Current Status of QoI and SDHI Mutations in *Alternaria* sp. in the USA

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Early Blight


Brown Spot

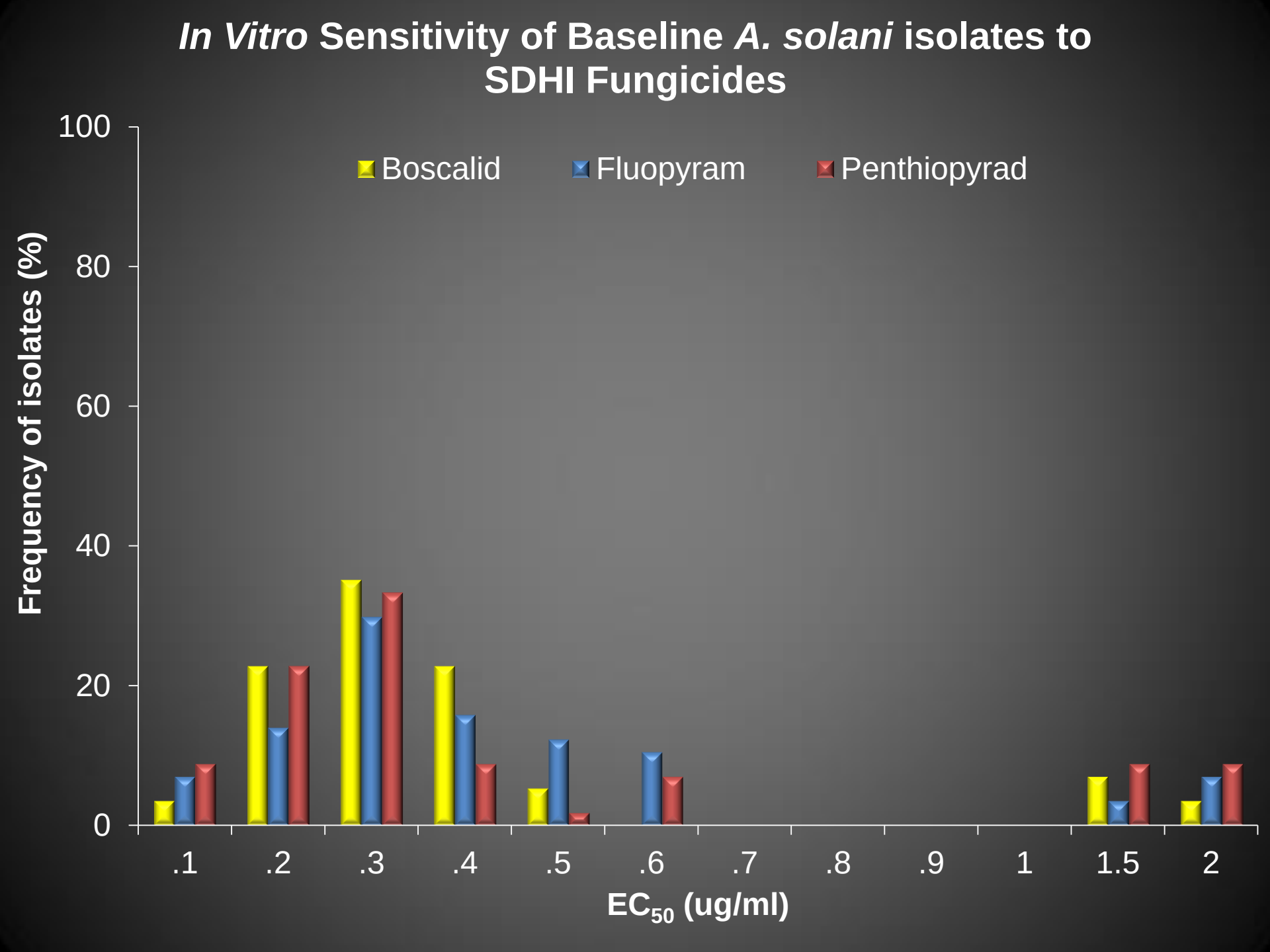

Botrytis


Black Dot

History of Fungicide Resistance in *As*

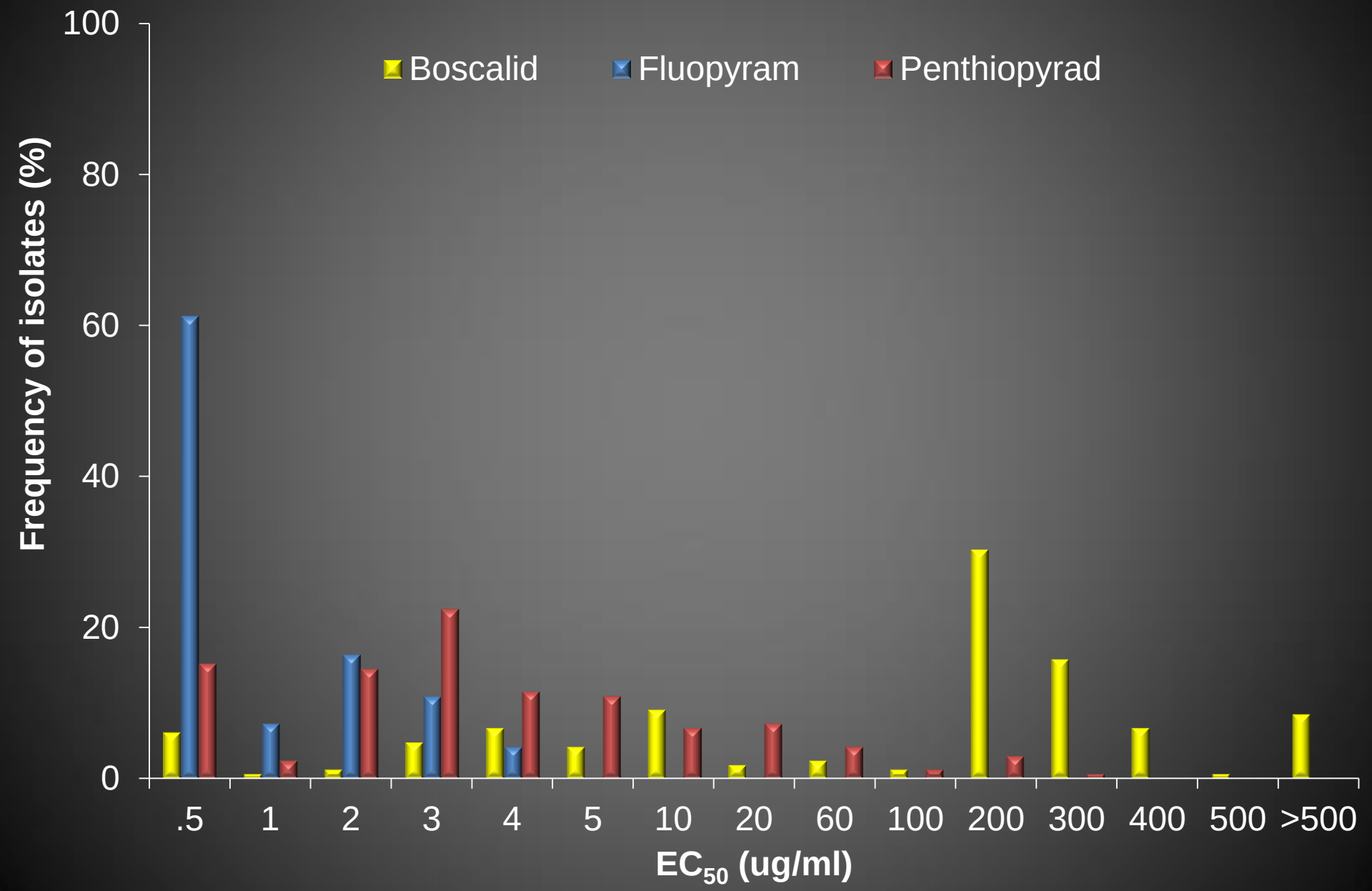
- First QoI (strobilurin) fungicide (azoxystrobin) registered on potato in 1999
- First QoI resistance in *A. solani* first reported in ND and NE in 2001 (Pasche, et al. 2004)
- First SDHI foliar fungicide (boscalid) registered on potato in 2005
- Poor early blight disease control reported in 2009 in ID; 2010 in ND & NE (Wharton, et al. 2012; Gudmestad, et al. 2013)

Baseline Sensitivity of *A. solani* to Endura, Luna & Vertisan


In Vitro Sensitivity of Baseline *A. solani* isolates to SDHI Fungicides
Boscalid
Fluopyram
Penthiopyrad
Frequency of isolates (%)
100
80
60
40
20
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1
1.5
2
EC50 (ug/ml)

