Monitoring 2016

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Cercospora beticola

Sugar beet pathogen

Risk of evolving resistance Medium

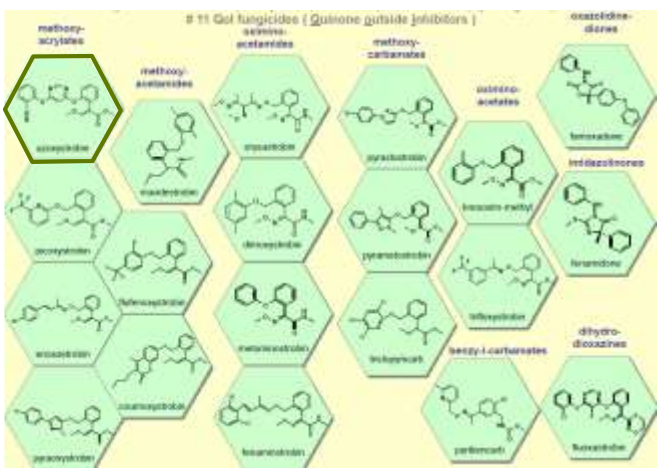
Cercospora beticola fungicide sensitivity monitoring 2016

Country	Isolates
Austria	0
Belgium	5
Croatia	0
Czech Republic	15
France	229
Germany	107
Netherlands	39
Poland	40
Romania	15
Russia	20
Slovakia	0
Sweden	26
Switzerland	30
13 Countries	526

- A total of 526 isolates were isolated from 190 samples collected in 13 European countries. From samples collected in Austria, Croatia and Slovakia we were not able to isolate Cercospora beticola

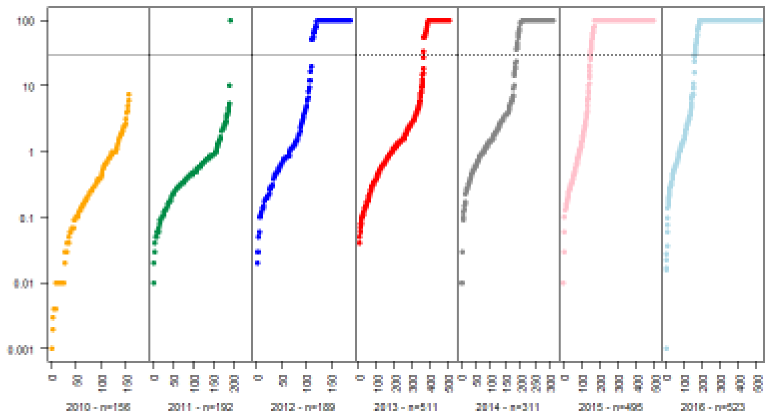
Cercospora beticola fungicide sensitivity monitoring 2016
Samples locations

