Mechanisms of fungicide resistance and actions to reduce risk

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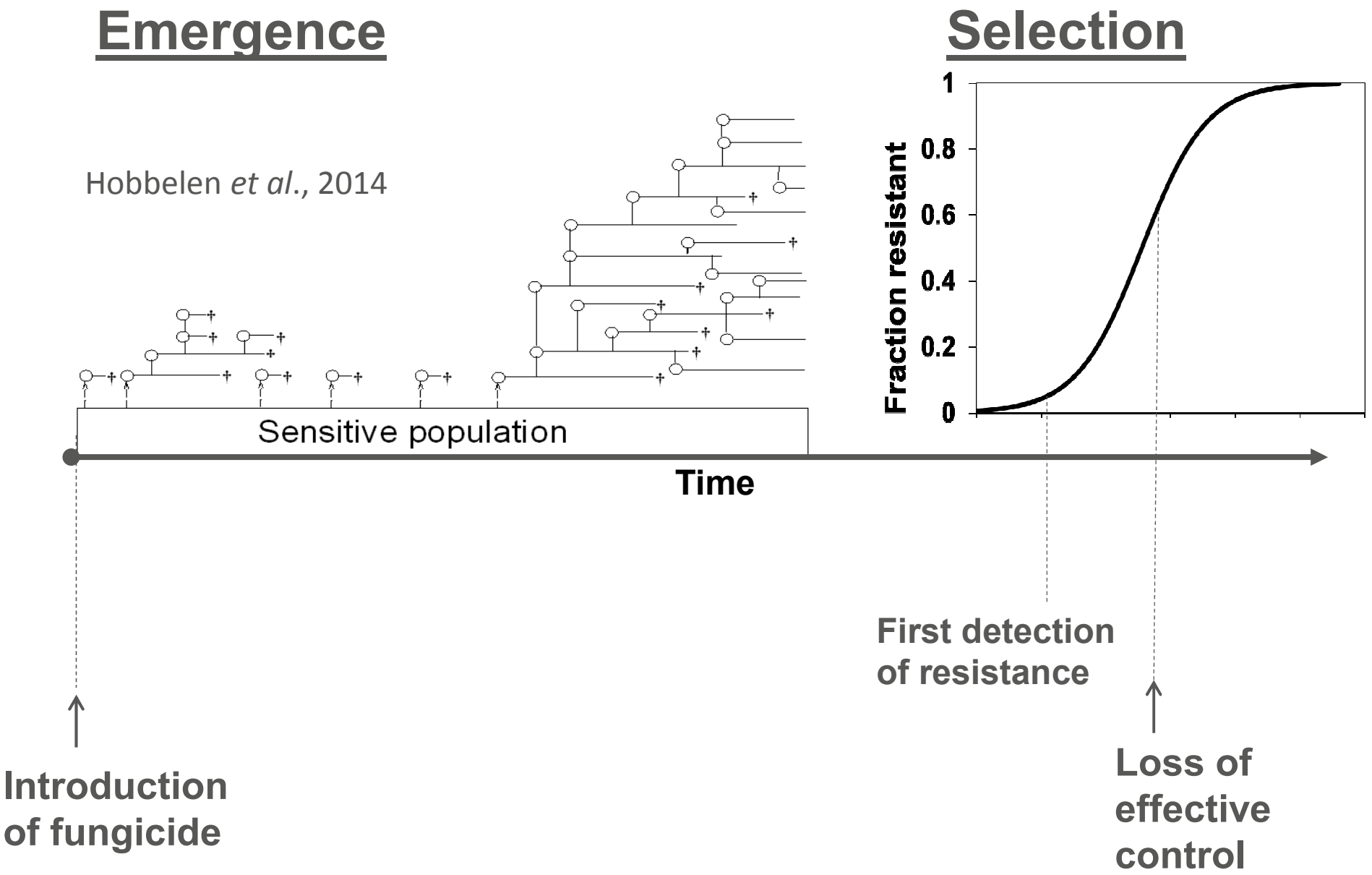
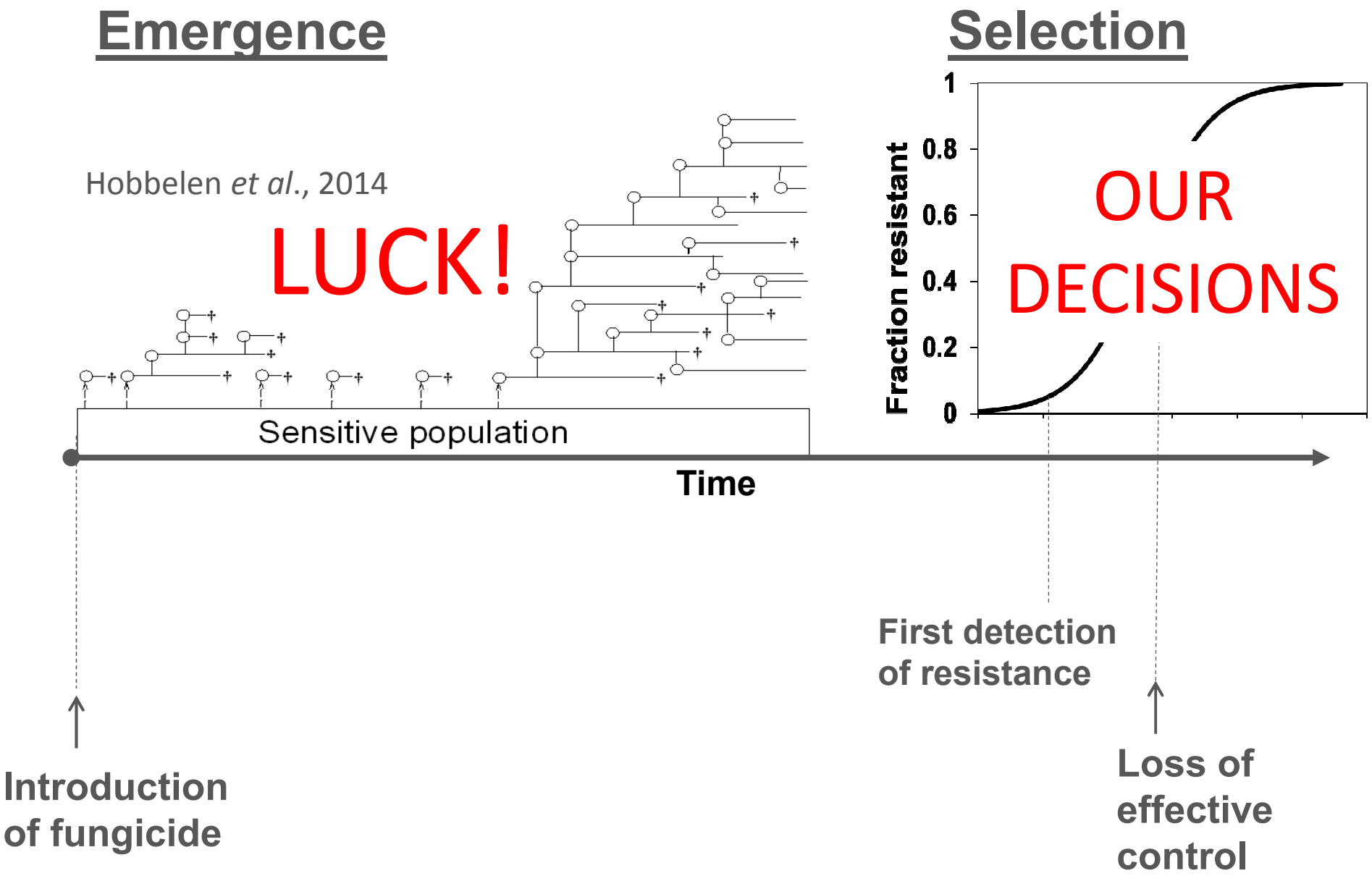
Frank van den Bosch & Bart Fraaije, Rothamsted