In Vitro Assays- 2010 & 2011 *A. solani* isolates

In Vitro Sensitivity of 2010 and 2011 *A. solani* isolates to SDHI Fungicides



Characterization of SDHI Mutation Genes in *Alternaria solani*

SDHI Resistance Mutation Genes

- We have characterized five mutation genes in *A. solani* (Mallik et al. 2014):
  - **H278Y** in SDHB gene- VH resistance to boscalid/penthiopyrad; fluopyram S
  - **H278R** in SDHB gene- M resistance to boscalid; S to penthiopyrad/fluopyram
  - **H134R** in SDHC gene- H resistance to boscalid/penthiopyrad; S to fluopyram
  - **H133R** in SDHD gene- VH resistance to boscalid; H to penthiopyrad; S to fluopyram
  - **D123E** in SDHD gene- very high resistance to boscalid; H to penthiopyrad; S to fluopyram

SDHI Resistance

- In original 2010 & 2011 surveys ~72% As isolates resistant to SDHIs, but resistance was spatially diverse
- Some farms or regions had resistance to boscalid, others did not
- For example, W-NE did not have resistance, but it was widespread in E-NE
- In ND, resistance widespread in central portion, but not in SE or NE portion of state

SDHI Resistance Mutation Genes

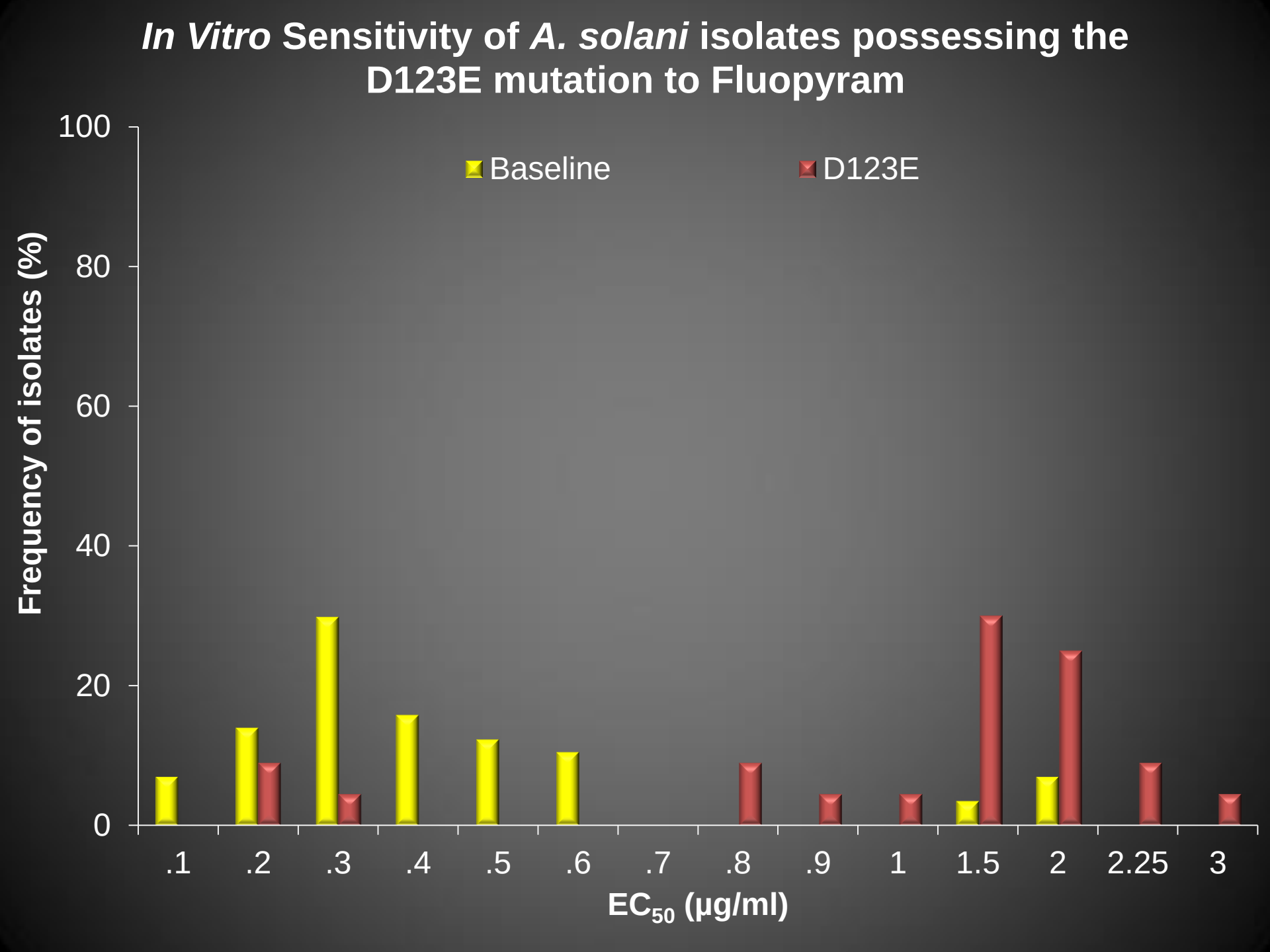
- Mutations in SDHB gene (H278Y & H278R) are most evenly distributed in USA- initially most common (2010-2011)
- The mutation in SDHC gene (H134R) are most common in the USA
- Most of the mutations in SDHD gene (H133R) found in ID and MN
- The D123E in SDHD gene was once very rare, only one isolate found in original survey but it may convey reduced sensitivity to fluopyram (EC_{50}=2.89 µg/ml)

SDHI/QoI Resistance in the U.S.A. 2013-2015

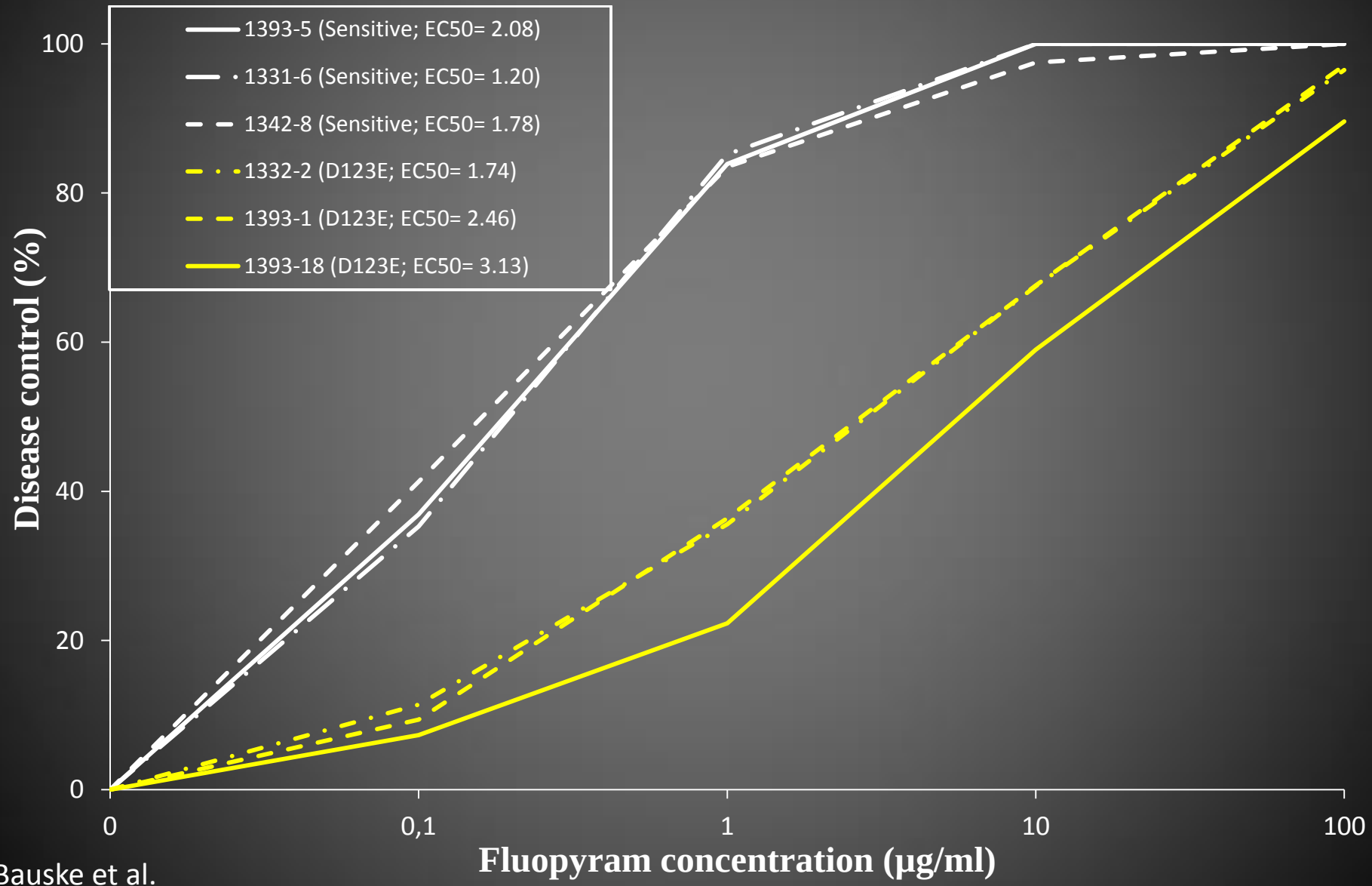
Year	No. of Isolates	H278 (VHR) %	H278R (MR) %	H134R (HR) %	H133R (VHR) %	D123E (VHR) %	SDHI (S) %	F129L %
2010-2011	67	48	19	7.5	15	1.5	9	92
2013	466	18	13	50	14	4	1	94
2014	295	38	2	36	15	7	2	90
2015	225	40	0	27	12	14	7	99
Mean[#]	986*	32	4	38	14	9	3	95

