



Fundamental Aspects for the Development of Resistance to Fungicides

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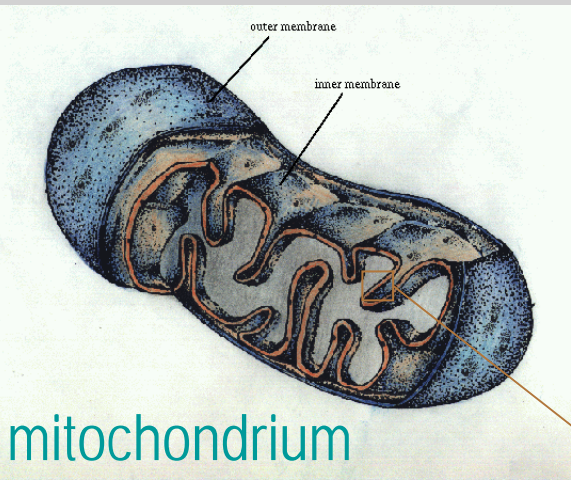


Many of the current ASR fungicides are either strobilurins or DMI's

Azoxystrobin	S		Tetraconazole	D
Boscalid			Prop & Azoxy	D&S
Chlorothalonil			Cyproconazole	D
Pyraclostrobin	S		Azoxy & Cypro	S&D
Propiconazole	D		Bosc & Pyraclo	A&S
Tebuconazole	D		Flusilazole	
Myclobutanil			Flutriafol	
Tri & Propi	S&D		Metconazole	D

S = strobilurin; D = DMI

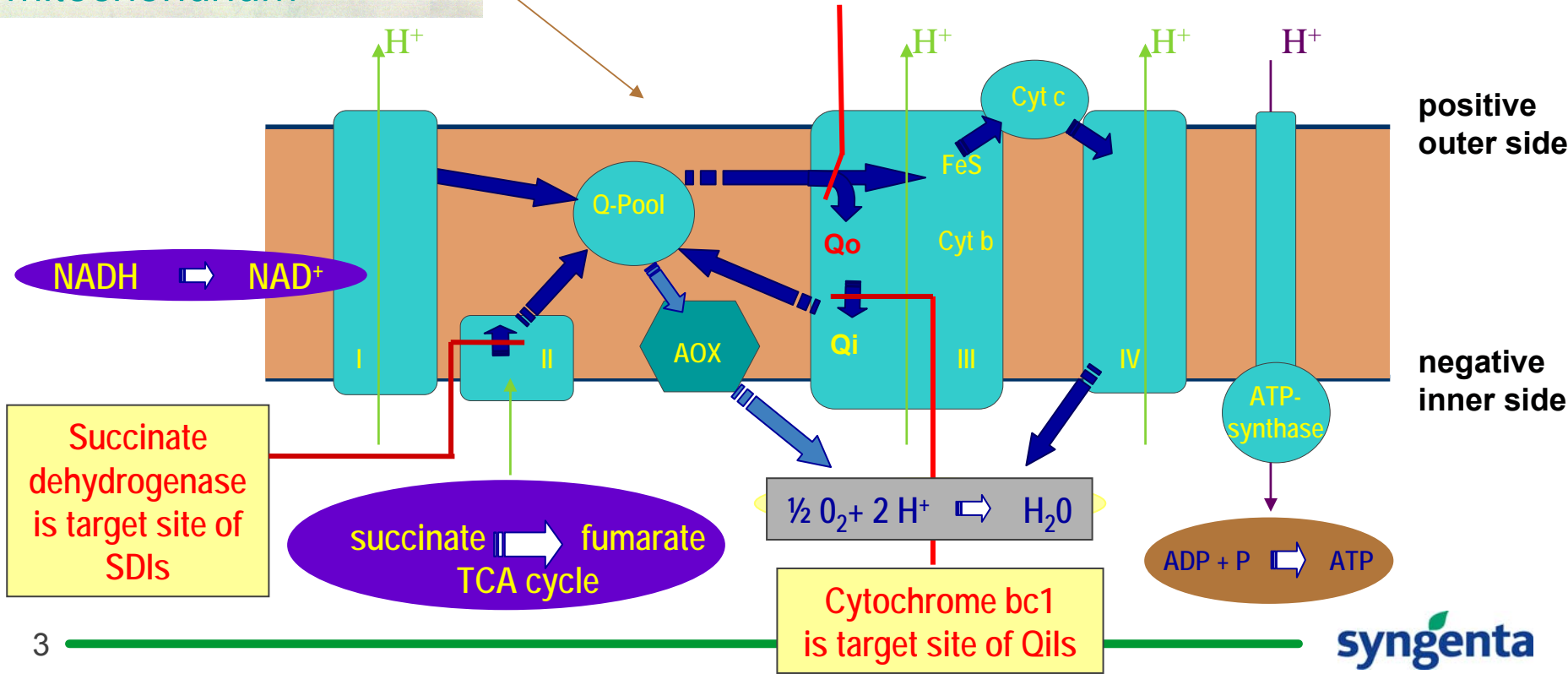
Schematic diagram of mitochondrial respiration and flow of electrons and protons



Cytochrome b gene is part of mitochondrial genome

Cytochrome bc1 is target site of Qols

Strobilurins



Mode of action and mechanism of resistance for Qols

Mode of action (single site inhibition):

Inhibition of cytochrome bc1 (complex III) at Qo pocket in mitochondrial respiration (encoded by the *cyt b* gene)

Mechanisms of resistance (monogenic, separation s/r):