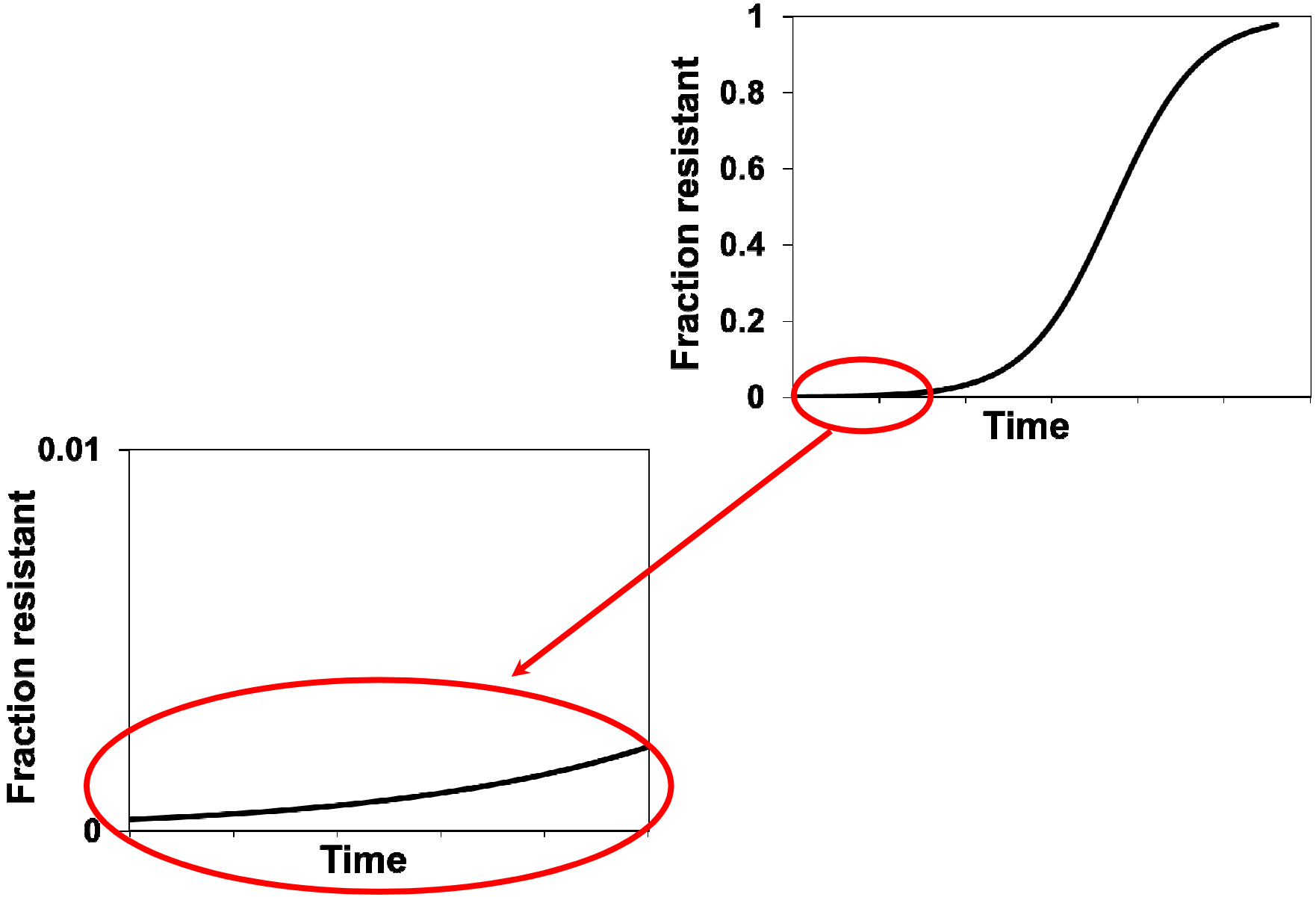
Acknowledgements

Phases of resistance evolution



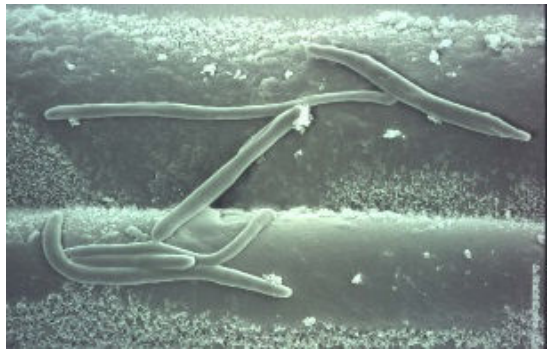
Phases of resistance evolution



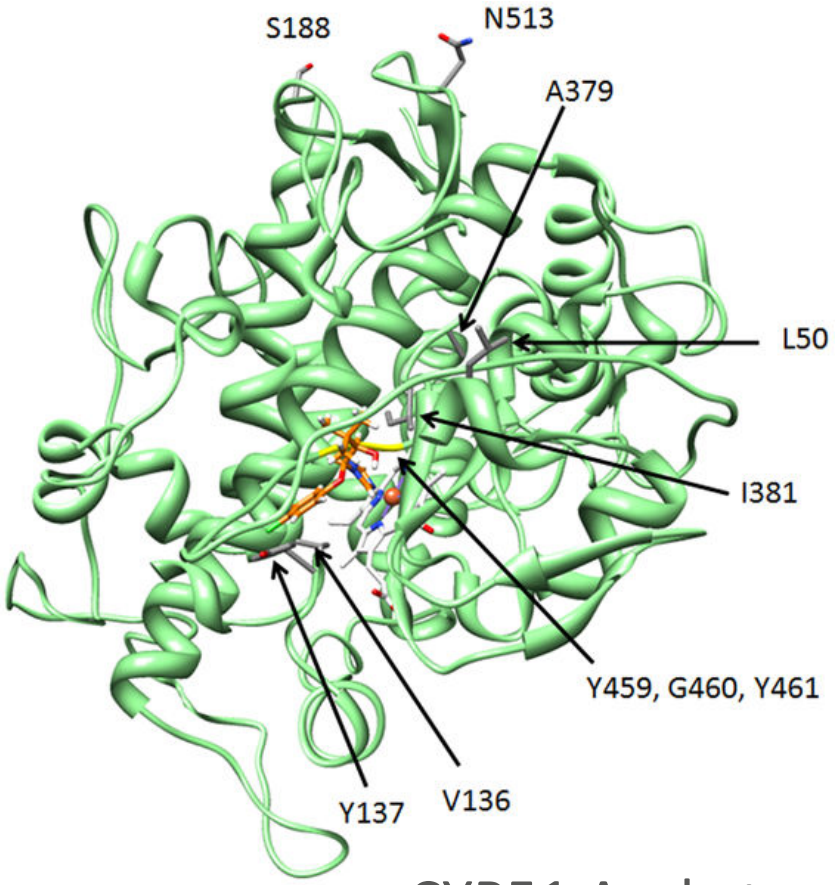

1
0.8
0.6
0.4
0.2
0
Fraction resistant
Time
0.01
0
Fraction resistant
Time

Fungicide resistance mechanisms:

- Target site mutations (MOA specific)
- Over-expression (MOA specific)
- Non-target site (MOA specific)
- Enhanced efflux (single-site MOA)



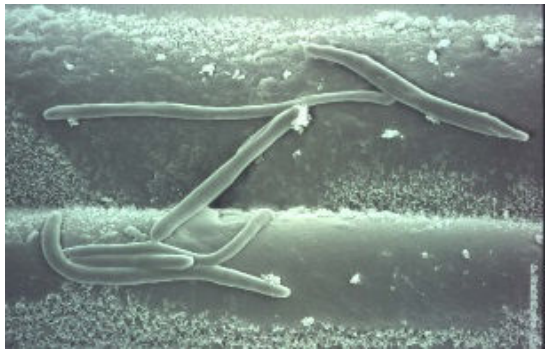
Zymoseptoria tritici



CYP51 Azole target site

Resistance mechanism and genotype

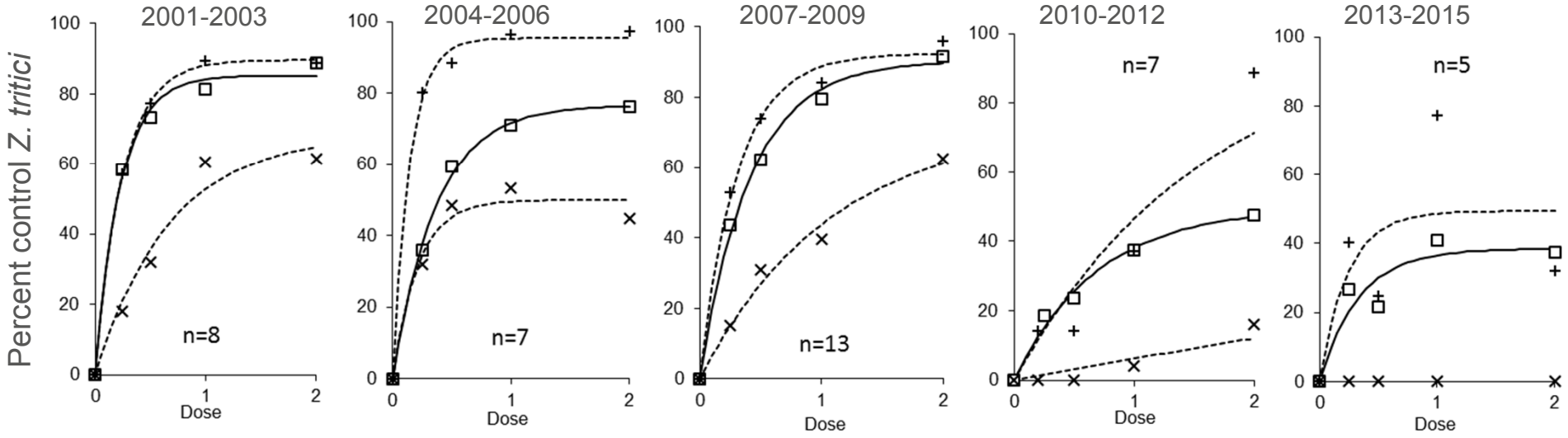
***Zymoseptoria tritici* CYP51 target site variants**



Wild-type
Y137F
Y137F & S524T
L50S, V136A & Y461H
L50S, I381V & Y461H
L50S, S188N, I381V, Δ & N513K
L50S, S188N, A379G, I381V, Δ & N513K
L50S, S188N, I381V, Δ & N513K + CYP51 over-expression
L50S, V136A, Y461S & S524T
V136C, I381V, Y461H & S524T
L50S, D134G, V136A, Y461S & S524T
L50S, D134G, V136A, I381V & Y461H
L50S, V136A, I381V, Y461H & S524T
L50S, V136C, S188N, I381V, Y461H, S524T
L50S, S188N, A379G, I381V, Δ, N513K & S524T
L50S, S188N, H303Y, A379G, I381V, Δ & N513K
L50S, D134G, V136A, I381V, Y461H & S524T
L50S, V136A, S188N, A379G, I381V, Δ & S524T
L50S, V136C, S188N, A379G, I381V, Δ & S524T
L50S, V136A, S188N, A379G, I381V, Δ, N513K & S524T
L50S, S188N, I381V, Δ & N513K + CYP51 over-expression + efflux

Data courtesy Bart Fraaije, Rothamsted

Prothioconazole field efficacy *Zymoseptoria tritici*



Blake *et al*. (2017) *Pest Management Science;* van den Bosch *et al. (2018) Plant Pathology*