QoIs



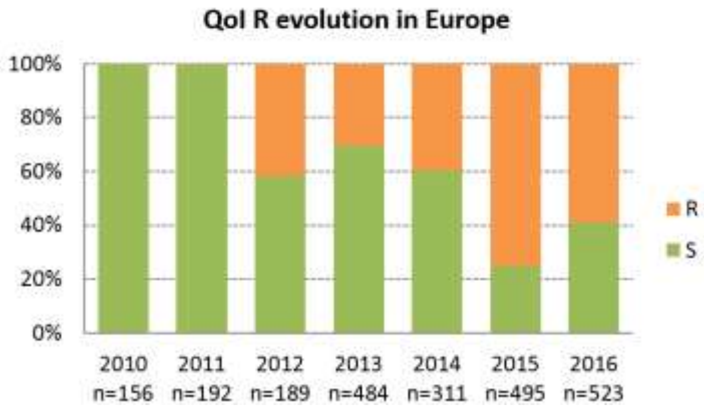
 Pyrenophora teres

C. beticola AZ sensitivity evolution from 2010 to 2016



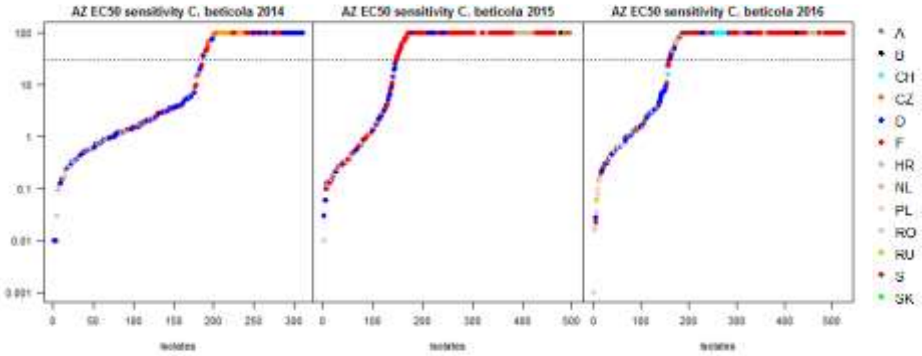
The first QoI resistant isolate was monitored in 2011
General stability of AZ resistance frequency in Europe in 2016 compared to 2015

A general AZ stability was monitored in Europe in 2016



- The first QoI resistant isolate was monitored in 2011
- In 2016 QoI sensitivity stabilized at the levels of 2015
- QoI sensitivity is highly heterogeneous between countries

QoI sensitivity of C. beticola populations from 2014, 2015, 2016



The mutation G143A is possible for C. beticola and it explain QoI resistance

In 2016, medium to high frequency of resistance monitored in B, CH, CZ, F, NL, S
In 2016, lower frequency of AZ resistance was monitored in D, PL, RO, RU

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QoI sensitivity of C. beticola populations from 2016

Country	QoI-R	QoI-S
Austria		
Belgium	4	1
Croatia		
Czech Republic	15	0
France	207	19
Germany	38	69
Netherlands	33	6
Poland	8	32
Romania	5	10
Russia	1	19
Slowakia		
Sweden	26	0
Switzerland	28	2
n=523	365	158

In 2016, medium to high frequency of resistance monitored in B, CH, CZ, F, NL, S
In 2016, lower frequency of AZ resistance was monitored in D, PL, RO, RU

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C. beticola AZ sensitivity geographical distribution 2014 - 2016

AZ resistant isolates were found in all tested European countries

Increased frequency of QoI resistance in most monitored countries from 2014 to 2015. In 2016 general stabilization on the level of 2015

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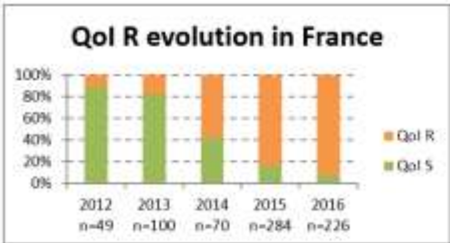
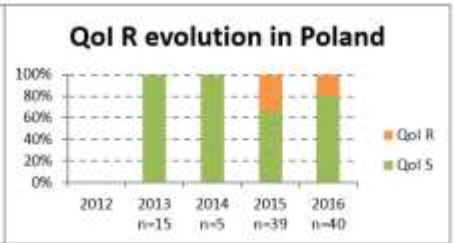
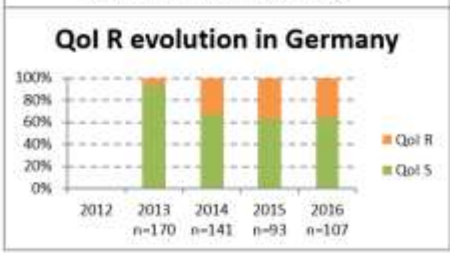
C. beticola QoI sensitivity evolution in European Countries







- Monitoring 2016 is showing a general stability in AZ sensitivity in Europe
- France assessed AZ resistance at high levels. Low frequency of AZ resistance was monitored in both Germany and Poland

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Cercospora beticola AZ sensitivity 2016 (by regions) - biotest

High heterogenous situation in Europe monitored in 2016 (e.g. Romania)
In Germany, Poland, Romania and Russia better sensitivity situtation
In F, B, CH, D, NL, PL, RO and RU QoI sensitive isolates were monitored

Cercospora beticola AZ sensitivity 2016 (by regions) - molecular

The molecular approach aimed to quantify the resistance allele G143A generally confirmed the results inferred by biotest.