1. G143A mutation (complete resistance, loss of disease control if Qol solo)

Blumeria (= *Erysiphe*) *graminis*, wheat and barley

Mycosphaerella graminicola (= *Septoria tritici*), wheat

Pyrenophora (= *Drechslera*, *Helminthosporium*) *tritici-repentis* (DTR), wheat

Plasmopara viticola, grape

Alternaria alternata, pistacio

2. F129L mutation (“partial“ resistance, reduced disease control if Qol solo)

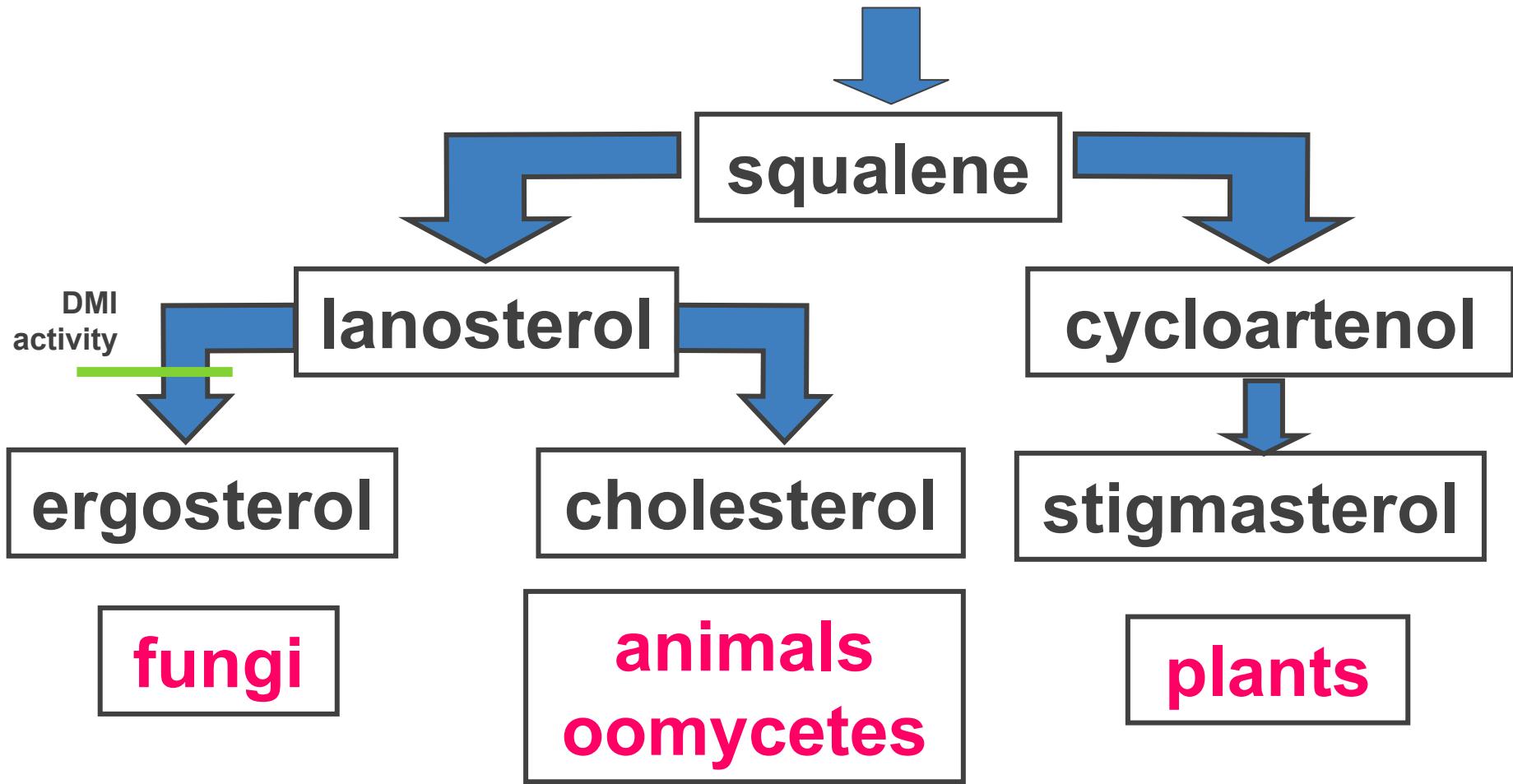
Pyrenophora (= *Drechslera*, *Helminthosporium*) *teres*, barley

Alternaria solani, potato (USA)

3. NO mutations (no resistance)

Puccinia, *Uromyces*, *Phakopsora*, *Hemileia*, different crops

Sterol biosynthesis and site of action of DMIs



Mode of action and mechanism of resistance for DMIs

Mode of action (single site inhibition):

Inhibition of cytochrome P 450-dependent lanosterol-C14 α -demethylase in the biosynthesis of fungal sterols such as ergosterol (encoded by the *cyp51* or *erg11* gene)

Mechanisms of resistance (polygenic, multi-allelic, sensitivity shift):

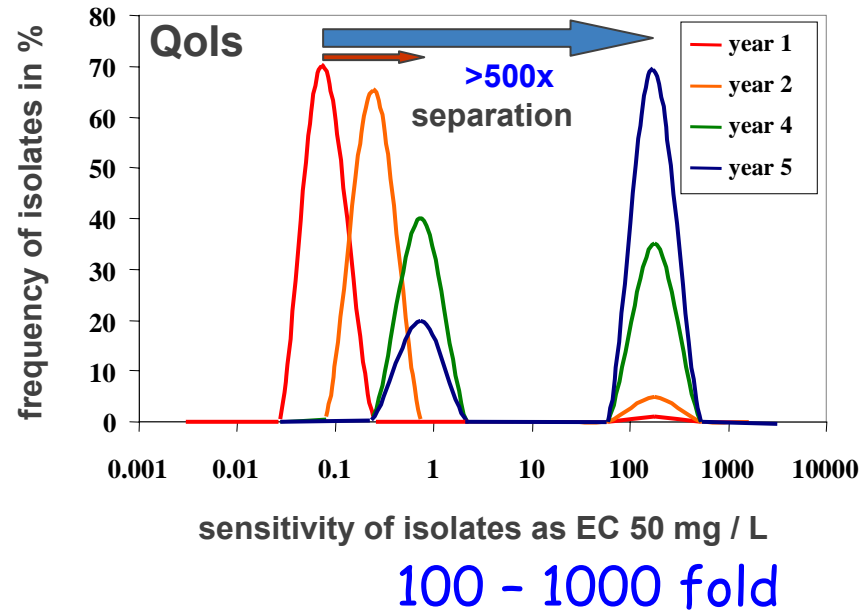
1. Mutations in *cyp51* gene: V136A, Y137F, A379G, I381V (and others and combinations thereof) and thus altered binding
2. Over-expression of *cyp51* gene and thus, increased production of target enzyme
3. Up-regulation of transporter genes and thus, increased activity of specific membrane (ABC-) pumps exporting DMIs out of cells

All 3 mechanisms of resistance can co-exist and contribute to decreasing sensitivity

Fungicide resistance development: Selection models for QoI and DMI fungicides



monogenic, single allelic
resistance at target site,
disruptive selection, high
risk



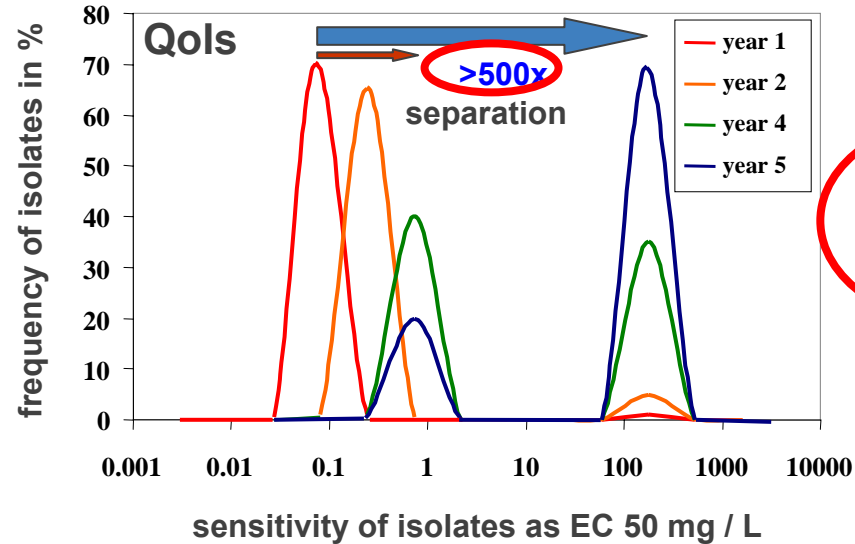
Loss of
Control

- Always starts with a gradual shift but rapid disruptive selection only occurs with a monogenic mechanism of resistance
- Therefore, the risk is **HIGH**