[#] Mean based on 2013-2015 survey only

* Total isolates from states, CO, ID, IL, MI, MN, ND, NE, NM, TX, WI, WA

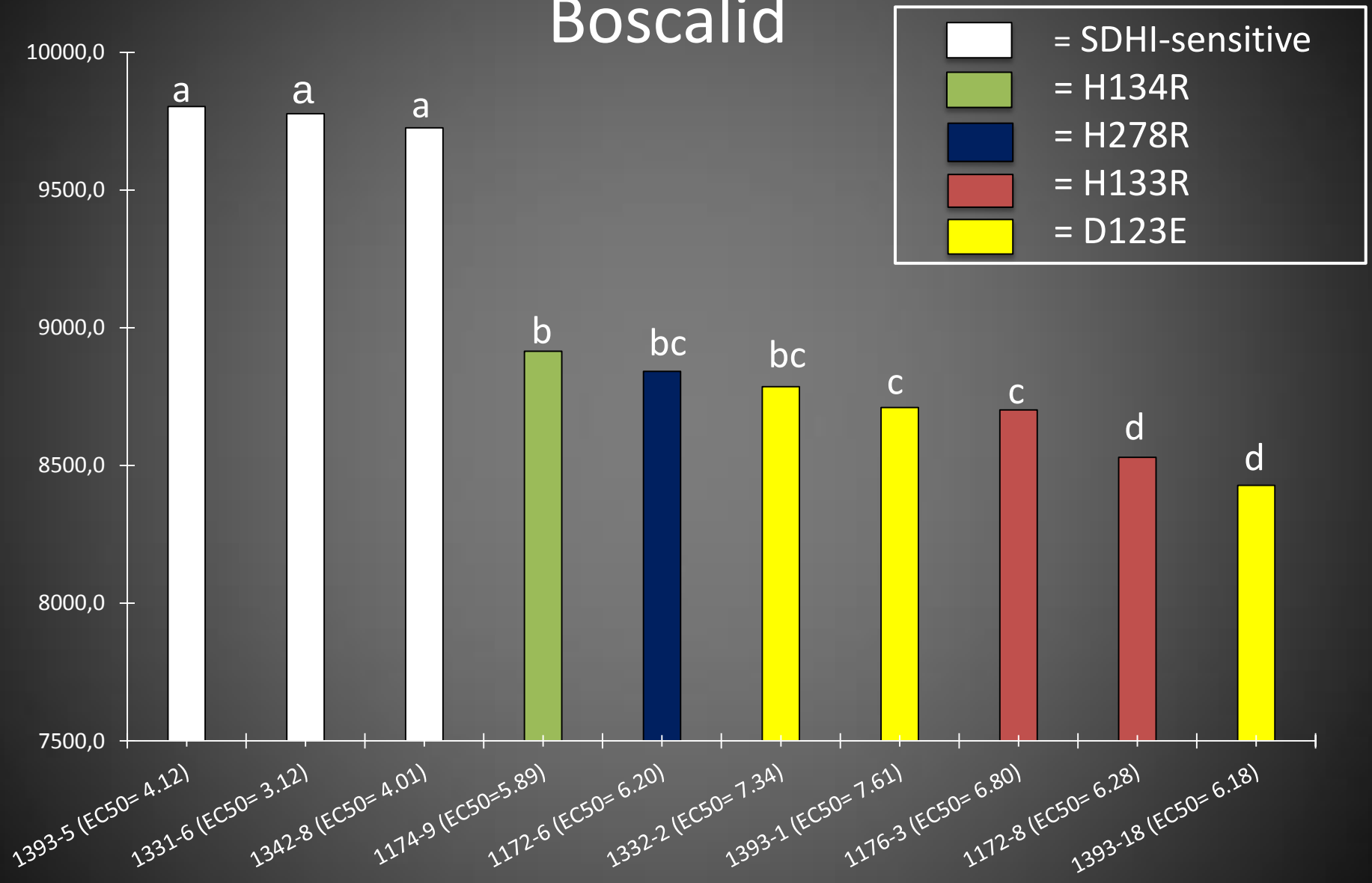

In Vitro Sensitivity of *A. solani* isolates possessing the D123E mutation to Fluopyram
Baseline
D123E
Frequency of isolates (%)
100
80
60
40
20
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1
1.5
2
2.25
3
EC50 (µg/ml)

Percentage disease control for SDHI-sensitive and D123E mutant *A. solani* isolates to fluopyram



Bauske et al.

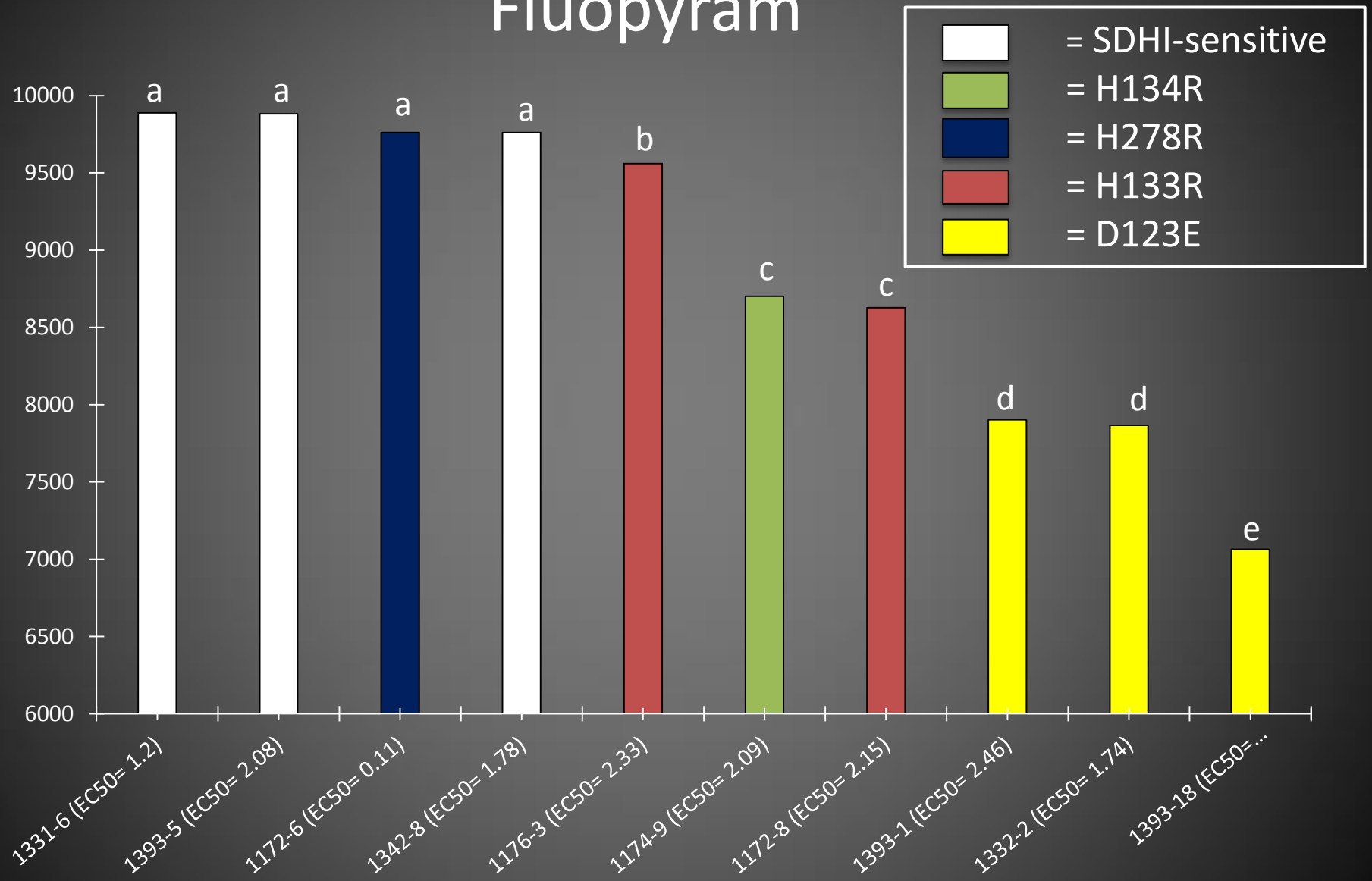
Area Under Dose Response Curve For Boscalid



Bauske et al.