In Some regions still high heterogenous situation with sample showing high frequency of resistance close to sensitive populations

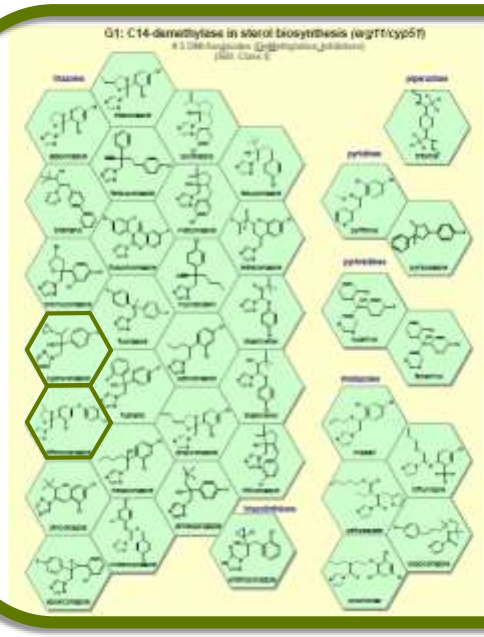
Preliminary results 2016 Cercospora beticola by BASF, Bayer, Syngenta (not yet online)

Intensive monitoring was carried out across Europe in 2016. The levels of resistance found were:
High levels UK, France, Belgium, Czech Republic, Sweden, Switzerland, The Netherlands
Heterogeneous from zero to high in Germany, Poland and Romania
Low levels Russia

new data not yet communicated to FRAC

FRAC WG QoI Minutes 2015 by BASF, Bayer CropScience, Du Pont, Syngenta
High levels: Austria, Croatia, Hungary, Italy, Slovakia, and Slovenia
Medium to high levels: Denmark, France (heterogeneous situation)
No to low levels: Poland, Romania, Sweden
No resistance: Lithuania, Russia
Moderate levels but rather heterogeneous: Czech Republic
Low levels but very heterogeneous: Belgium, Germany, Netherlands and Switzerland

- Apply QoI fungicides according to **manufacturer's recommendations** for the target disease (or complex) at the specific crop growth stages indicated. **Effective disease management** is a critical parameter in delaying the build-up of resistant pathogen populations
- QoI fungicides must be applied only in mixture with partners **from a different cross-resistance group**, contributing to the effective control of the target pathogens
- Apply QoI fungicides **preventatively**. Under high disease pressure the spray interval should not be extended.
- Do not exceed **50% of the total number of sprays** with QoI containing products. In low disease pressure situations **where only 1 fungicide application** is required for disease control then a QoI – containing **mixture** (as defined above) may be used.
- **Where QoI fungicides are used targeting other sugar beet diseases** (e.g. rust, powdery mildew, Rhizoctonia, Ramularia, Stemphylium) then the potential impact of applications on the **resistance management of Cercospora beticola should be considered**. Where Cercospora beticola is not a disease of importance (e.g. in a certain geography) then the general guidelines for QoI fungicides apply.