Fungicide resistance development: Selection models for QoI and DMI fungicides

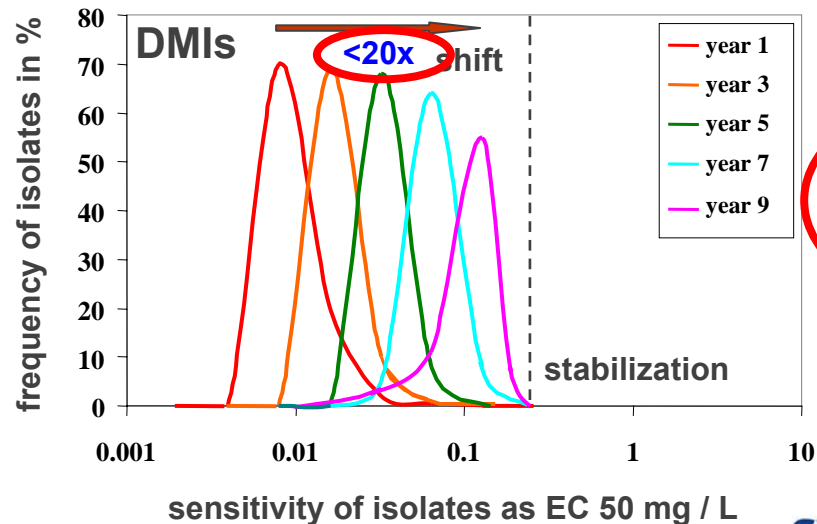


monogenic, single allelic
resistance at target site,
disruptive selection, **high
risk**

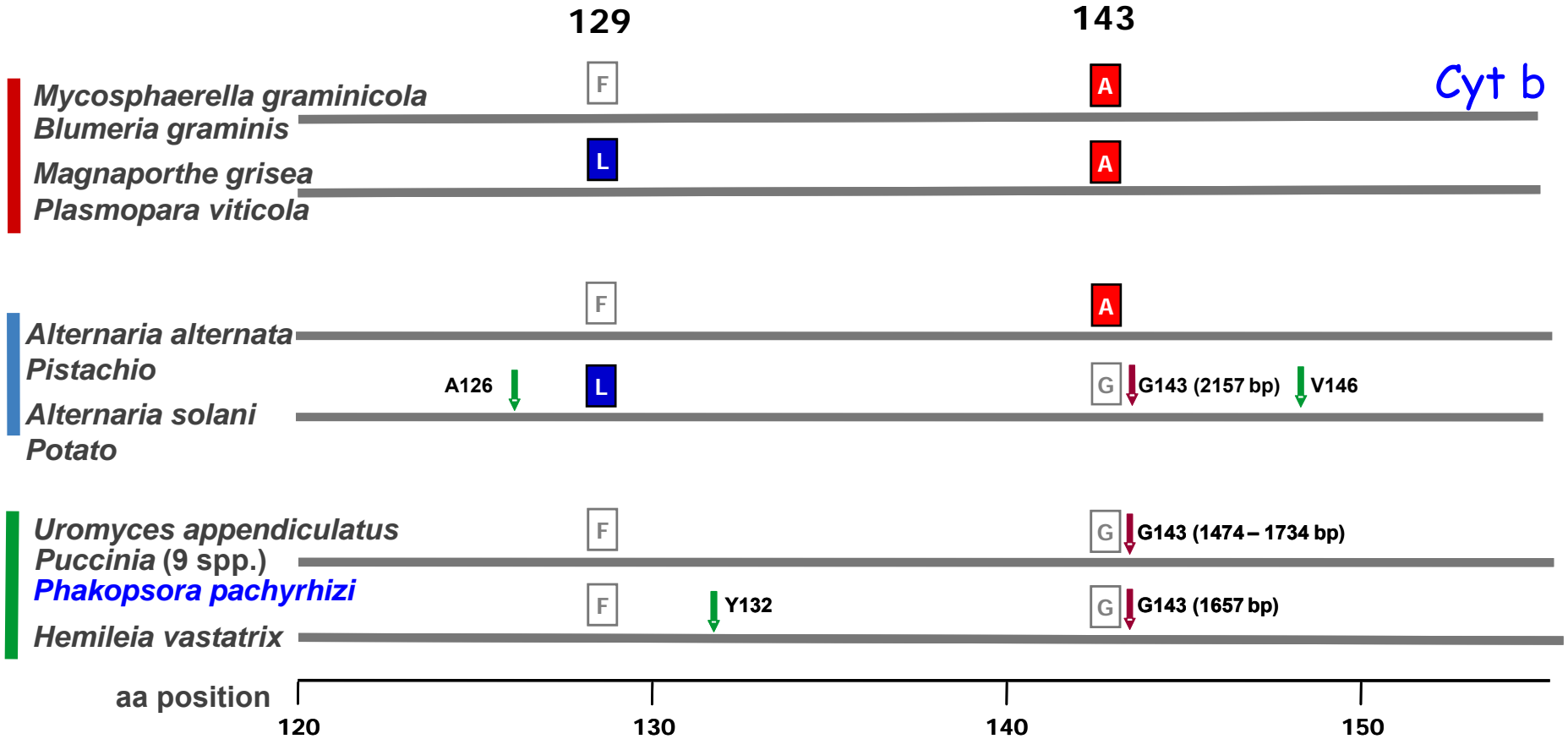


polygenic, multi allelic
resistance at target site,
continuous selection,
moderate risk

- Fitness cost
- Polygenic inheritance (dilution)