* Bars with the same letter are not significantly different

Area Under Dose Response Curve For Fluopyram



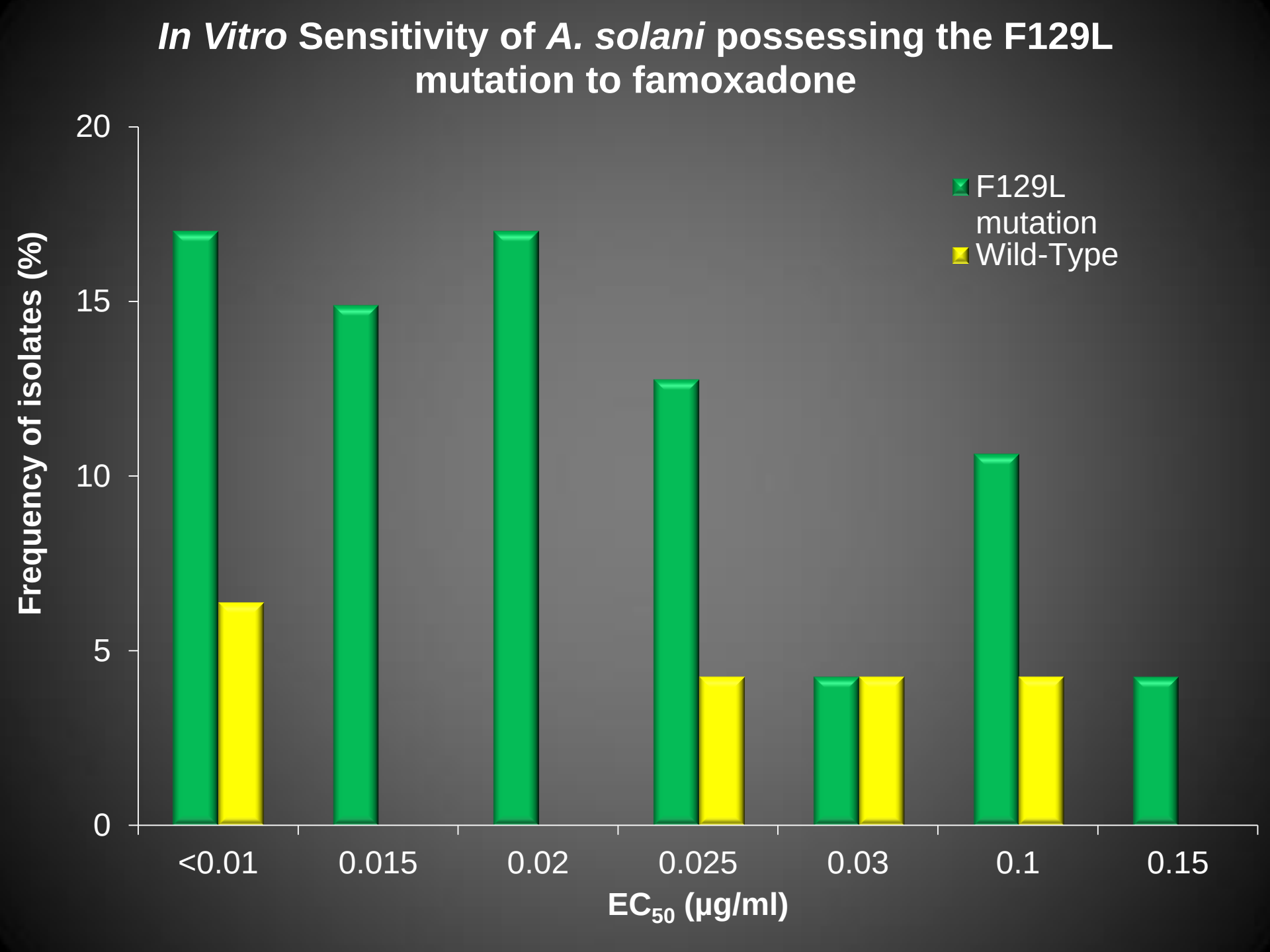
Bauske et al.

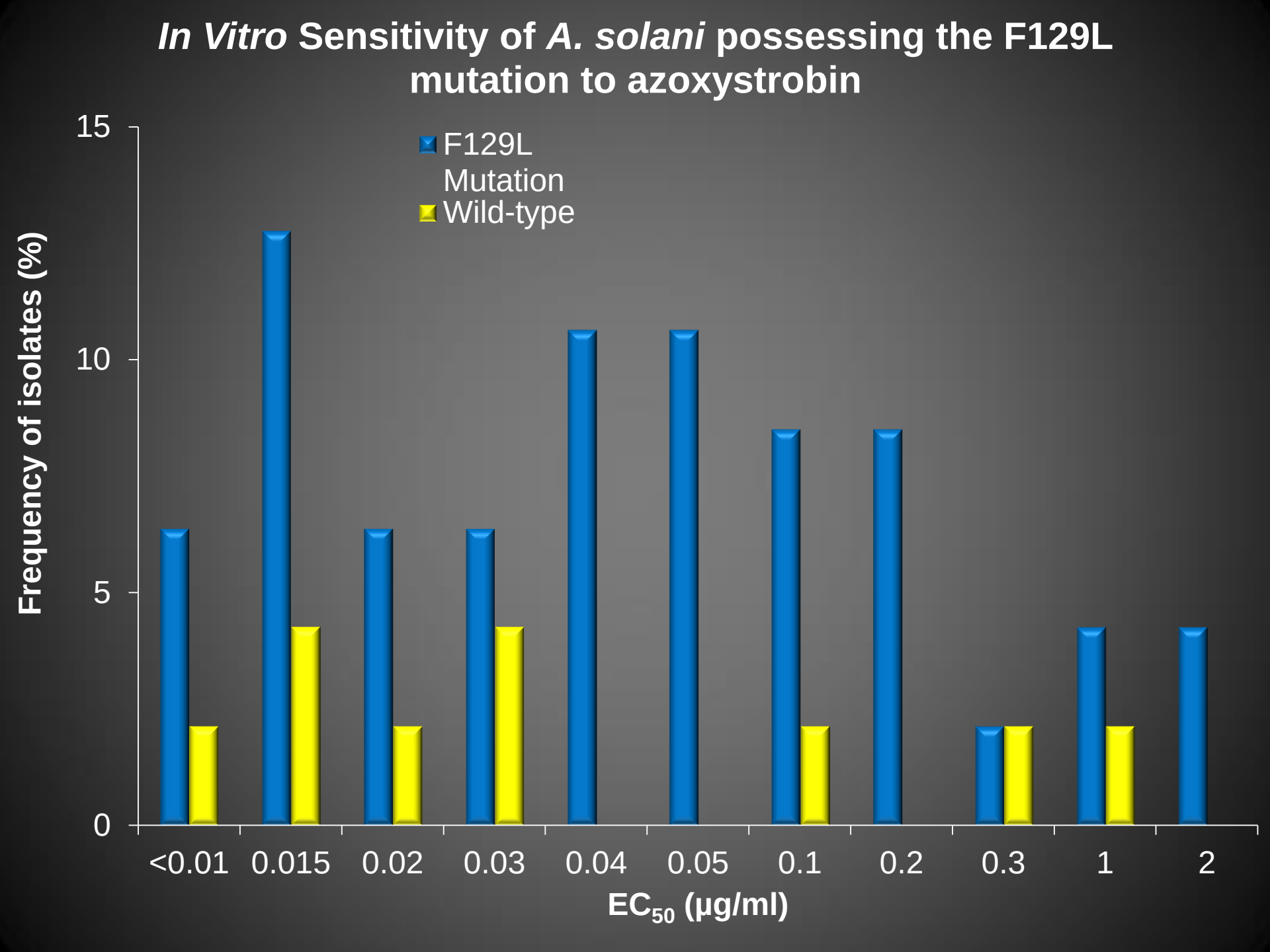
* Bars with the same letter are not significantly different