Resistance mechanism and genotype

Fitness phenotype

***Zymoseptoria tritici* CYP51 target site variants**

Wild-type
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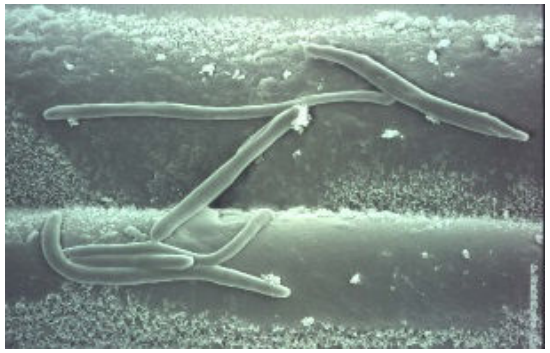
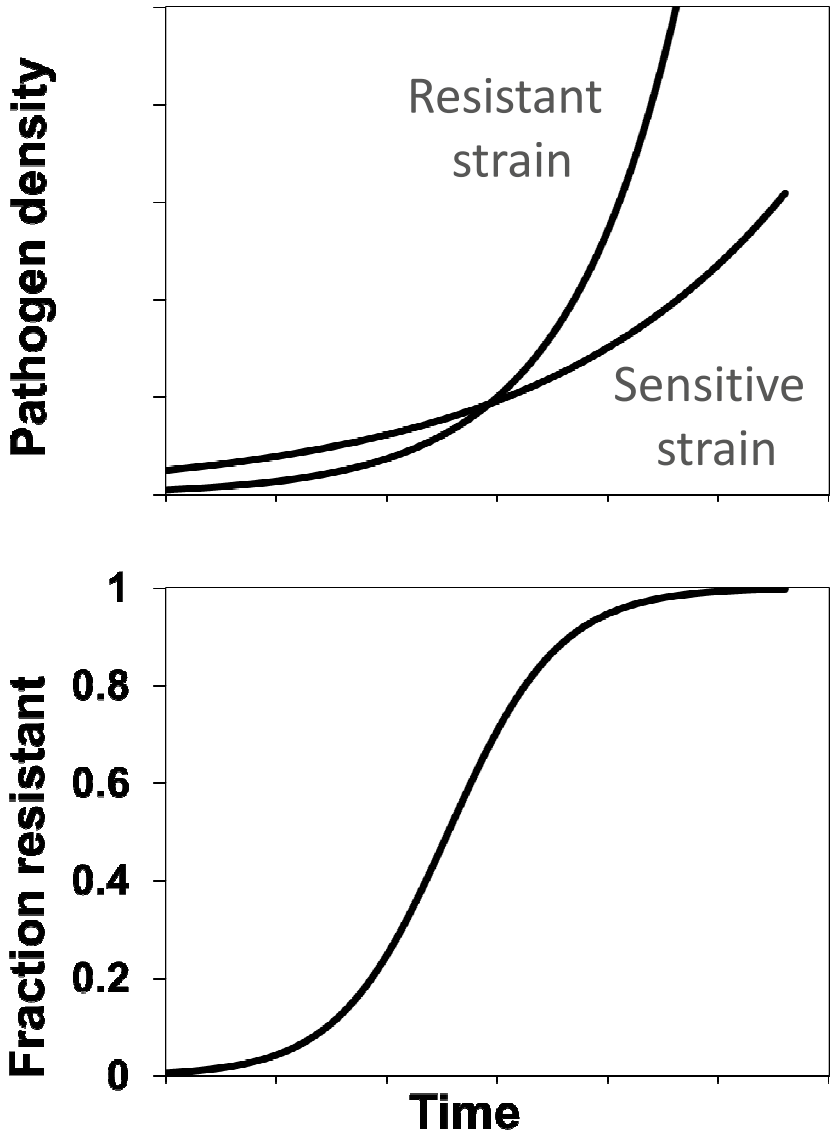
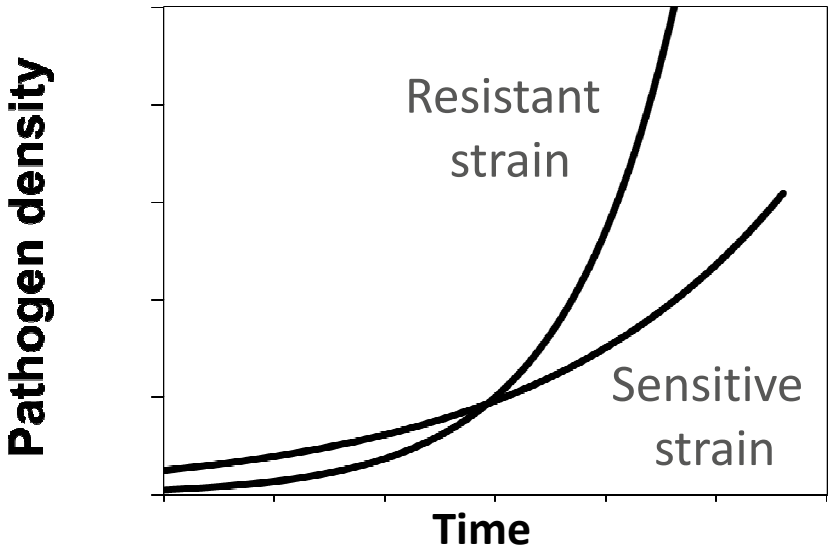


Per capita rate of increase (*r*)

Data courtesy Bart Fraaije, Rothamsted



Van den Bosch *et al*. 2011 *Plant Pathology* 60, 597-606



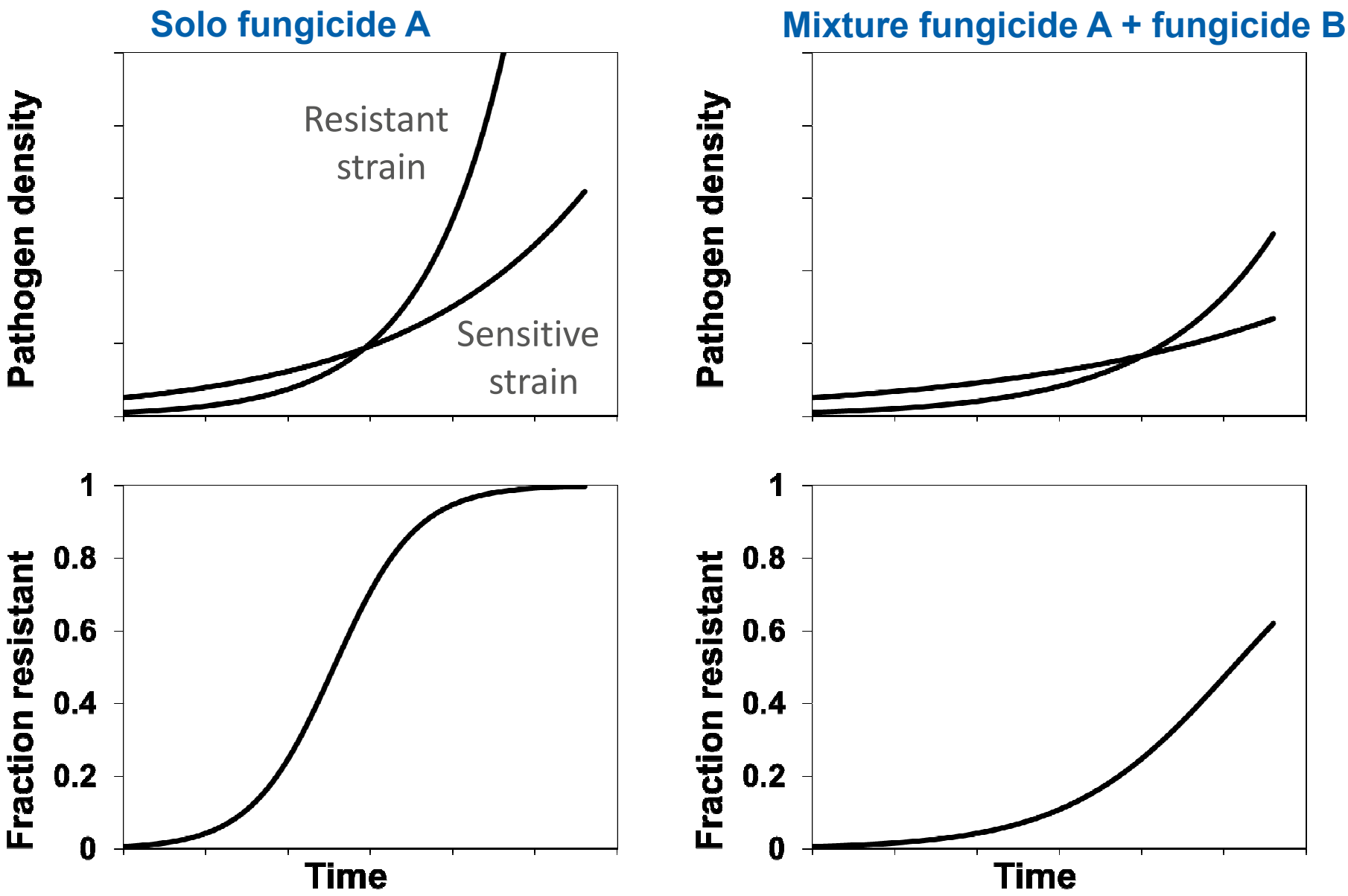
Selection rate =
Difference in *per capita* rate of increase of resistant and sensitive strains