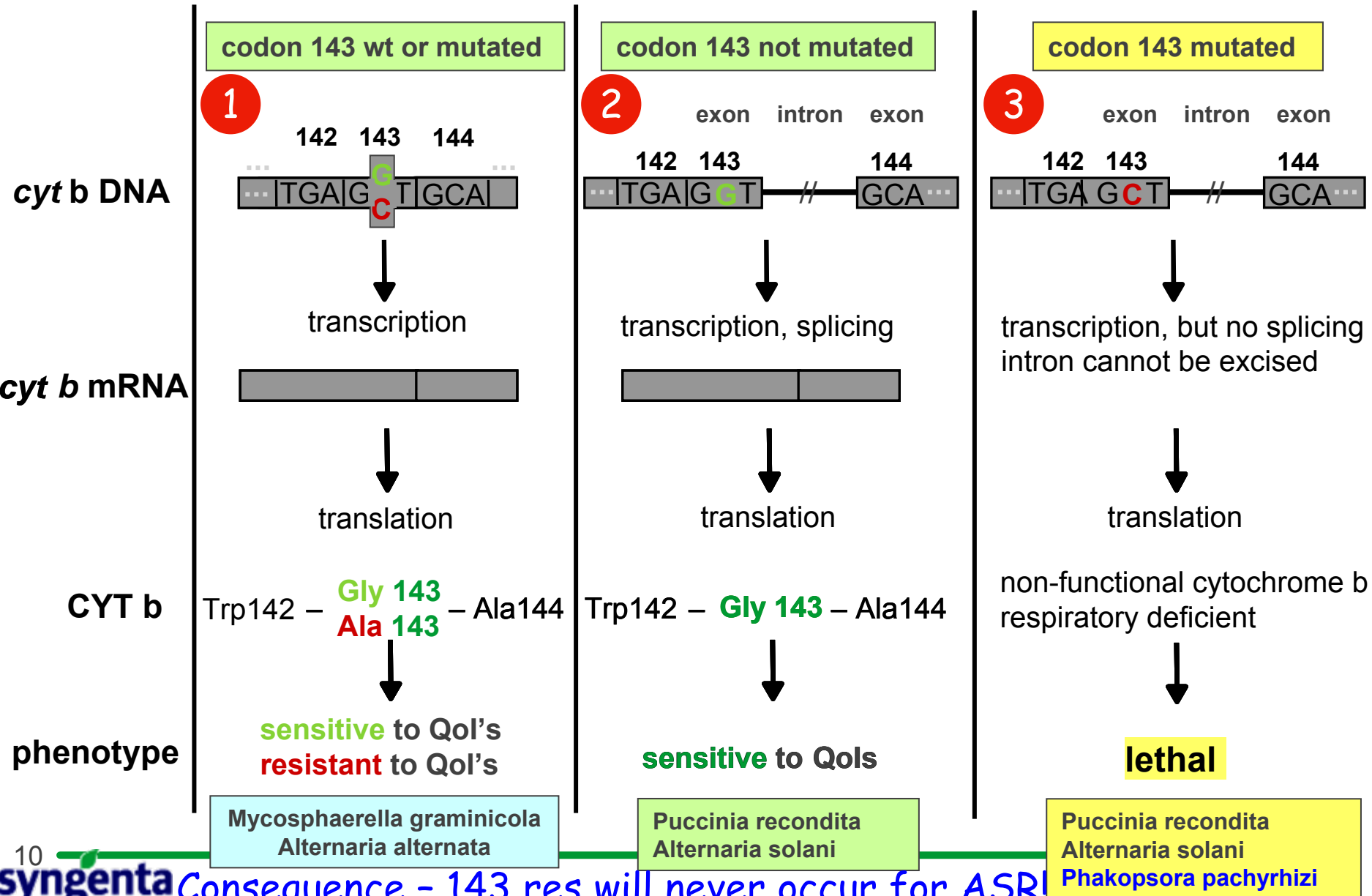


QoI Structure of cytochrome b gene and position of introns (arrow) and G143A and F129L mutations*



- F = wild type (phenylalanine)
- A = mutation from glycine to alanine
- L = mutation to lysine

Structure of cytochrome b gene fragment coding for sensitivity to Qol fungicides (with/without intron)



Summary: Molecular mechanisms of Qol resistance



***Alternaria solani*: Intron at 143, NO G143A mutation, but F129L was detected (moderate risk) (Pasche et al., 2002; Rosenzweig et al., 2006).**

***Alternaria alternata*: NO Intron at 143, G143A possible (high risk). G143A was detected in resistant isolates (Ma & Michailides, 2004).**

All rusts (*Puccinia*, *Uromyces*, *Phakopsora*, *Hemilia*) as well as *Alternaria solani* and *Pyrenophora teres* CANNOT acquire Qol resistance (based on G143A), because specific gene structure does not allow it (intron at position 143): **Low resistance risk (Grasso et al., 2006).**

The F129L mutation was not found in rust species. In bioassay, NO Qol resistance found in rusts (more than 5000 *P.recondita* isolates tested – past 10 years).

For the first time, it can be predicted, based on molecular information, whether resistance may appear or not in a particular pathogen.

Now have a molecular tool

Published cases of decreased sensitivity to DMIs and molecular explanations in plant pathogens



Mutations in *cyp51* gene

Uncinula necator, grape, Delye et al. 1997, Y136F

Erysiphe (Blumeria) graminis fsp. hordei, barley, Delye et al. 1998, Y136F

Erysiphe (Blumeria) graminis fsp. tritici, wheat, Wyand & Brown 2005, Y136F, K147Q

Mycosphaerella graminicola, wheat, Cools et al. 2007, Leroux et al., 2007, Chassot et al., 2007,

V136A, Y137F, A379G, I 381V (also detected but less important are L50S, V136C, S188N and several deletions and exchanges at aa positions 459, 460, 461)

Over-expression of *cyp51* gene (insert or repeats in promotor)

Venturia inaequalis, apple, Schnabel & Jones 2001

Penicillium digitatum, citrus, Hamamoto et al. 2000

Mycosphaerella graminicola, wheat, Stergiopoulos et al. 2003, Chassot et al. 2007

Blumeriella jaapii, cherry, Ma et al. 2006

Up-regulation of ABC transporter genes

Botrytis cinerea, grape, Hayashi et al. 2003

Mycosphaerella graminicola, wheat, Stergiopoulos et al. 2003; Cools et al. 2005; Chassot et al. 2007

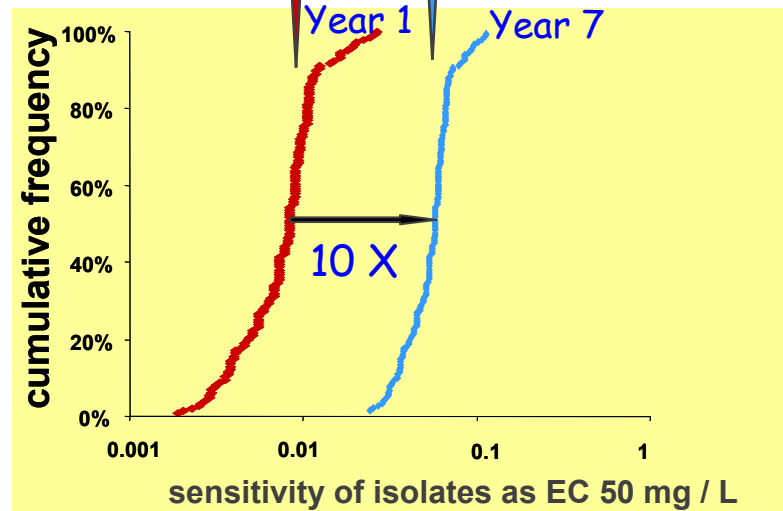
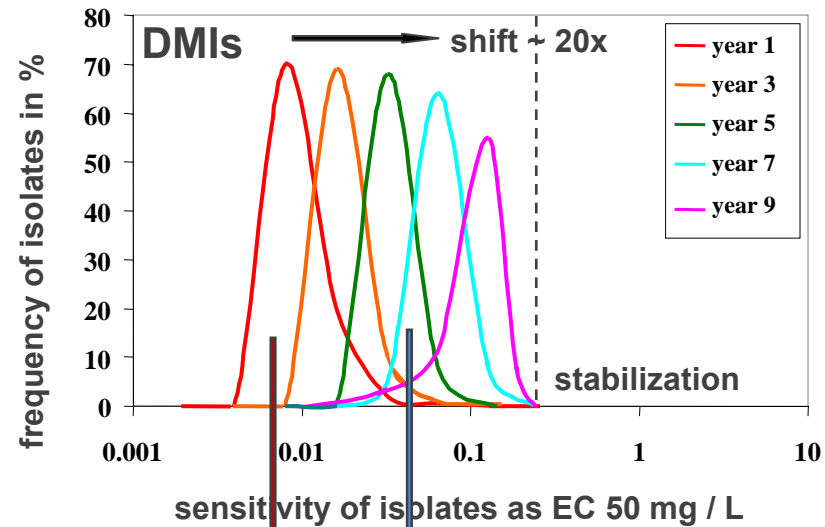
Monilinia fructicola, peach, Schnabel et al. 2003