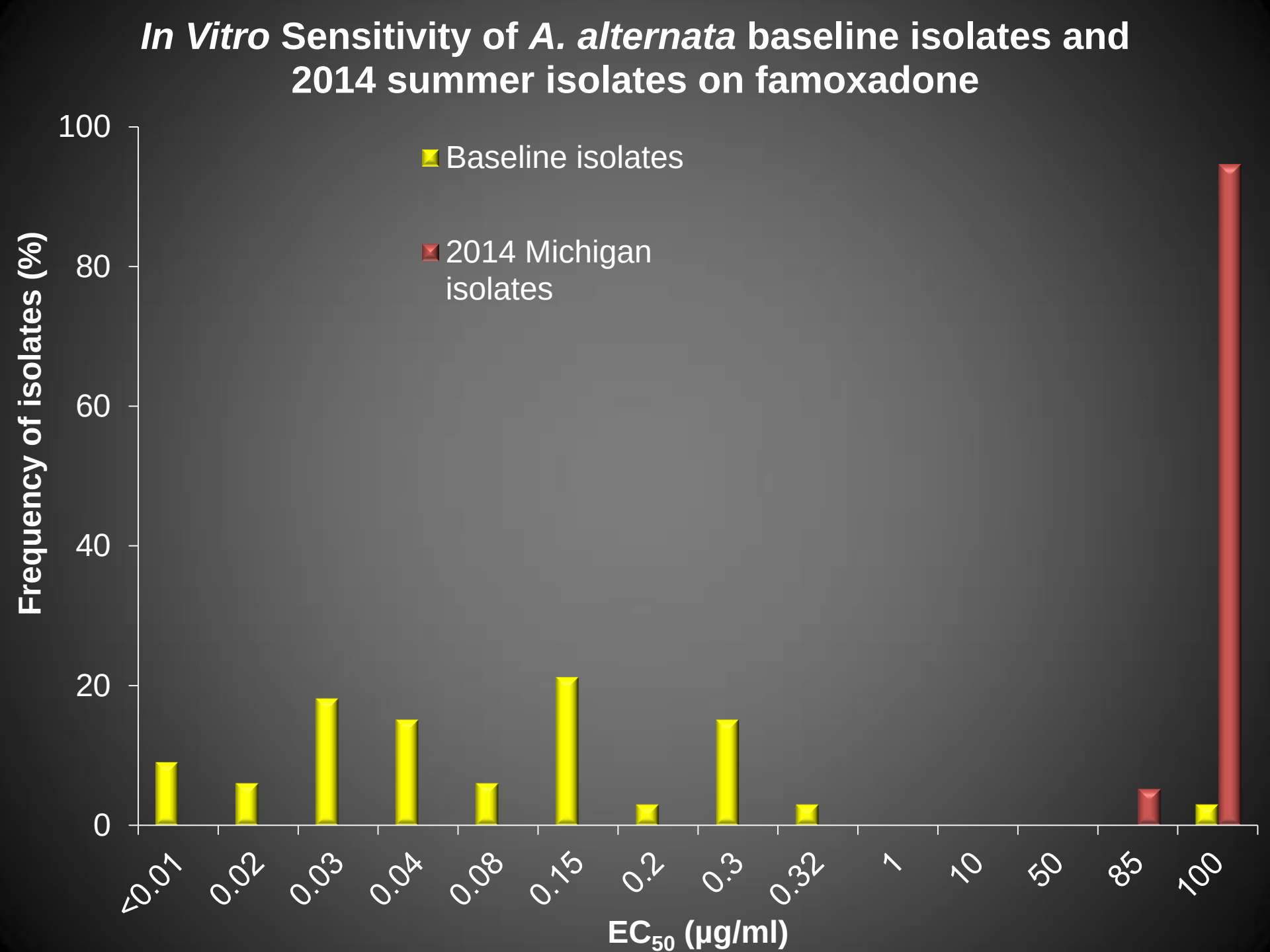
Status of Sensitivity of *Alternaria* sp Affecting Tomato in MI & IN

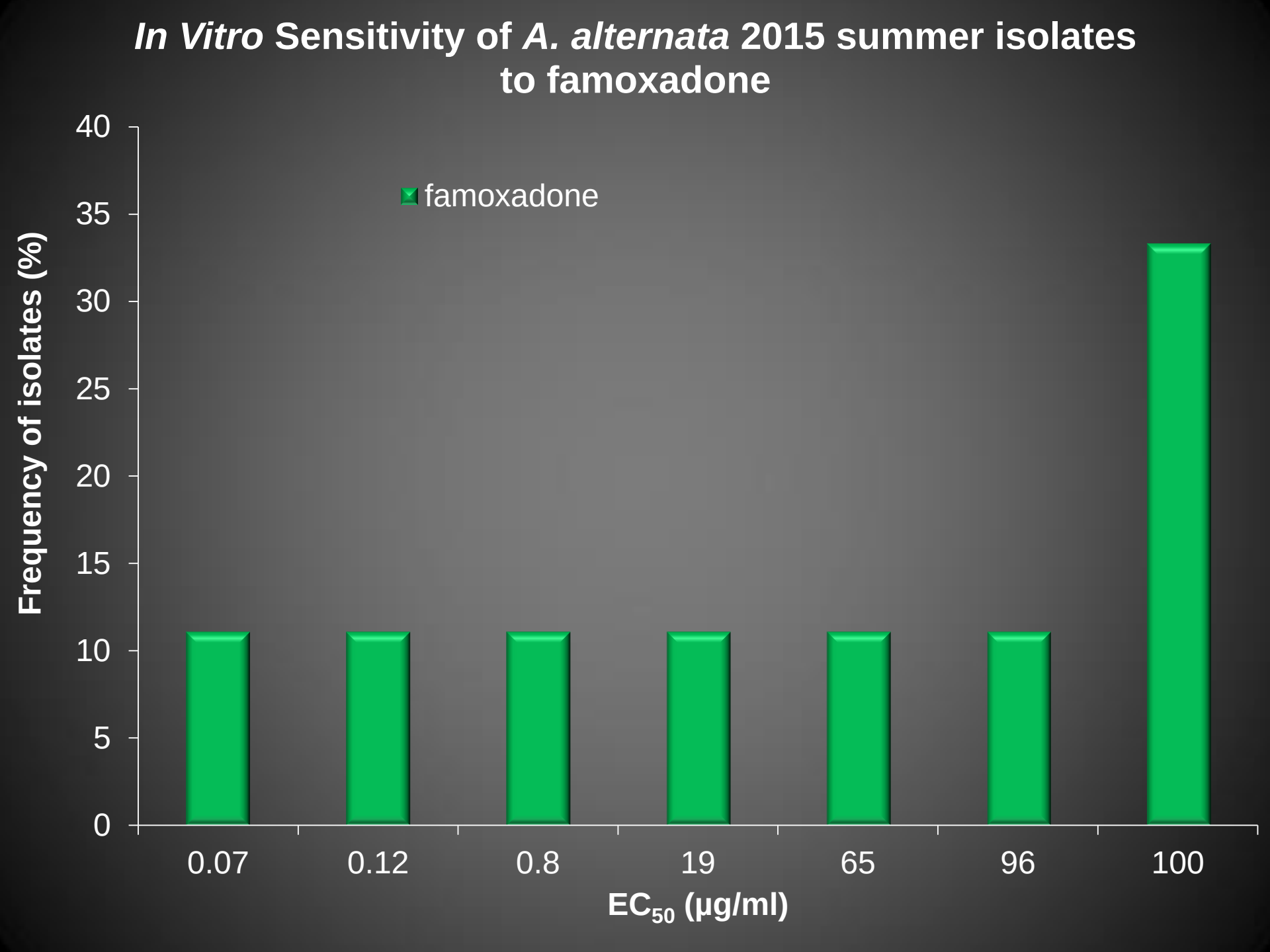
Alternaria sp. Affecting Tomato

- In 2014 a leaf spot epidemic caused significant economic losses to tomato growers in MI and IN despite heavy fungicide use
- *A. tomatophila*, *A. solani*, and *A. alternata* isolates obtained from 2014 & 2015
- All *Alternaria* sp. involved in the leaf spot complex in 2014; *A. alternata* in 2015
- In vitro assays and genome sequencing used to investigate the problem


