Impacts of fungicide resistance and product loss
(*Zymoseptoria tritici* case study)

Neil Paveley, ADAS

Disease control sustainable if:

- Unlimited supply of new disease resistance genes and fungicide modes of action that are:

  - accessible (financial & technical constraints)

  - safe (regulatory & political constraints)

and

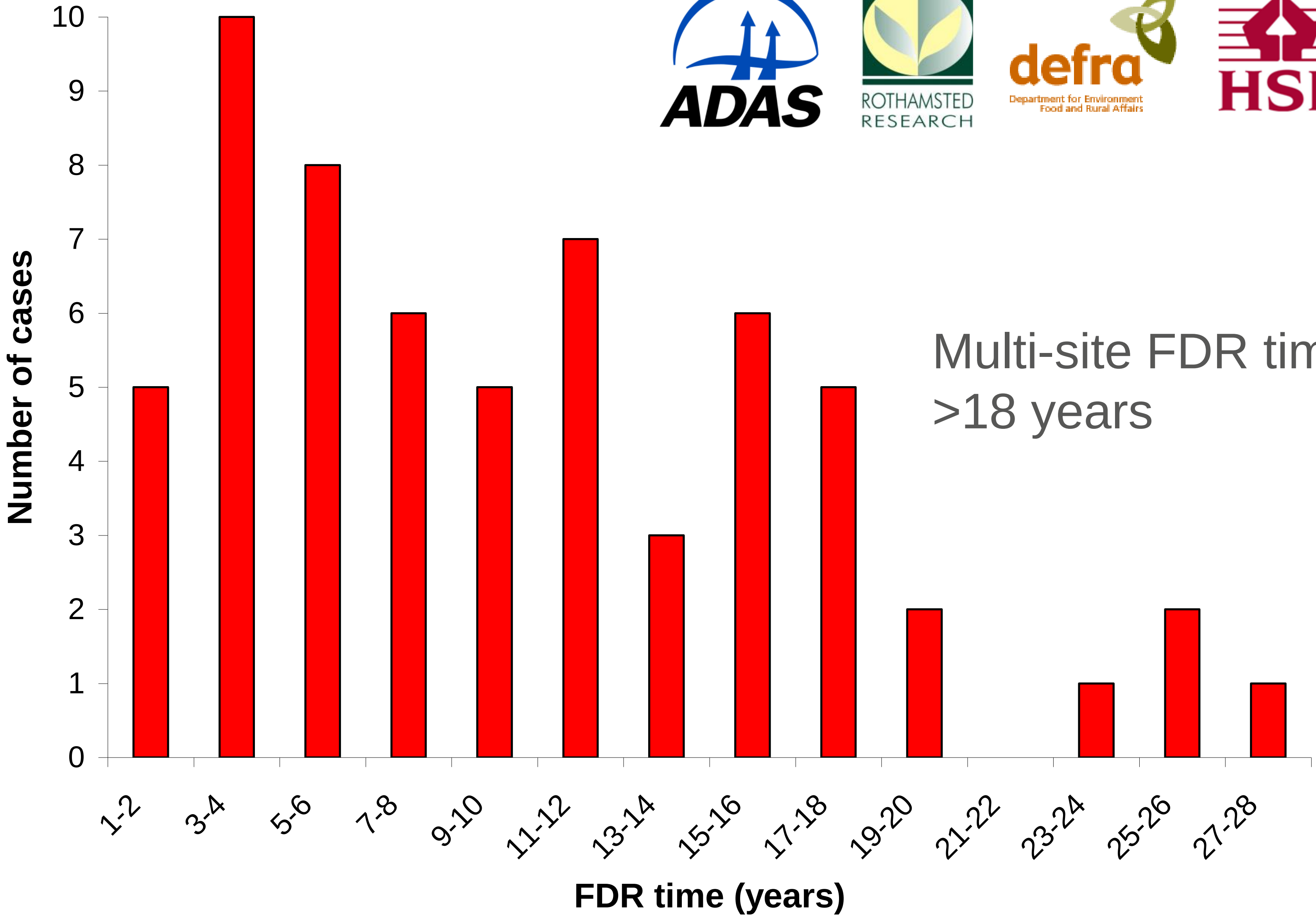
- Introduce new disease resistance genes and fungicide modes of action faster than pathogens defeat them

Broad-spectrum systemic modes of action:

• Azoles	1970s
• Strobilurins	1990s
• SDHIs (new generation)	2010s
• Next new mode of action	??
	Regulatory uncertainty?