Fungicide resistance development: Shift in sensitivity to DMI fungicides

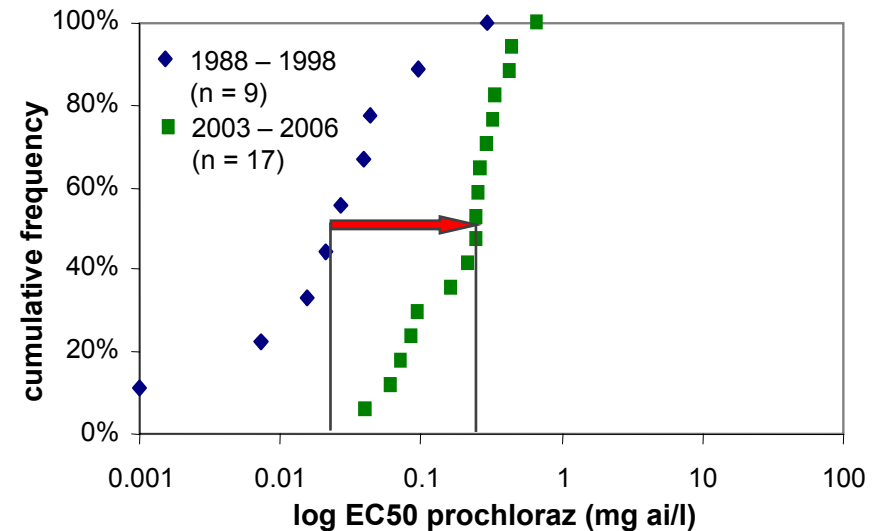
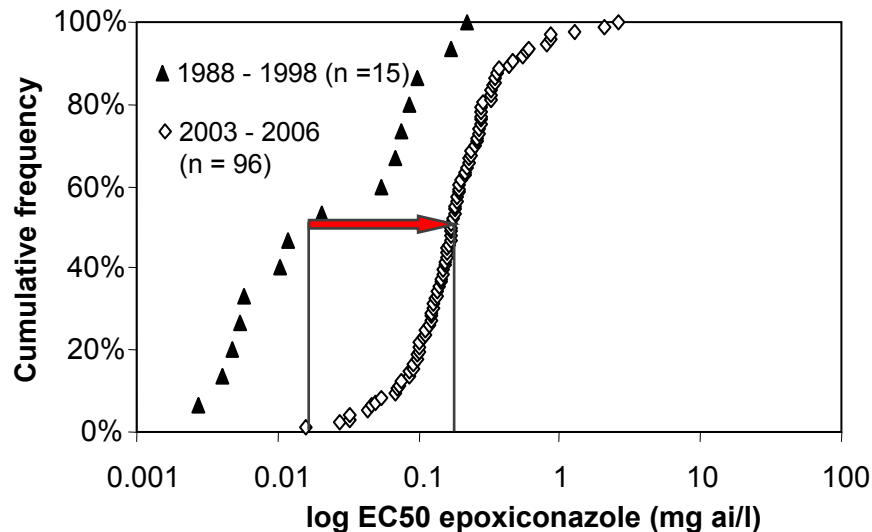
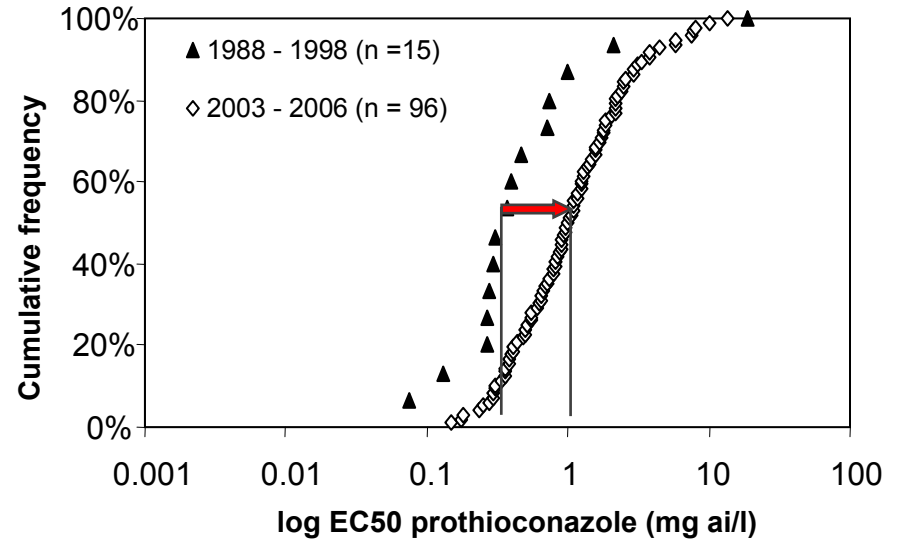
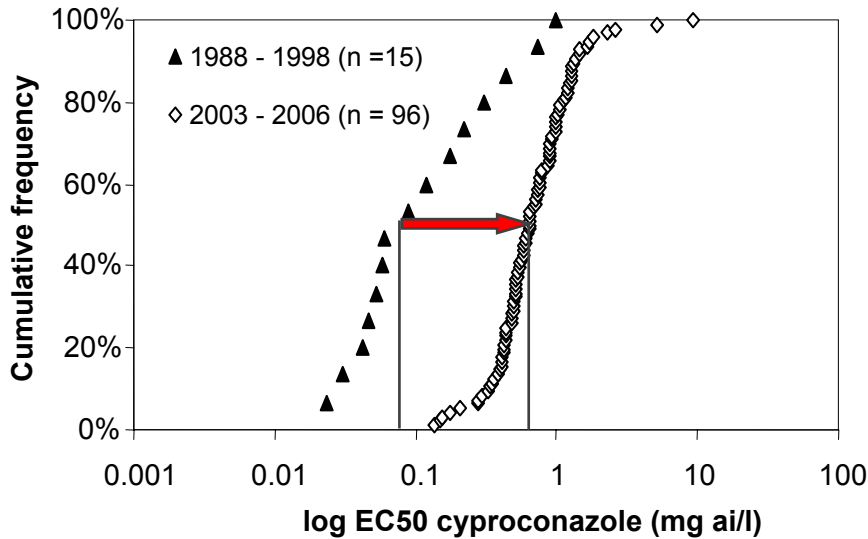