In Vitro Sensitivity of *A. solani* possessing the F129L mutation to famoxadone
Frequency of isolates (%)
20
15
10
5
0
F129L mutation
Wild-Type
<0.01
0.015
0.02
0.025
0.03
0.1
0.15
EC50 (µg/ml)


In Vitro Sensitivity of *A. solani* possessing the F129L mutation to azoxystrobin
F129L Mutation
Wild-type
Frequency of isolates (%)
15
10
5
0
<0.01
0.015
0.02
0.03
0.04
0.05
0.1
0.2
0.3
1
2
EC50 (µg/ml)


In Vitro Sensitivity of *A. alternata* baseline isolates and 2014 summer isolates on famoxadone
Frequency of isolates (%)
100
80
60
40
20
0
Baseline isolates
2014 Michigan isolates
<0.01
0.02
0.03
0.04
0.08
0.15
0.2
0.3
0.32
1
10
50
85
100
EC50 (µg/ml)


In Vitro Sensitivity of A. alternata 2015 summer isolates to famoxadone
famoxadone
Frequency of isolates (%)
40
35
30
25
20
15
10
5
0
0.07
0.12
0.8
19
65
96
100
EC50 (µg/ml)

Frequency of G143A mutants and wild-types amongst *A. alternata* baseline, 2014 Michigan isolates and 2015 Midwest isolates

***A. alternata* isolates**	**Wild-Type**	**Mutant**
Baseline Isolates (n=41)	97.56%	2.44%
2014 Michigan Isolates (n=19)	5.26%	89.47%
2015 Midwest Isolates* (n=36)	13.89%	83.33%

*States sampled includes: IN, MI, and OH

Summary of QoI/SDHI Resistance

- QoI resistance widespread in all Alternaria sp. affecting solanaceous crops
  - F129L mutation in *A. solani*; G143A in *A. alternata* and *A. tomatophila*
- It does not appear that the level of resistance conveyed by F129L in *A. solani* is a significant factor in tomato isolates
- With G143A prevalence 35-100% in *A. alternata*, this is suspected to be the primary cause of the economic losses

Summary of QoI/SDHI Resistance

- SDHI resistance in *As* is conveyed by one of five different mutations:
  - All mutations affect boscalid
  - Some mutations affect penthiopyrad, none affect in vitro sensitivity of fluopyram
- D123E mutation has increased from ~1% of the population to >10%, probably in response to increase useage of fluopyram
- D123E isolates fall within fluopyram baseline, but have higher EC_{50} value

Future Studies

- SDHI fungicide group continues to be developed by basic manufacturers
- Two important molecules in potato, both from Syngenta:
  - Solatenol (benzovindiflupyr)- registered 2016
  - Adepidyn- in development
- Both molecules unaffected by SDHI mutations in *A. solani* based on field trials
- In vitro trials in progress with particular attention to *A. solani* isolates with D123E

