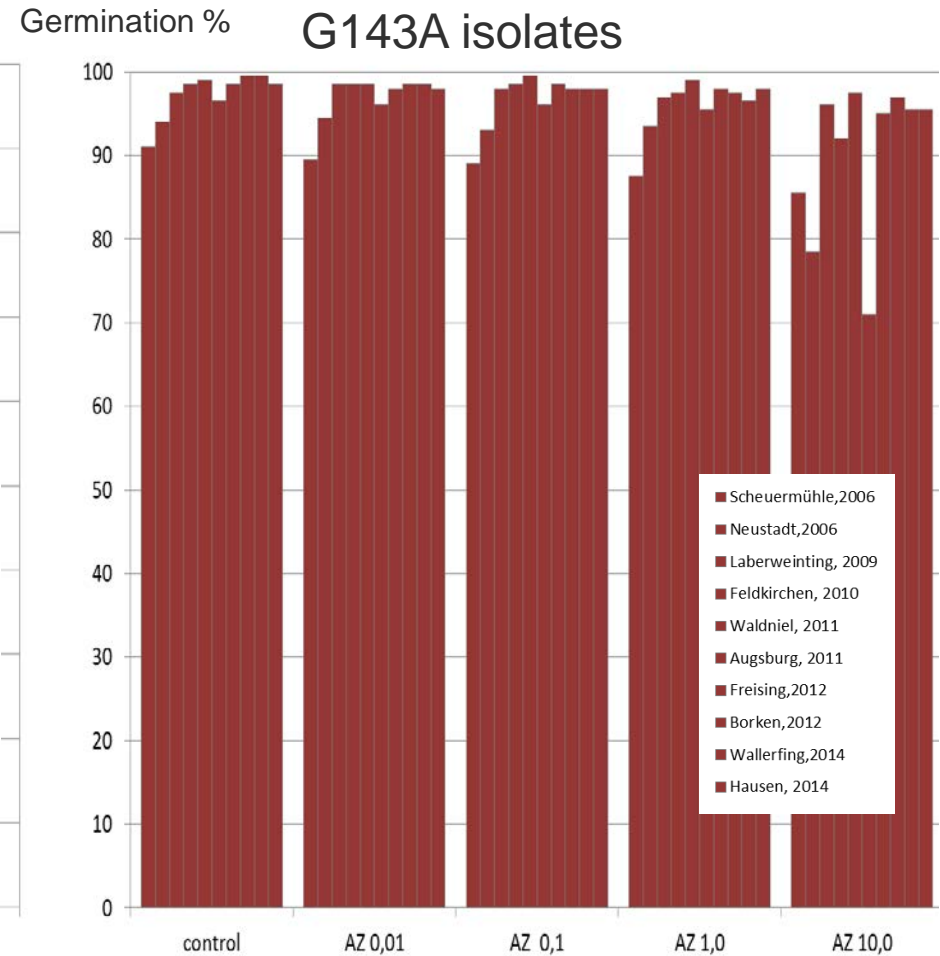
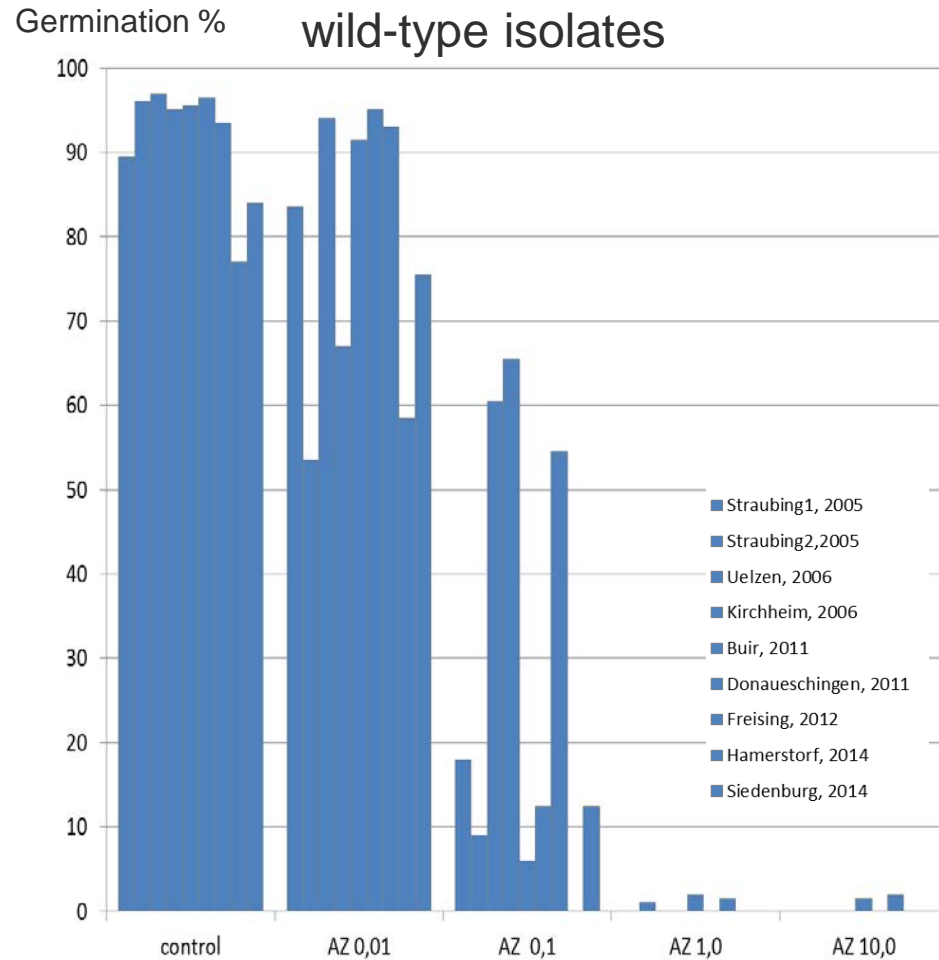
A. alternata
2005-2014

location with

-  G143A mutant
-  wild-type
-  wild-type and G143A mutants



evaluation of fungicide sensitivity of *A. alternata* wild-type and G143A mutants



- prevalence of the F129L and G143A mutation in Germany
- actually no loss in fungicide sensitivity (field)
 - further specific field trials in 2015 (artificial inoculation)
- use of QoI fungicides to GAP:
 - limitation of Strobilurin-containing products
 - Use products/mixtures with high efficacy
 - Full dosage
 - Use of „EB fungicides“
 - first spray: 7-8 weeks after the crop emerge
 - further applications: according to disease development and weather condition