summation, cumulative
frequency of log-normal
distribution

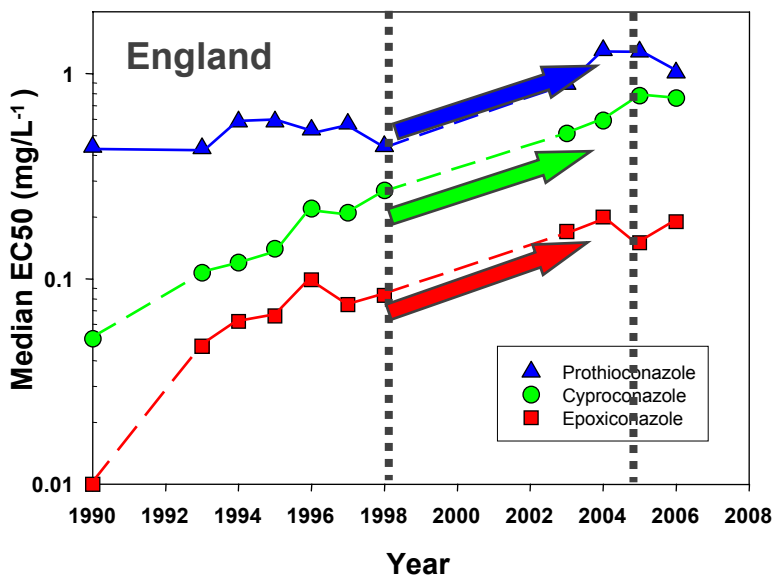
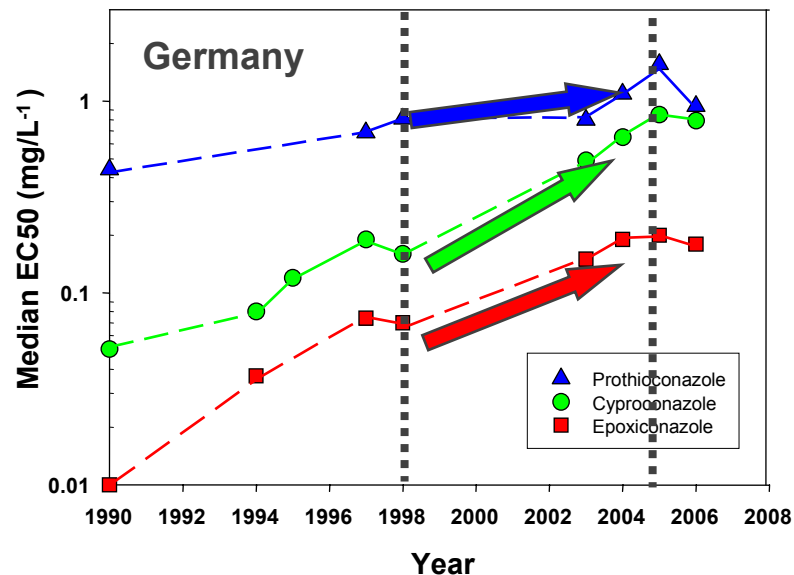
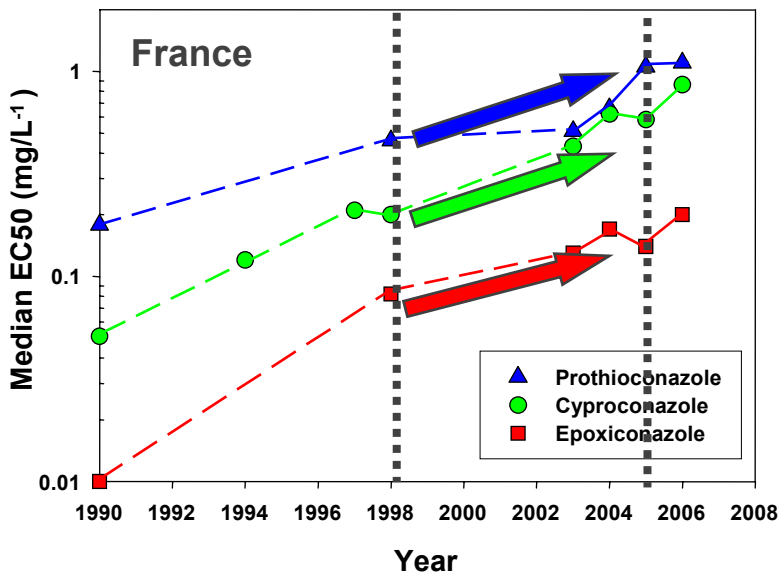
log-normal distribution



Shift in sensitivity to DMIs for *Mycosphaerella graminicola* isolates collected in Europe between 1988 and 2006



Shift of sensitivity (mean EC 50 of population) in *Mycosphaerella graminicola* to cyproconazole, epoxiconazole and prothioconazole in Europe between 1990 and 2006



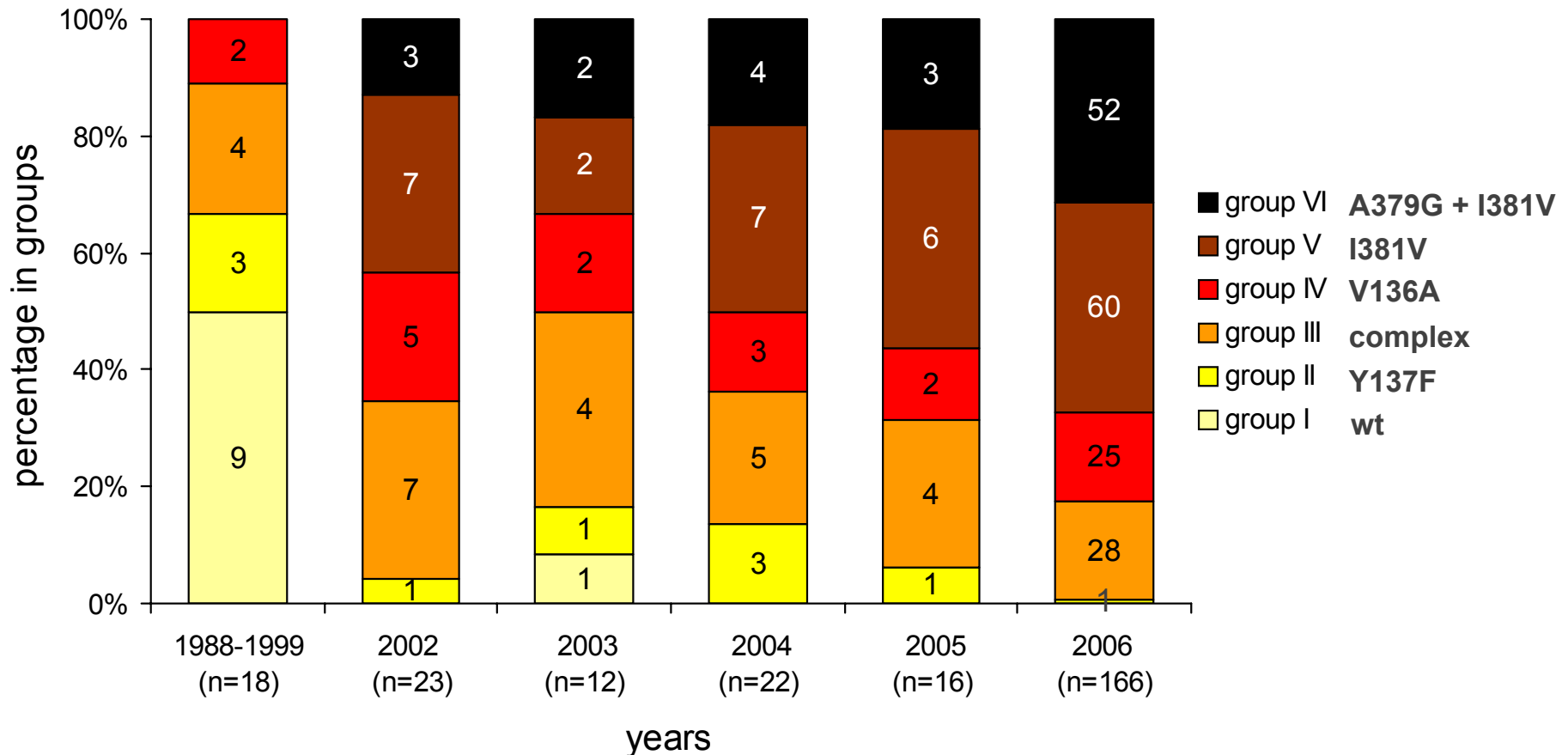
Slow but continuous shift in sensitivity between 1990 and 2006. Stabilization since 2005? Sensitivities of recent populations are lower than in the 1990's for all DMIs in all countries.