Acknowledgements

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  - Mitchell Bauske- PhD candidate
  - Sarah Budde- PhD student
  - Ipsita Mallik- molecular diagnostics
  - Chris Johnson- genome sequencing
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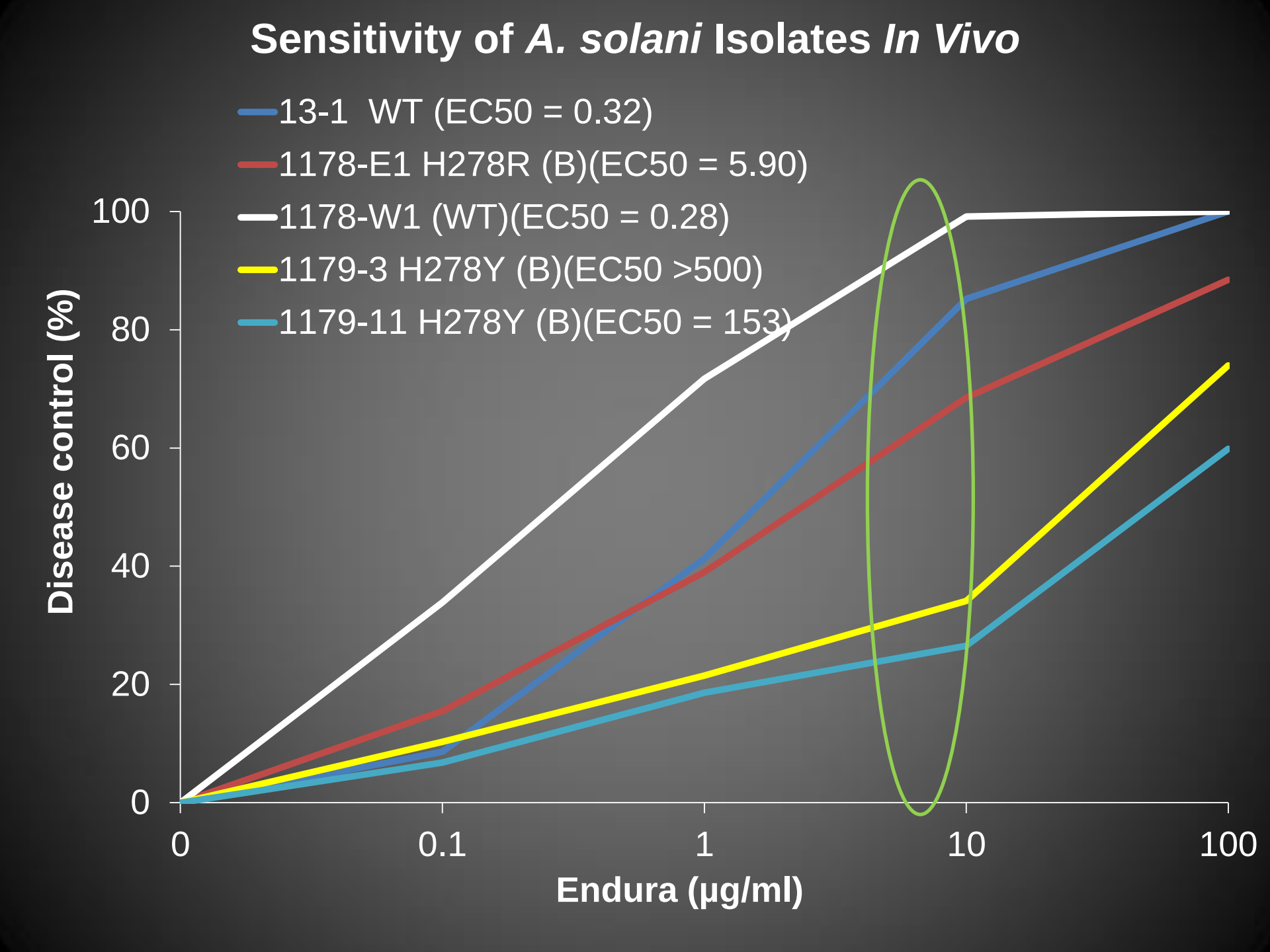
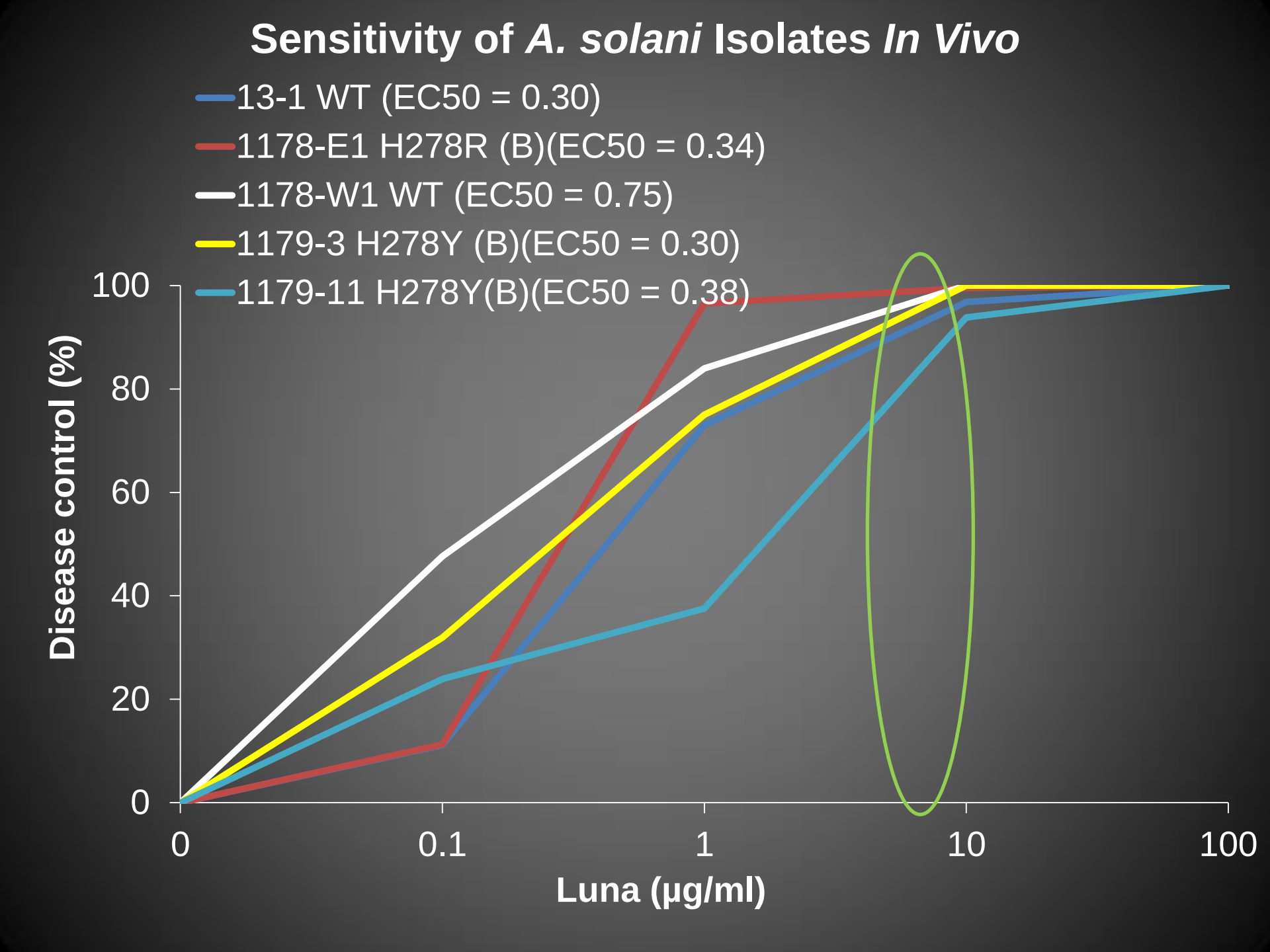
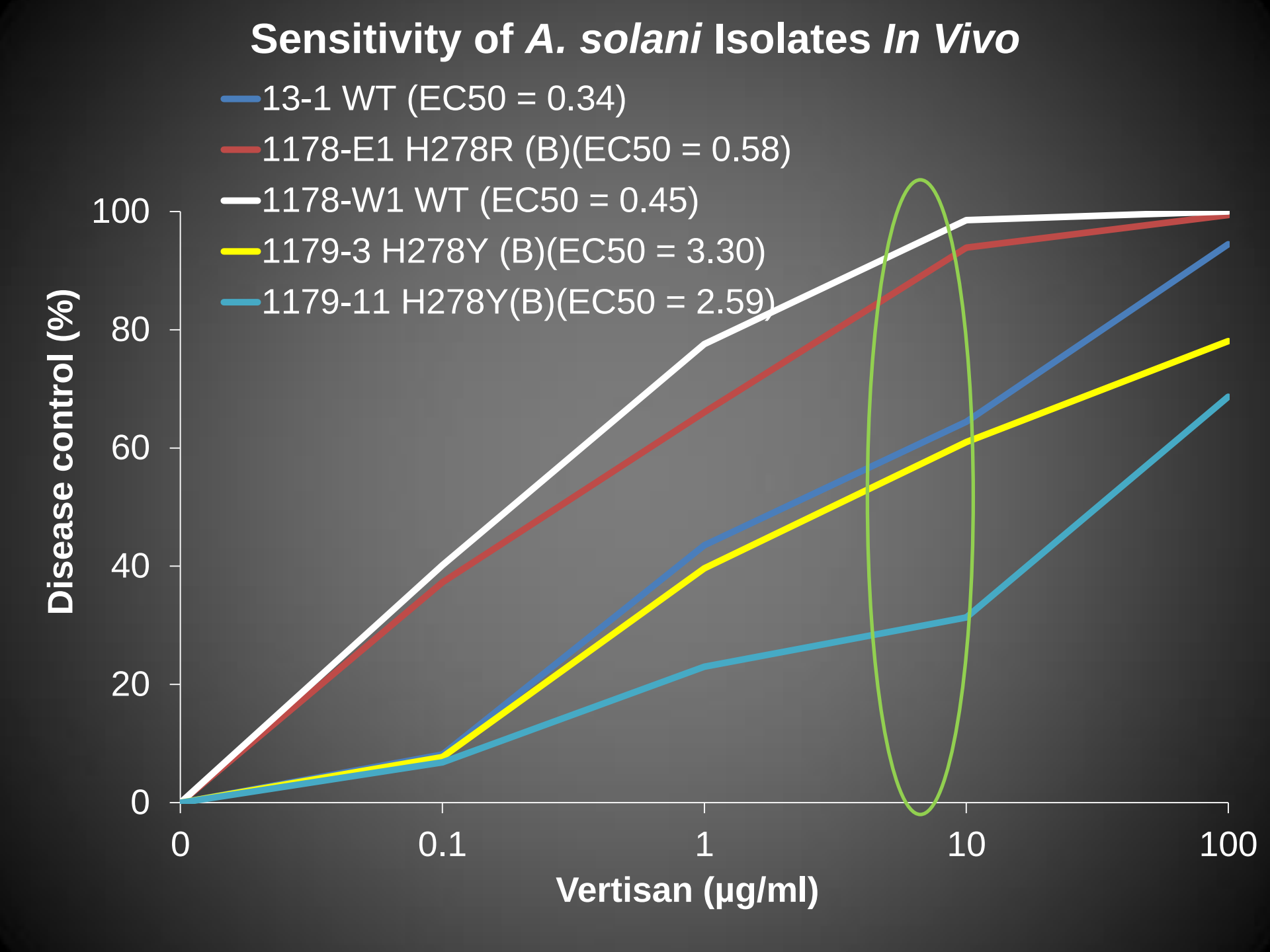
  - Bayer CropScience
  - Syngenta
  - DuPont

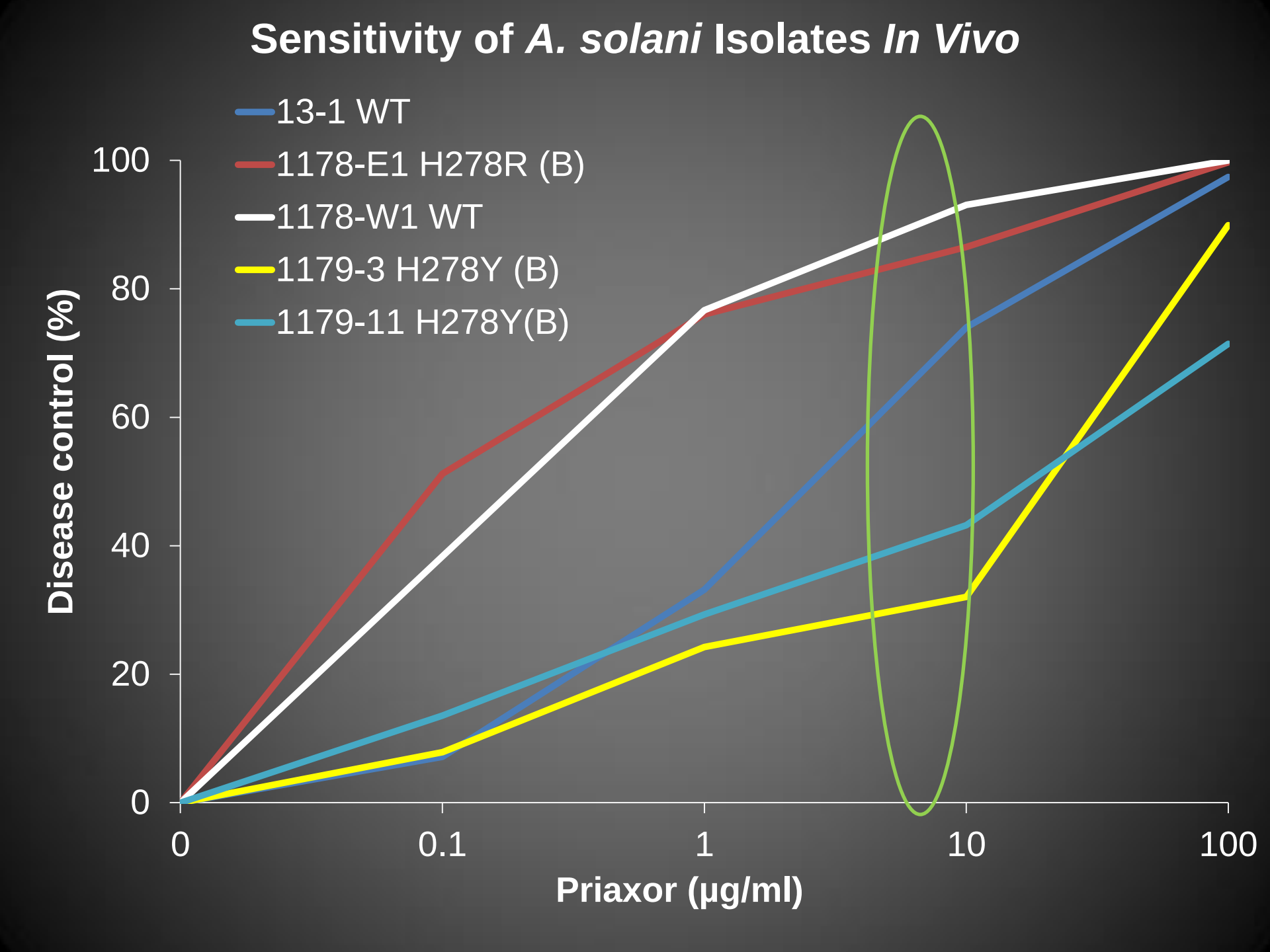
Thank you!
Questions?