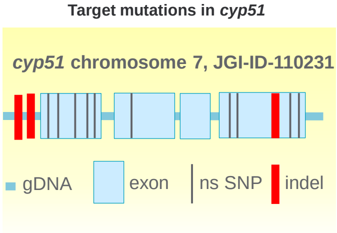
DMI

DFZ, CCZ

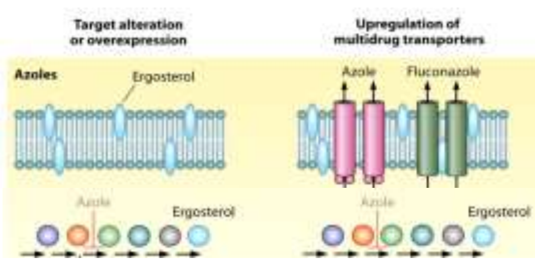
DMI mechanisms of shifting and cyp51

- **Mutations in *cyp51* gene** - principal MoR in several plant pathogen
- **Overexpression of the *cyp51* gene** - *C. beticola, V. inaequalis,. M. fructicola*
- **Efflux of fungicides (ABC transporters)** - *B. cinearea*

Target mutations in *cyp51*



E.g. cyp51 Z. tritici, JGI-ID-110231



Shapiro et al. 2011. Microbiol. Mol. Biol. Rev. DOI: 10.1128/MMBR.00045-10

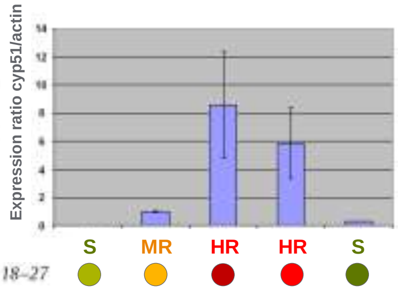
Recurring cycles of **recombination** coupled with **selection** imposed by the DMIs increase the frequency of novel mutants or recombinants with **higher resistance**

Brunner et al. 2008. Mol Plant Pathol 9:305-316 – Zymoseptoria tritici DMI sensitivity evolution

Cercospora beticola DMI sensitivity

- In several plant pathogens **overexpression of *cyp51*** has been identified as a mechanism of azole resistance (e.g. *V. inaequalis, M. fructicola*)
- Sequence comparison between *C. beticola* sensitive and resistant isolates revealed 3 amino acid substitutions. However, no amino acid substitutions were found in some highly resistant isolates
- **Overexpression of cyp51 is correlating to DMI resistance**

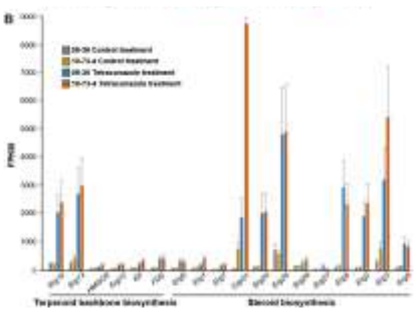
			Genotype		
Isolate	DMI sensitivity	Code	E297K	I330T	P384S
222s	Sensitive		-	-	-
131ve	Sensitive		-	-	-
45am	Moderately R		-	-	S
126ve	Highly R		K	-	-
156ve	Highly R		-	-	-

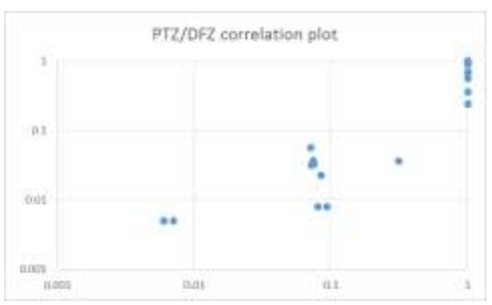


D. Nikou et al. / Pesticide Biochemistry and Physiology 95 (2009) 18–27

Mechanism of shift

no mutations or haplotypes were associated with DMI resistance or sensitivity. No evidence for alternative splicing or differential methylation of *CbCyp51* was found that might explain reduced sensitivity to DMIs. However, *CbCyp51* was overexpressed in isolates with high EC50 values