How to explain the shift? → Genotypes

Sequenced *cyp51* gene

Frequency of *cyp51* genotypes in *Mycosphaerella graminicola* in Europe between 1988 and 2006 (n = 257)

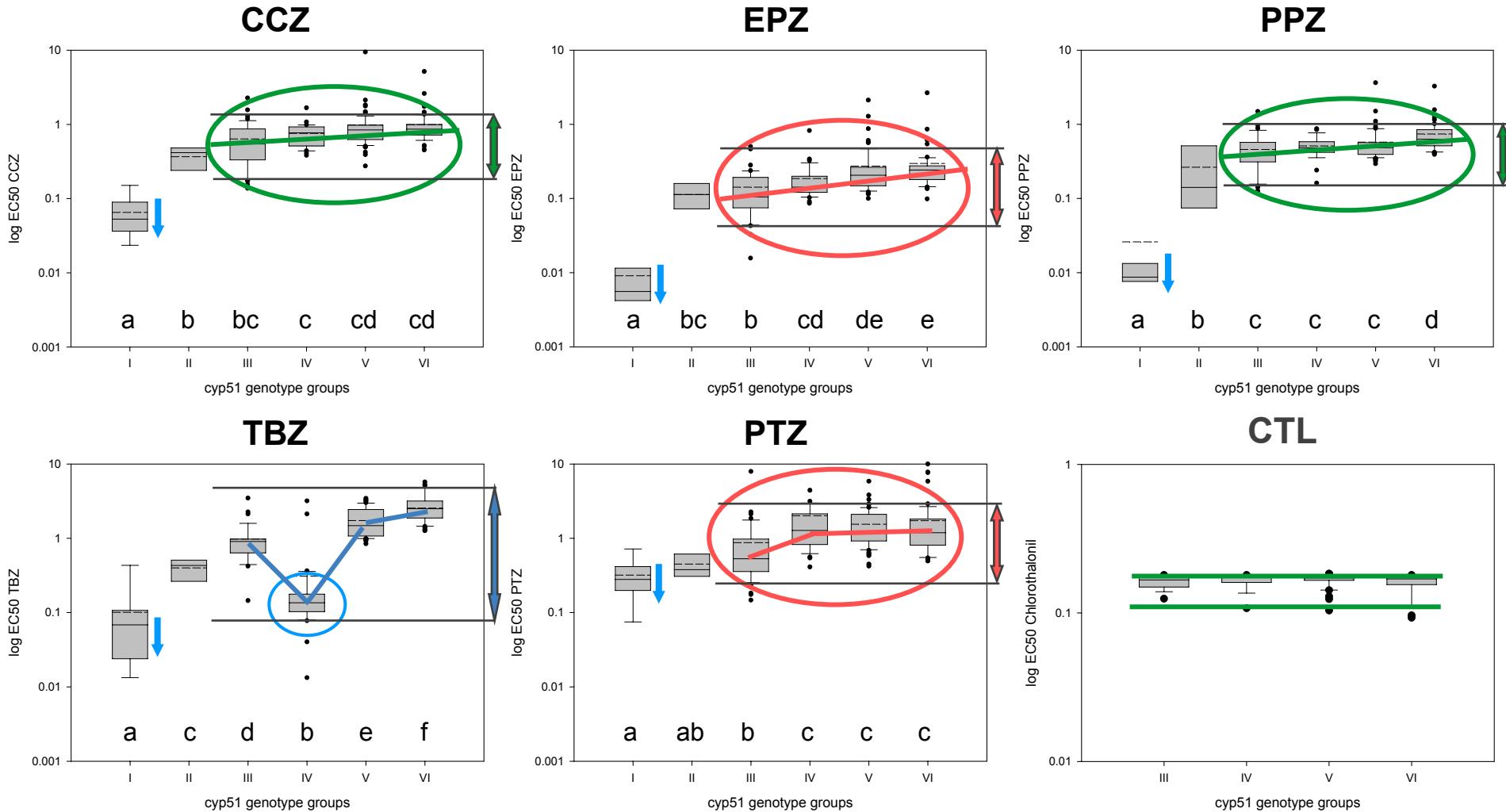
Relative genotype frequency



- ▶ wild type isolates disappeared from recent populations
- ▶ number of mutations increases over the years
- ▶ increase of isolates carrying I 381V and A379G mutations