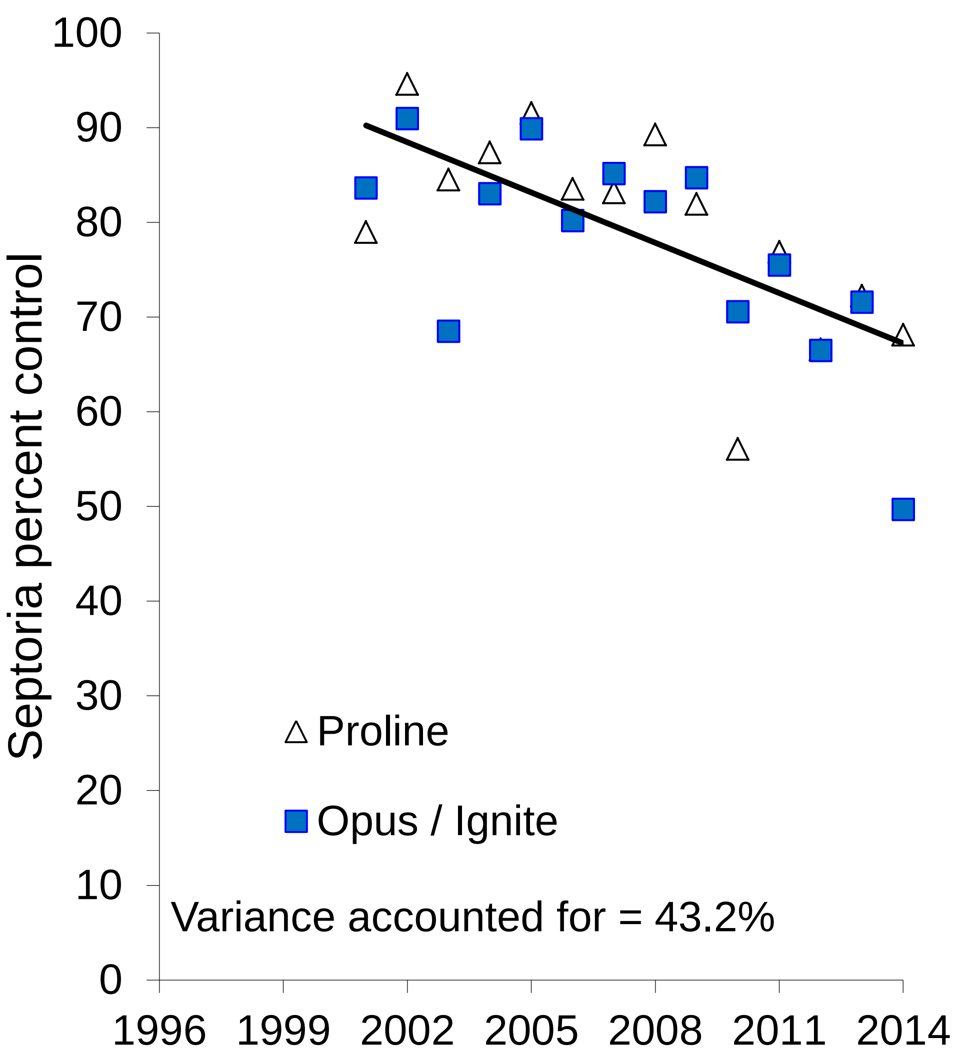
ADAS

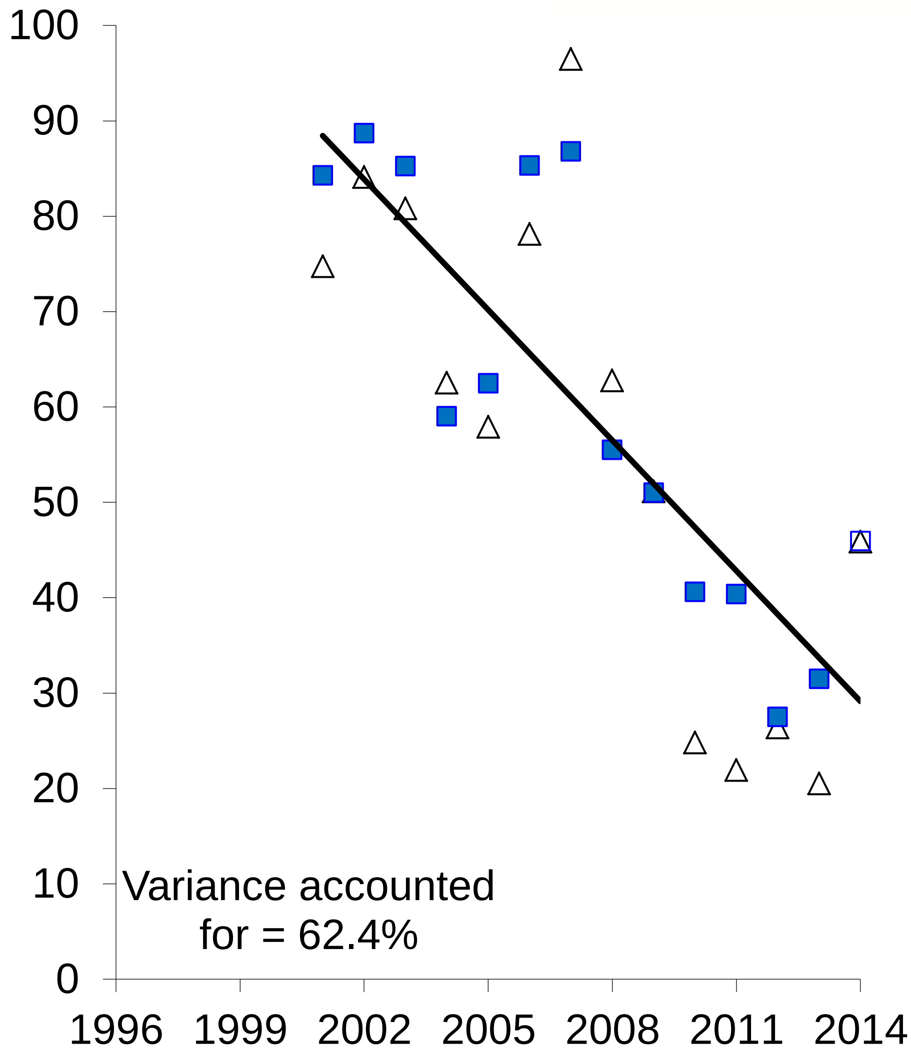
Years to First Detection of Resistance (n = 61) for single-site fungicides