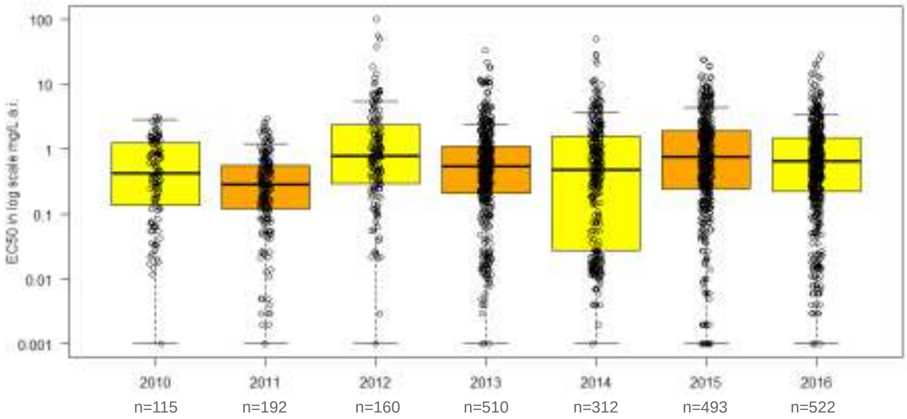




Bolton et al Fungal Genetics and Biology 92 (2016) 1–13

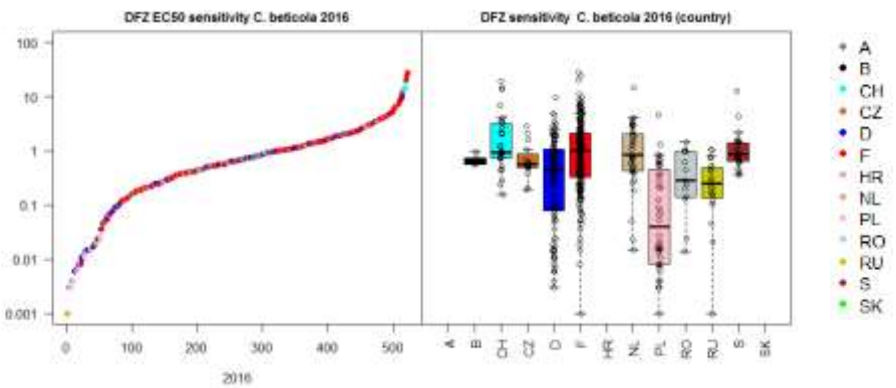
Bolton, M. D., Birla, K., Rivera-Varas, V., Rudolph, K. D., and Secor, G. A. 2012. Characterization of *CbCyp51* from field isolates of *Cercospora beticola*. Phytopathology 102:298-305

C. beticola sensitivity evolution to DFZ from 2010 EUROPE



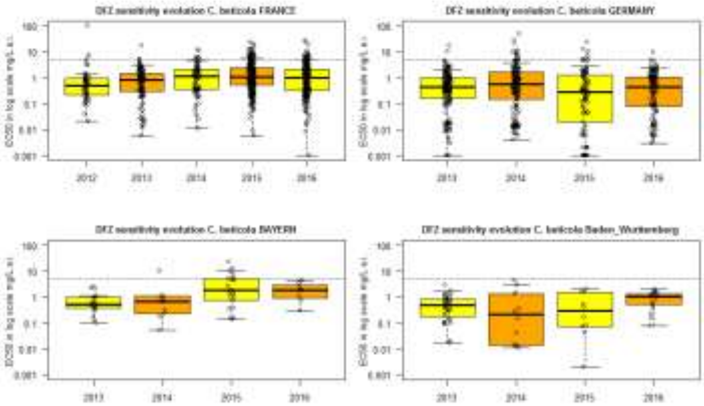
No variation in the sensitivity of Cercospora beticola to DFZ from 2010
From 2012 outliers showing decreased sensitivity can be found at low frequency

C. beticola sensitivity distribution to DFZ in 2016



No major geographical variation reported were monitored for DFZ sensitivity
In 2015 and 2016 outliers showing decreased sensitivity monitored in CH, D, F, NL, S

C. beticola sensitivity evolution to DFZ in regions



The presence of geographically defined populations showing DMI shifted phenotype cannot be excluded. Sensitivity shift might occur through accumulation of outliers showing DMI decreased sensitivity, locally. Further analysis are ongoing to determine the precise geographic distribution of DMI sensitivity and possible DMI shifts.