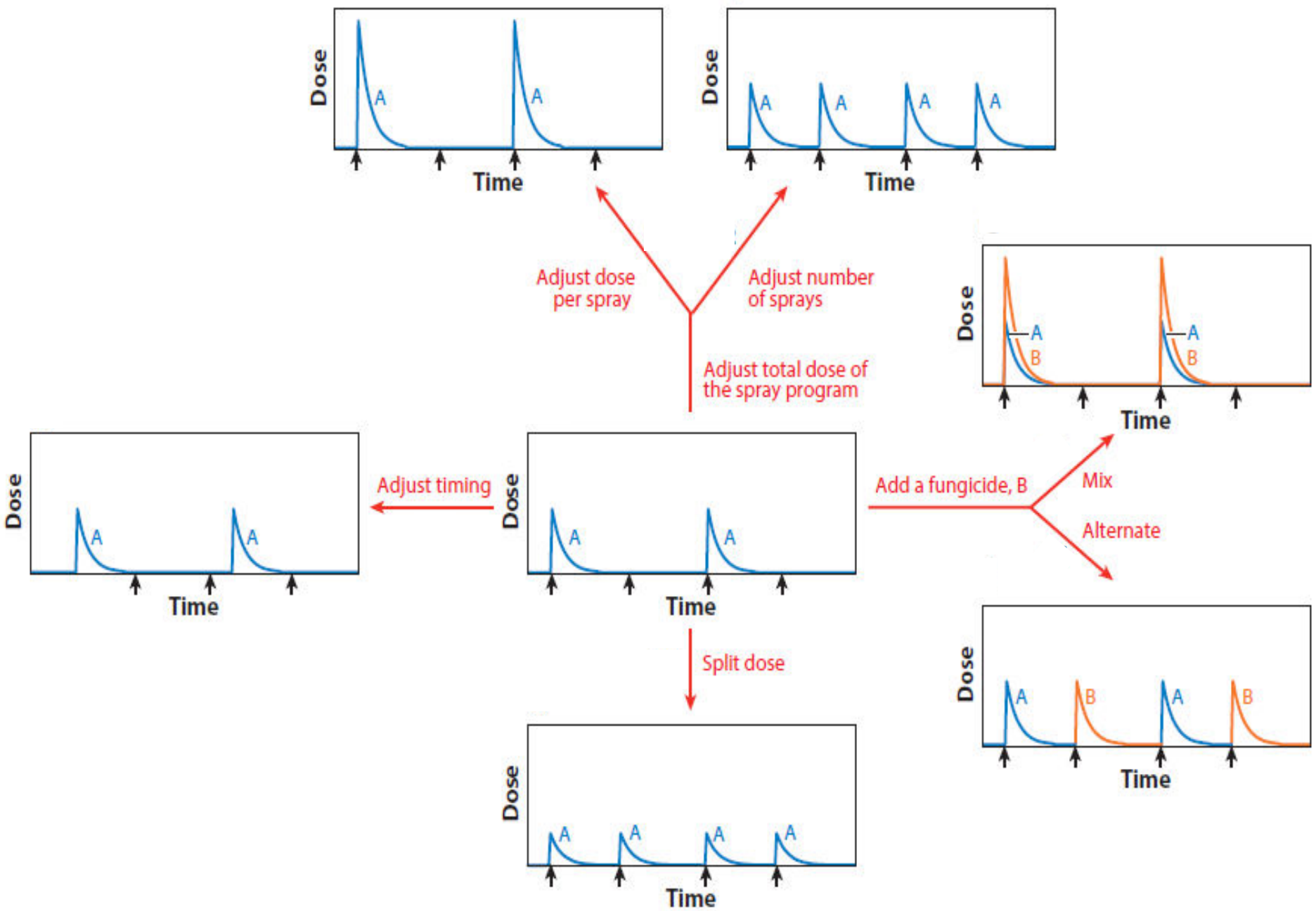
Strategy 1: **Reduce growth rates of resistant and sensitive strains**

Strategy 2: **Reduce growth rate of resistant strain relative to sensitive strain**

Strategy 3: **Reduce time pathogen exposed to fungicide**

Milgroon & Fry, 1988; van den Bosch *et al*., 2014


Solo fungicide A
Mixture fungicide A + fungicide B
Pathogen density
Resistant strain
Sensitive strain
Fraction resistant
1
0.8
0.6
0.4
0.2
0
Time



Systems providing experimental evidence:

Pathogen	Crop	Modes of action
Blumeria graminis f.sp. *hordei*	Barley	DMI, amines, QoI, pyrimidine
Zymoseptoria tritici	Wheat	DMI, QoI
Blumeria graminis f.sp. *tritici*	Wheat	DMI, amines
Venturia inequalis	Apple	DMI, MBC, QoI
Polyscytalum pustulans	Potato	MBC
Pythium aphanidermatum	Ryegrass	Phenylamide
Plasmopara viticola	Grapevine	QoI, phenylamide, cyanoacetamide
Botrytis cinerea	Strawberry, grapevine, geranium	Dicarboxamide, AP, hydroxyanilides
Podosphaera xanthii	Cucurbit	DMI, MBC
Phytophthora infestans	Potato, tomato	Phenylamide
Rhynchosporium secalis	Barley	DMI
Erwinia amylovora	Pepper	Glucopyranosyl antibiotic
Xanthomonas vesicatoria	Pepper	Glucopyranosyl antibiotic
Cercospora beticola	Sugar beet	MBC
Tapesia spp.	Wheat	MBC
Colletotrichum gleosporioides	Euonymus	MBC
Parastagonospora nodorum	Wheat	DMI, MBC
Pseudoperonospora cubensis	Cucumber	CAA
Helminthosprium solani	Potato	MBC