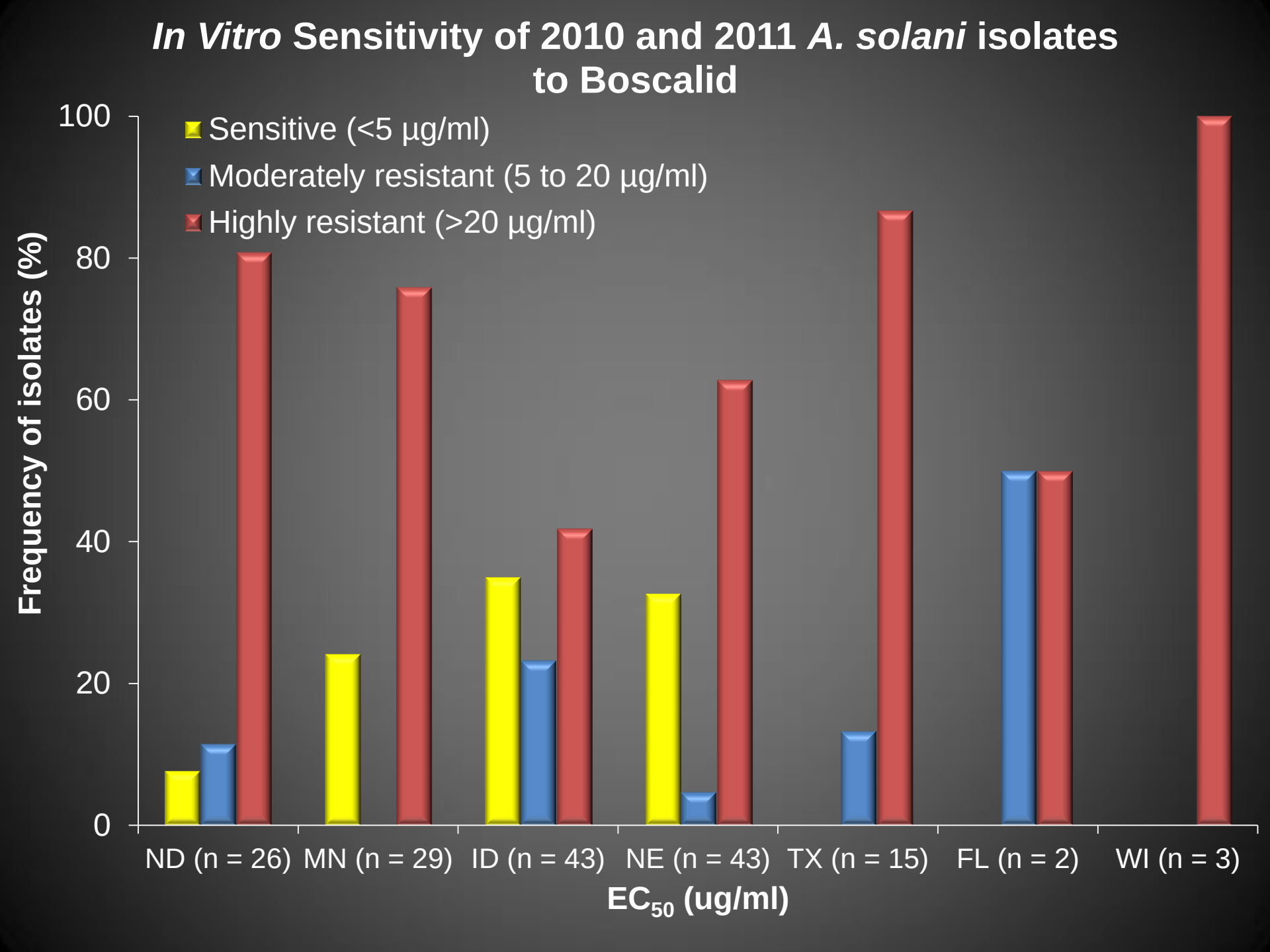
Impact of Endura Resistance on Disease Control


Sensitivity of A. solani Isolates In Vivo
13-1 WT (EC50 = 0.32)
1178-E1 H278R (B)(EC50 = 5.90)
1178-W1 (WT)(EC50 = 0.28)
1179-3 H278Y (B)(EC50 >500)
1179-11 H278Y (B)(EC50 = 153)
Disease control (%)
100
80
60
40
20
0
0
0.1
1
10
100
Endura (µg/ml)


Sensitivity of A. solani Isolates In Vivo
13-1 WT (EC50 = 0.30)
1178-E1 H278R (B)(EC50 = 0.34)
1178-W1 WT (EC50 = 0.75)
1179-3 H278Y (B)(EC50 = 0.30)
1179-11 H278Y(B)(EC50 = 0.38)
Disease control (%)
100
80
60
40
20
0
0
0.1
1
10
100
Luna (µg/ml)


Sensitivity of A. solani Isolates In Vivo
13-1 WT (EC50 = 0.34)
1178-E1 H278R (B)(EC50 = 0.58)
1178-W1 WT (EC50 = 0.45)
1179-3 H278Y (B)(EC50 = 3.30)
1179-11 H278Y(B)(EC50 = 2.59)
Disease control (%)
100
80
60
40
20
0
0
0.1
1
10
100
Vertisan (µg/ml)


Sensitivity of A. solani Isolates In Vivo
13-1 WT
1178-E1 H278R (B)
1178-W1 WT
1179-3 H278Y (B)
1179-11 H278Y(B)
Disease control (%)
100
80
60
40
20
0
0
0.1
1
10
100
Priaxor (µg/ml)


In Vitro Sensitivity of 2010 and 2011 *A. solani* isolates to Boscalid
Sensitive (<5 µg/ml)
Moderately resistant (5 to 20 µg/ml)
Highly resistant (>20 µg/ml)
Frequency of isolates (%)
100
80
60
40
20
0
ND (n = 26)
MN (n = 29)
ID (n = 43)
NE (n = 43)
TX (n = 15)
FL (n = 2)
WI (n = 3)
EC50 (ug/ml)

SDHI Resistance in TX, NE, CO- 2013

Frequency of SDHI Resistance Mutation in Each State

State	No. of Isolates	H278Y (VHR) %	H278R (MR) %	H134R (HR) %	H133R (VHR) %	D123E (VHR) %	Sensitive Endura %
Texas	86	12	1	72	7	6	2
Neb.	47	23	7	36	19	13	2
Colo.	80	7	15	64	10	3	1

SDHI Resistance in ND & MN- 2013

Frequency of SDHI Resistance Mutation in Each State

State	No. of Isolates	H278Y (VHR) %	H278R (MR) %	H134R (HR) %	H133R (VHR) %	D123E (VHR) %	Sensitive Endura %
North Dakota	83	14	17	64	4	0	1
Minn.	51	4	2	53	41	0	0
Total*	466	18	13	50	14	4	1

* States sampled includes, CO, ID, IL, MI, MN, ND, NE, NM, TX, WI, WA