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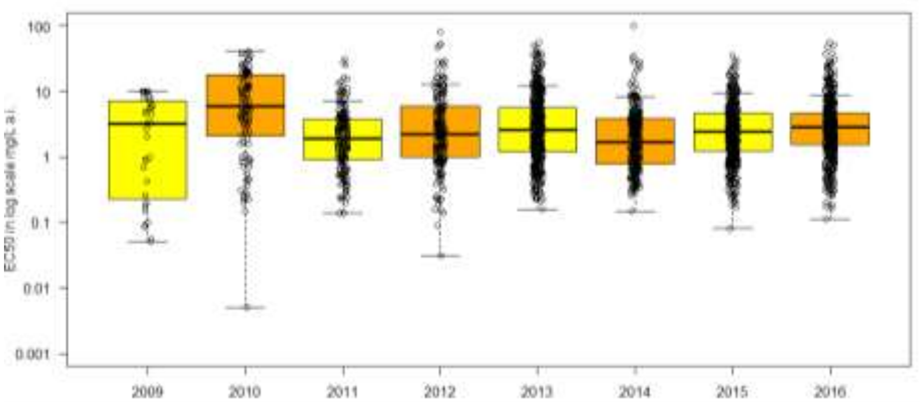
C. beticola sensitivity evolution to CCZ from 2010 EUROPE



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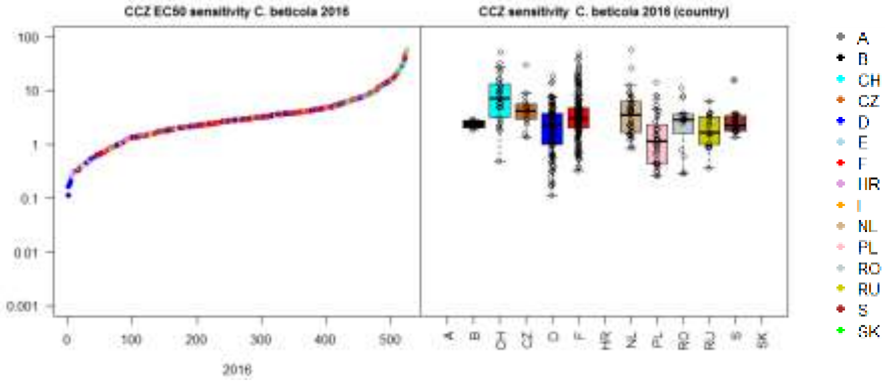


No major geographical variation reported for CCZ sensitivity
Outliers showing decreased sensitivity were monitored at low frequency since years

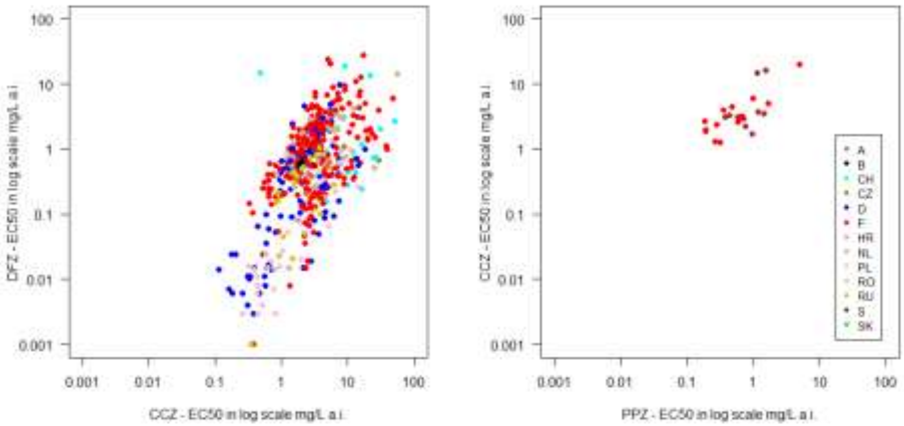
Geographic distribution of C. beticola sensitivity to DMIs