	Increase selection	No effect	Decrease selection
Increase dose	**16**	1	2
Increase spray number	**6**	0	0
Split the dose	**10**	0	1
Add mixture partner	1	6	**46**
Alternate (replace sprays)	1	2	**9**
Adjust timing	3	1	2

van den Bosch et al. 2014 Governing principles can guide resistance management tactics *Annual Review Phytopathology*

Possible exceptions:

- Monocyclic pathogens
  - MOA mixtures may not slow selection
- Small sensitivity differences between strains
  - may change effect of dose on selection
- Experimental evidence based on measuring selection against one MOA

Two single-site acting modes of action (MOA):

Mixtures provide good resistance management:

- Mutual protection of both MOA

Restricting number of treatments of a MOA provides good resistance management:

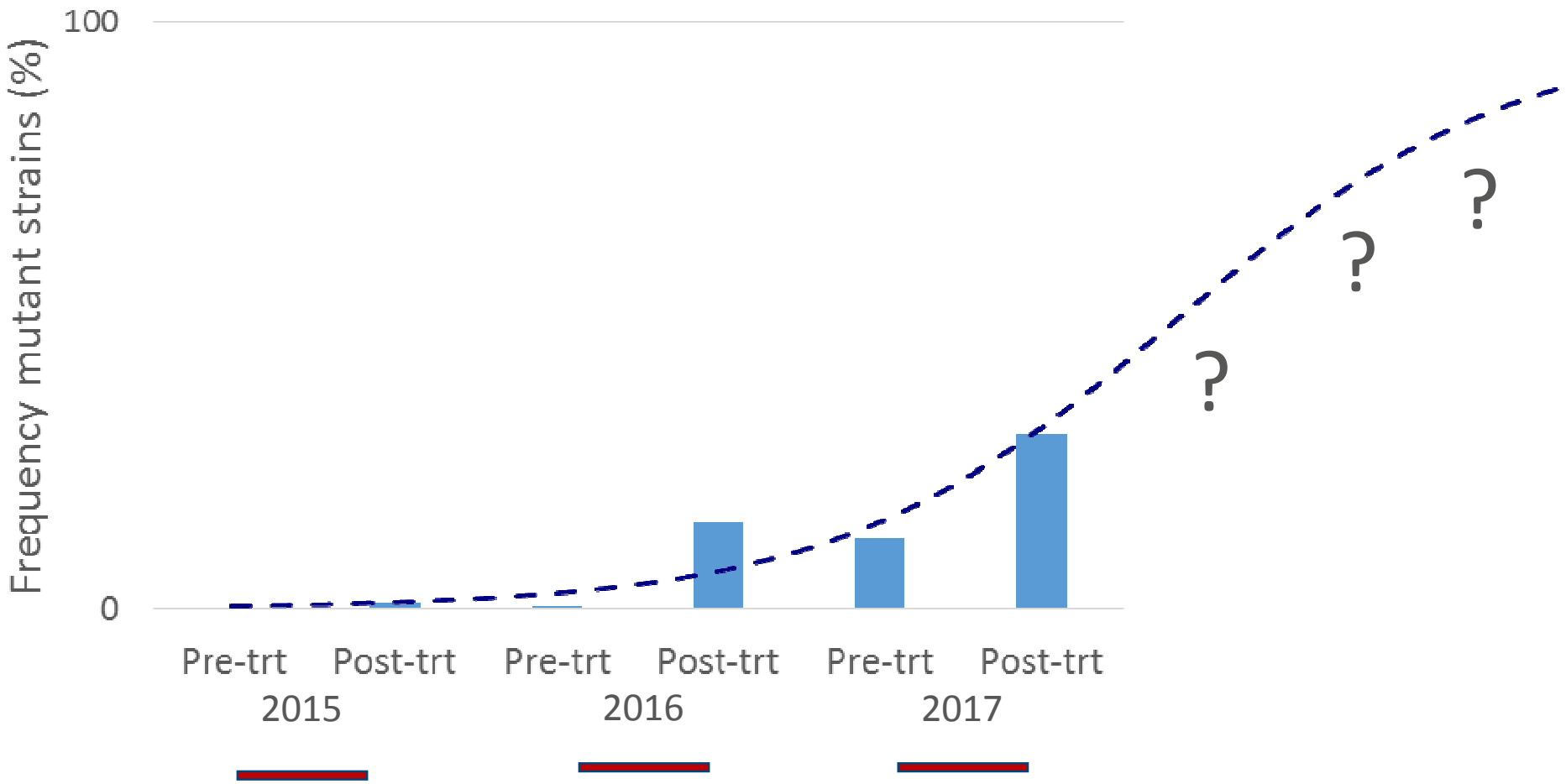
- Reduces exposure, which reduces selection

BUT: restricting number of treatments restricts use of mixtures

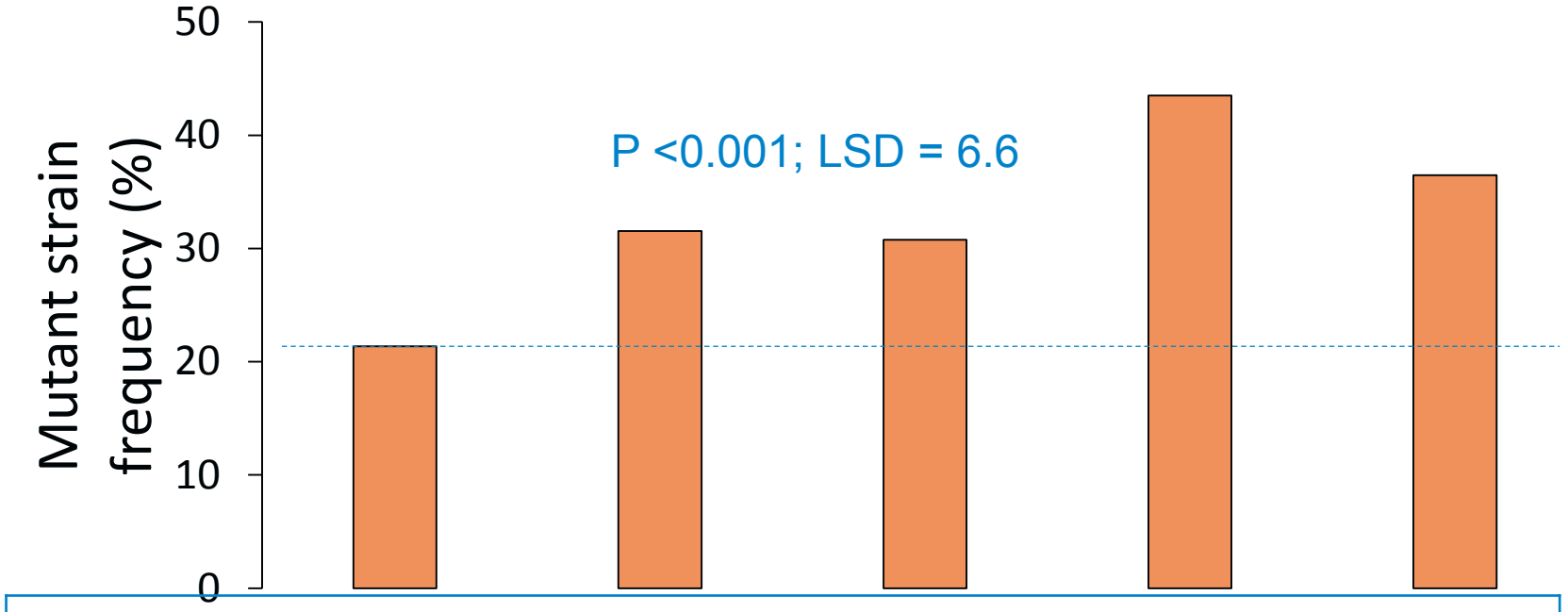
- protects one MOA
- leaves other MOA at higher risk