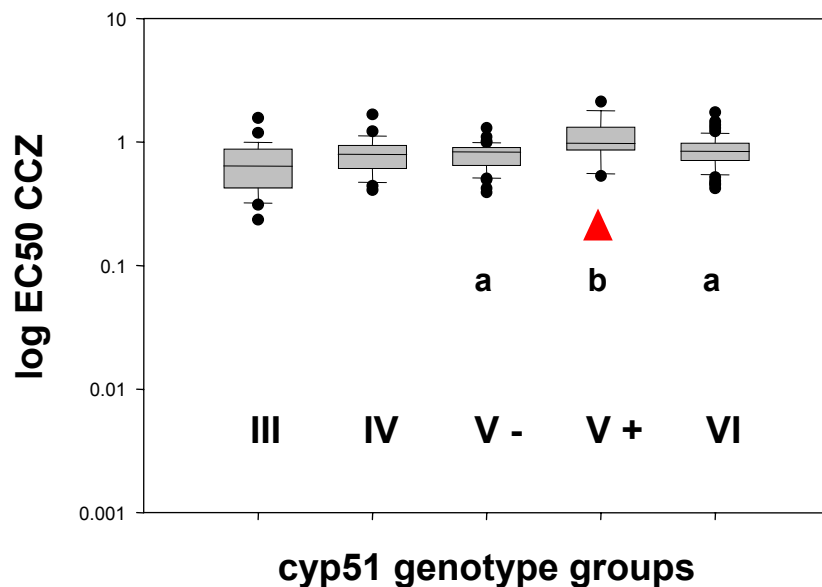
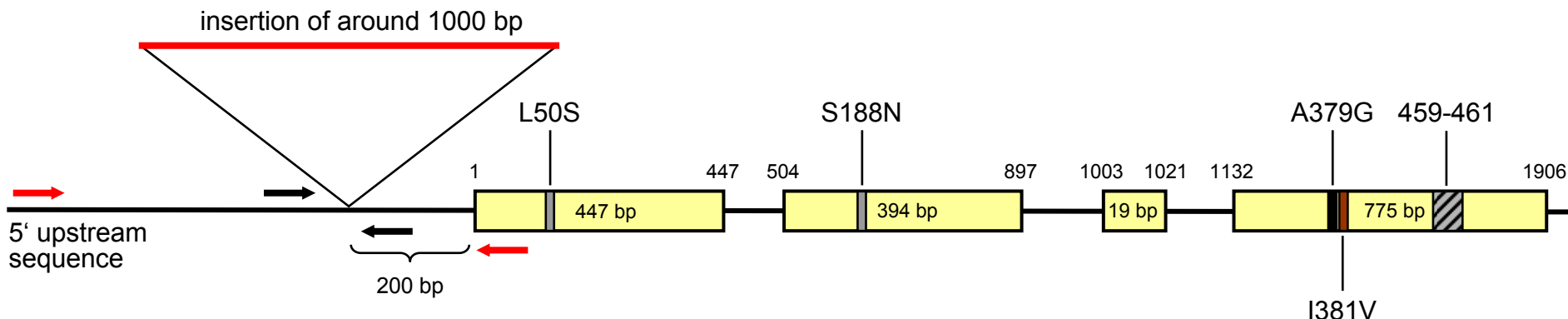
Sensitivity of the 6 *cyp51* genotypes of *M. graminicola* to different DMIs

CCZ: cyproconazole; EPZ: epoxiconazole; PPZ: propiconazole; TBZ: tebuconazole;
 PTZ: prothioconazole; CTL: chlorothalonil (n = 211, isolates from 1988 to 2006)



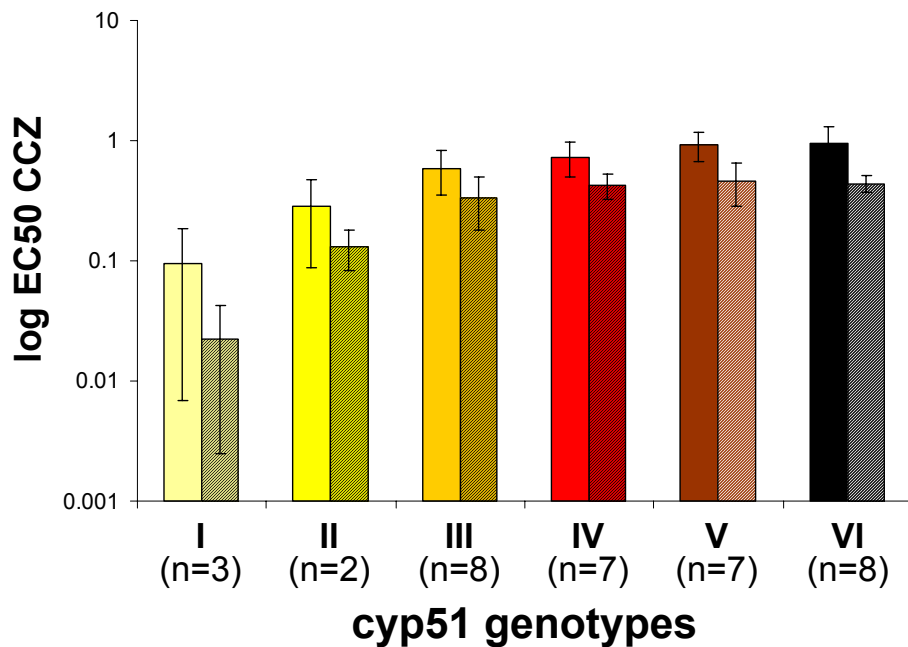
- ▶ range of variation (boxes): CTL << CCZ = PPZ < EPZ ~ PTZ << TBZ
- ▶ differences between genotypes: small for CCZ, PPZ, EPZ, PTZ; big for TBZ
- ▶ the combination of CCZ + PPZ + CTL is expected to impose low selection

Sequence analysis of *cyp51* promoter and sensitivity of genotypes to DMIs in *Mycosphaerella graminicola*



- ▶ insertion in promoter present only in genotype V isolates (in about half of the type V isolates and in very few isolates of type VI)
- ▶ an insertion in the *cyp51* gene results in an over-expression of the gene
- ▶ as a consequence, genotype V isolates with an insertion in the promoter are significantly less sensitive to DMIs

Influence of promazine (40 mg/ L, inhibitor of ABC transporters) on the sensitivity of *Mycosphaerella graminicola* genotypes to DMIs



in vitro growth assay

- - transporter protein inhibitor
- ▨ + transporter protein inhibitor (40 ppm promazine)

inhibitors of ABC transporters slightly increase the sensitivity to DMIs, suggesting that ABC transporters may be involved in the decrease of sensitivity to DMIs

Summary

- Strobilurins have specific, monogenic resistance mechanisms for resistance. Therefore, they are High Risk if this occurs.
- Resistance due to G143A mutation will never occur of *Phakopsora pachyrhizi* because this would be a lethal mutation due to non splicing of introns.
- Resistance to DMI's can occur via several mechanisms. However, this is a gradual shift which does not result in loss of control.

Summary



- A slow but continuous shift in sensitivity has been observed to all DMIs in many pathogen populations since the introduction of DMIs resulting in an erosion of DMI product performance
- In *Mycosphaerella graminicola*, the sensitivity shift to DMIs observed between 1988 and 2006 is due to a change in genotype distribution in field populations (mutations in *cyp51* gene)
- Because the more recent genotypes (V and VI) of *M. graminicola* are somewhat less sensitive to triazoles than isolates from the 1990's, fungicide rates must be kept high to ensure robust disease control

Summary

- The presence of a promotor in the cyp51 gene (over-expression of cyp51 gene) and the action of ABC transporters (up-regulation of ABC transporter genes) further decrease the sensitivity to DMIs
- Research results currently “In Press” support the notion that mixtures of DMI’s and/or DMI’s with other fungicides should provide a good resistance management strategy
 - I.E. Absolute; Alto and Quadris tank mix; Stratego; Quadris Xtra