Average EC50 for DFZ 2016 by admin Division

Average EC50 for CCZ 2016 by admin Division

Results presented in these maps should not be overestimated since these are highly depentent from sampling and sample size (not uniform through regions)

All DMIs should be considered as cross resistant



- All tested DMIs (DFZ, CCZ, PPZ) showed cross resistance
- cyp51 overexpression was the described mechanism to explain DMI sensitivity shift
- Cross resistance pattern DFZ vs CCZ needs more molecular investigations to investigate the evolution of putative new resistance mechanisms (e.g. SNPs)

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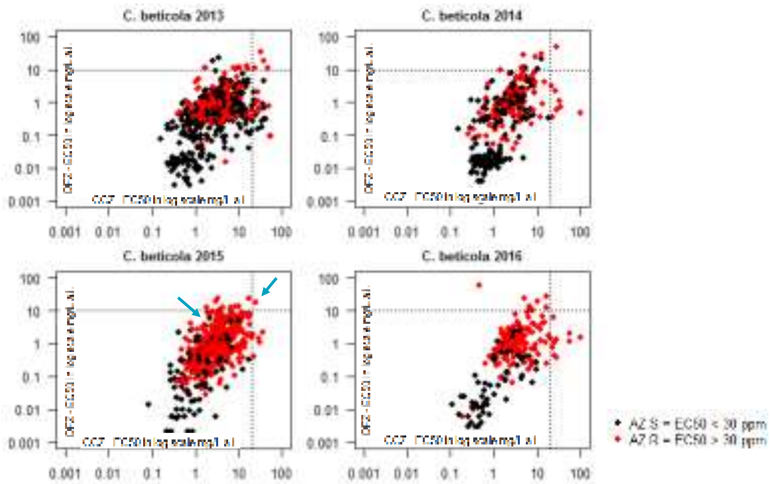
C. beticola CCZ, DFZ, AZT correlation plot 2013 - 2016



- The frequency of samples with multiple resistance (DMIs/QoI, top right panel in the cross resistance panels) is not increasing in the last years
- Glasshouse experiments using two samples showing high EC50 values to DFZ showed that DFZ+AZ showed 15 dai an activity between 78 to 90%

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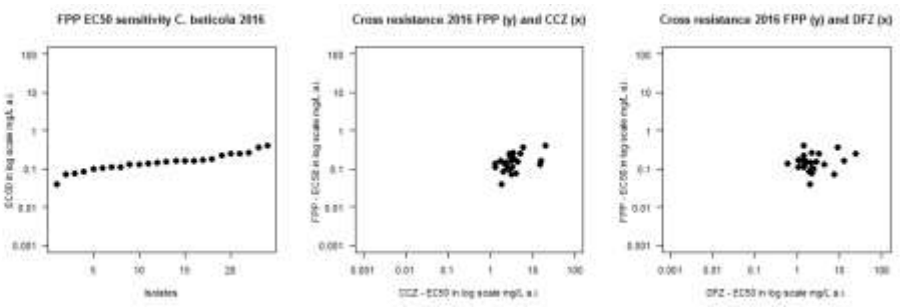
C. beticola sensitivity to Fenpropidin and non cross resistance to DMIs



C. beticola sensitivity of 10 isolates collected from Sweden and 14 from France was monitored

Fenpropidin EC50 values were narrow distrubuted suggesting a general sensitive situation

Fenpropidin didn't show cross resistance to DMI.

General Conclusions

- QoI resistance based on G143A mutation is present
  - QoI activity is affected
  - Other mechanisms, like F129L, can be found but are rare
- DMI sensitivity shift has been observed to a certain extend
  - Mechanism probably related to DMI compound independent overexpression of cyp51 – all DMI cross resistant
  - DMI activity in general not or only weakly affected, in specific cases stronger effects might be observed
- Other fungicide classes are not affected by the shift/resistance developments: multi-sites, Morpholines
- Use all possible fungicde classes in alternations of mixtures
- Consider the epidemic speed in relation to the threshold currently used