Moderately SDHI insensitive strains

Based on Rothamsted/AHDB monitoring of FP and other sites (illustrative as high variability between sites)



Effect of SDHI fungicide programs on selection

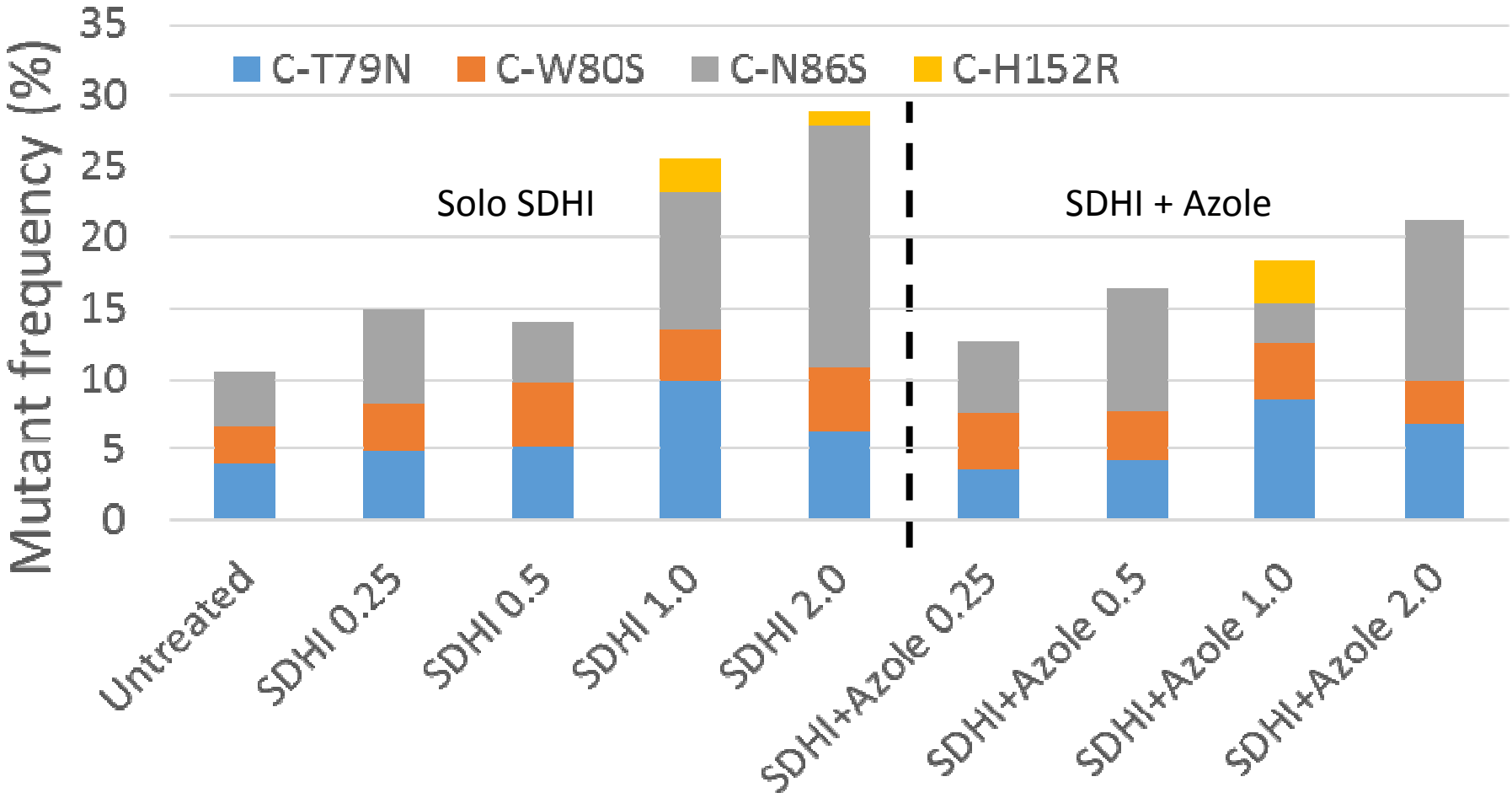


GS32	**-**	**-**	**-**	**SDHI**	**SDHI**
	-	**Azole**	**Azole**	**Azole**	**Azole**
	-	**-**	**MS**	**-**	**MS**
GS39	**-**	**SDHI**	**SDHI**	**SDHI**	**SDHI**
	-	**Azole**	**Azole**	**Azole**	**Azole**
	-	**-**	**MS**	**-**	**MS**

(3 UK trials, 2017) Sampled after treatment. Non-SDHI treatments applied at T0 and T3
SDH mutant strains: T79N, W80S, N86S, and H152R

SDHI dose and & azole mixture effect (2016)

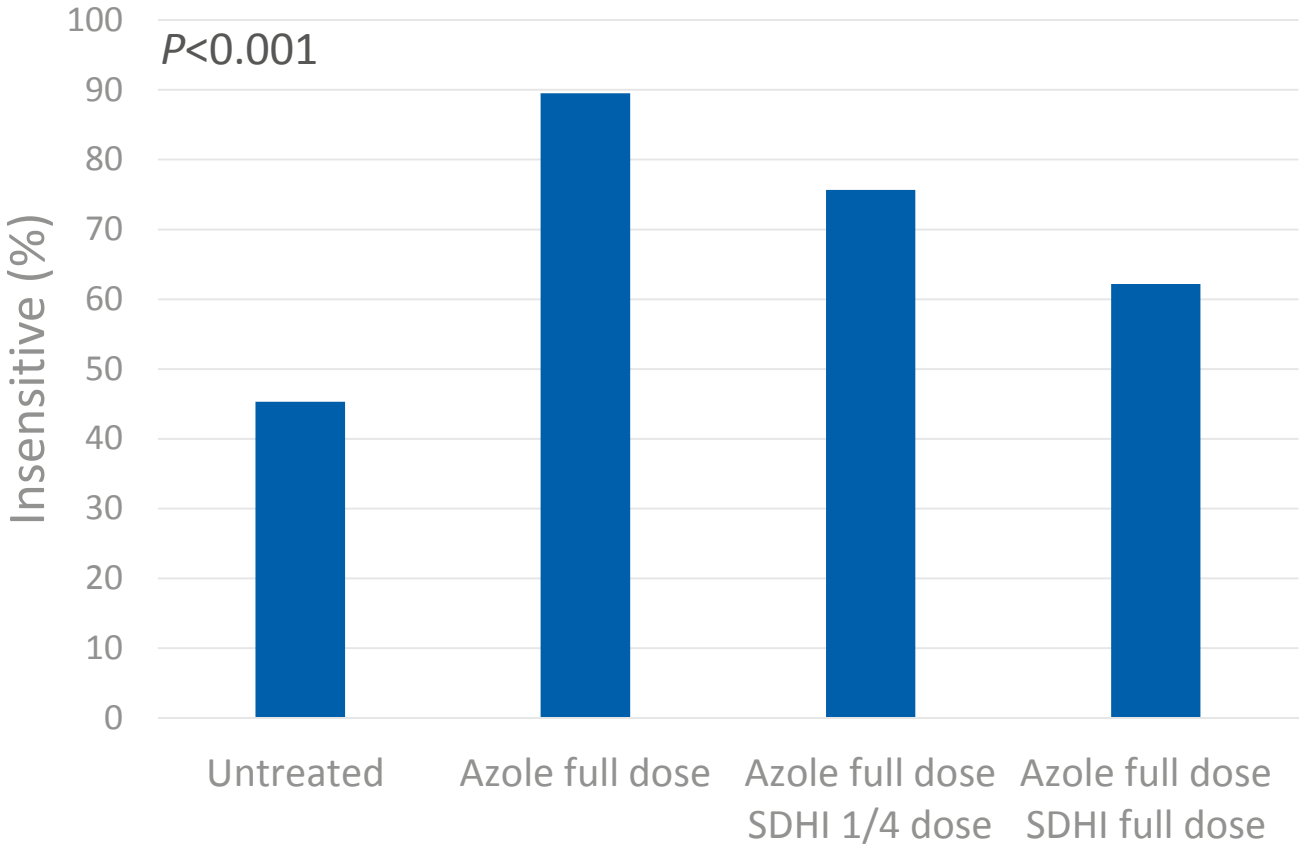
(Detection threshold approx. 4% per mutation)



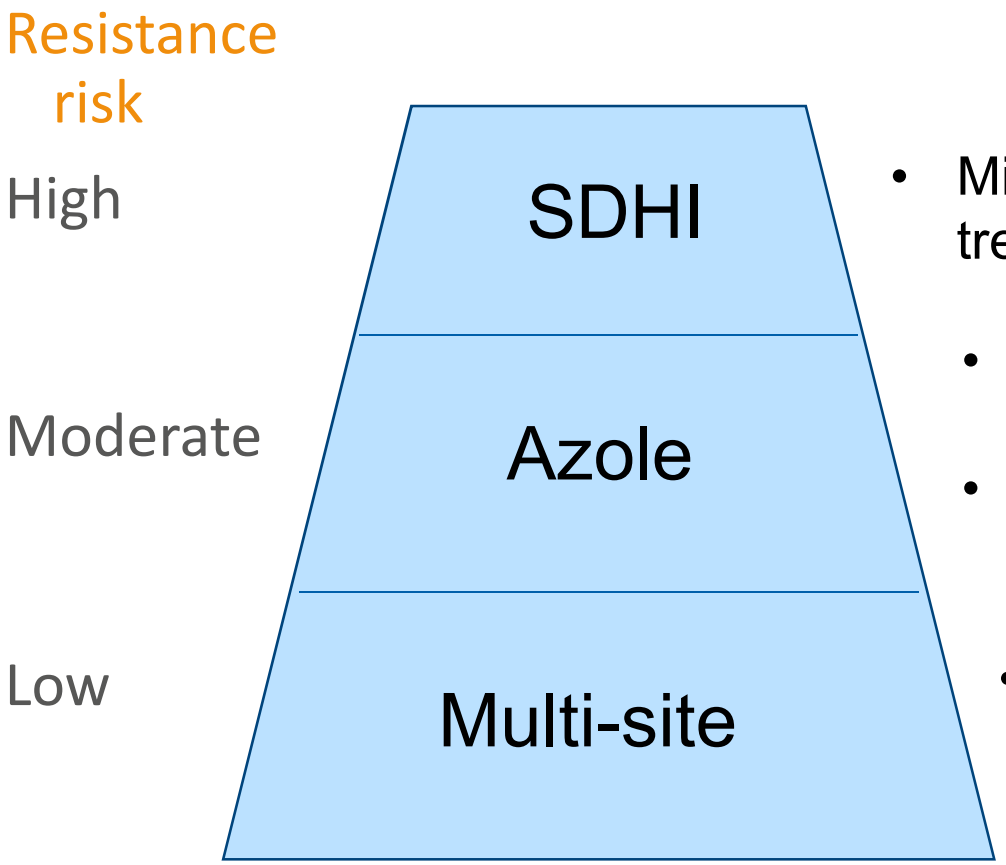
SDHIs must not be used solo or at more than the maximum permitted dose

One application Mean of three field trials, England and Wales, 2016

Selection for tebuconazole insensitive *Z. tritici*
(field experiments 2012-2013)



Effective septoria control & slow resistance:
build mixtures from the foundation up



- Minimum SDHI dose & number of treatments for effective control
  - Limit number of azole treatments
  - Robust azole doses at T1/T2 when mixing with SDHIs
  - Use multi-sites throughout spray programme

Maximum permitted 2 applications of SDHI containing products

Resistance management guidance: https://cereals.ahdb.org.uk/frag

Conclusions

Use IPM e.g. disease resistance in wheat varieties:

- Reduces per capita epidemic growth rates (strategy 1)
- Reduces economic optimum fungicide input (strategy 3)
- Improves economics of disease forecasting (strategy 3)

Managing selection of resistant strains:

- Determined by fitness phenotype and our treatment decisions
- Implement resistance management at introduction of new MOA
- Time fungicide applications when can judge need for treatment and for maximum efficacy
- Balance fungicide programmes according to risk of resistance against each MOA

“Don’t be too keen to be clean” (John Lucas)