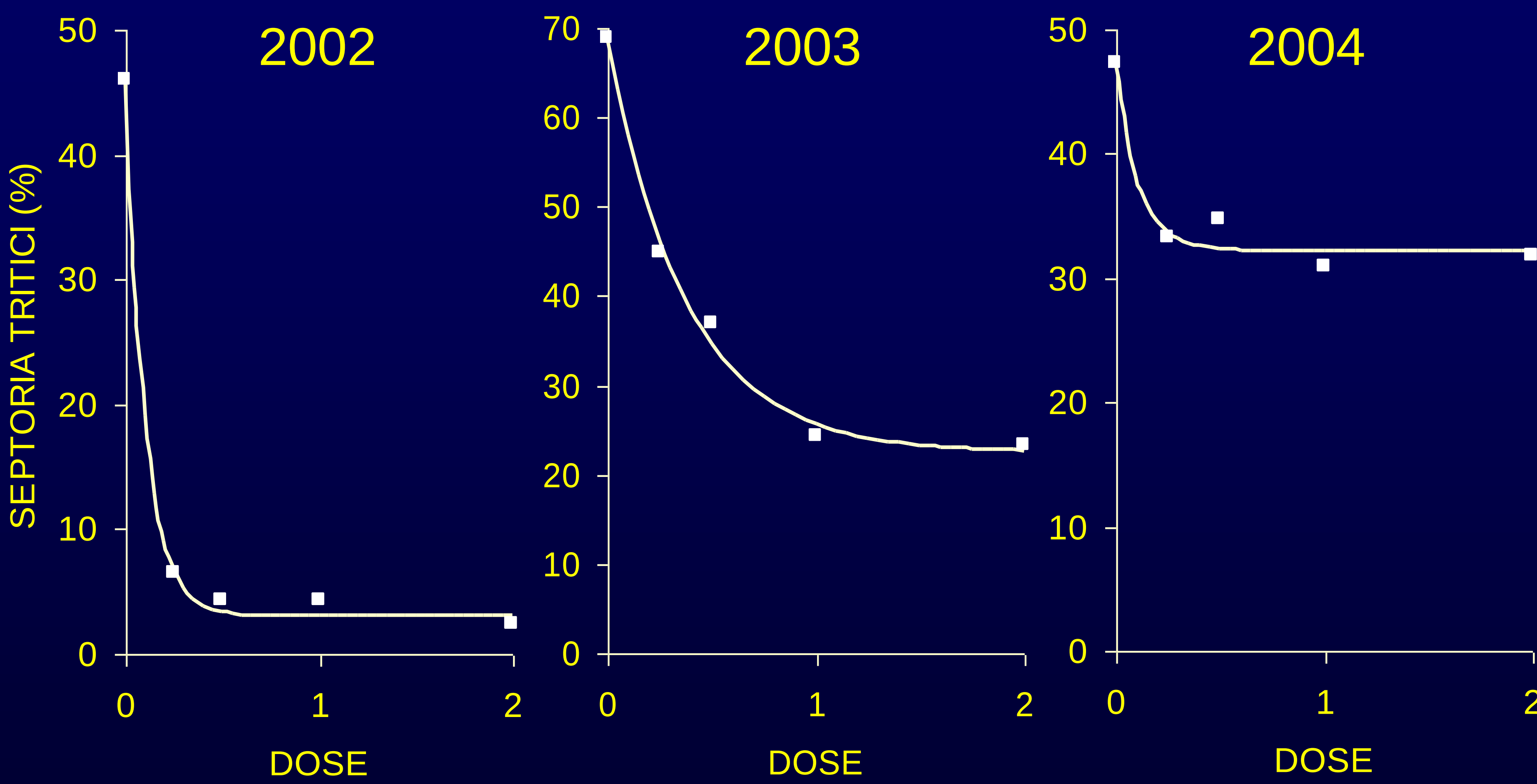
Grimmer *et al.*, 2014. Pest Man. Sci.

Azole efficacy (full label dose)



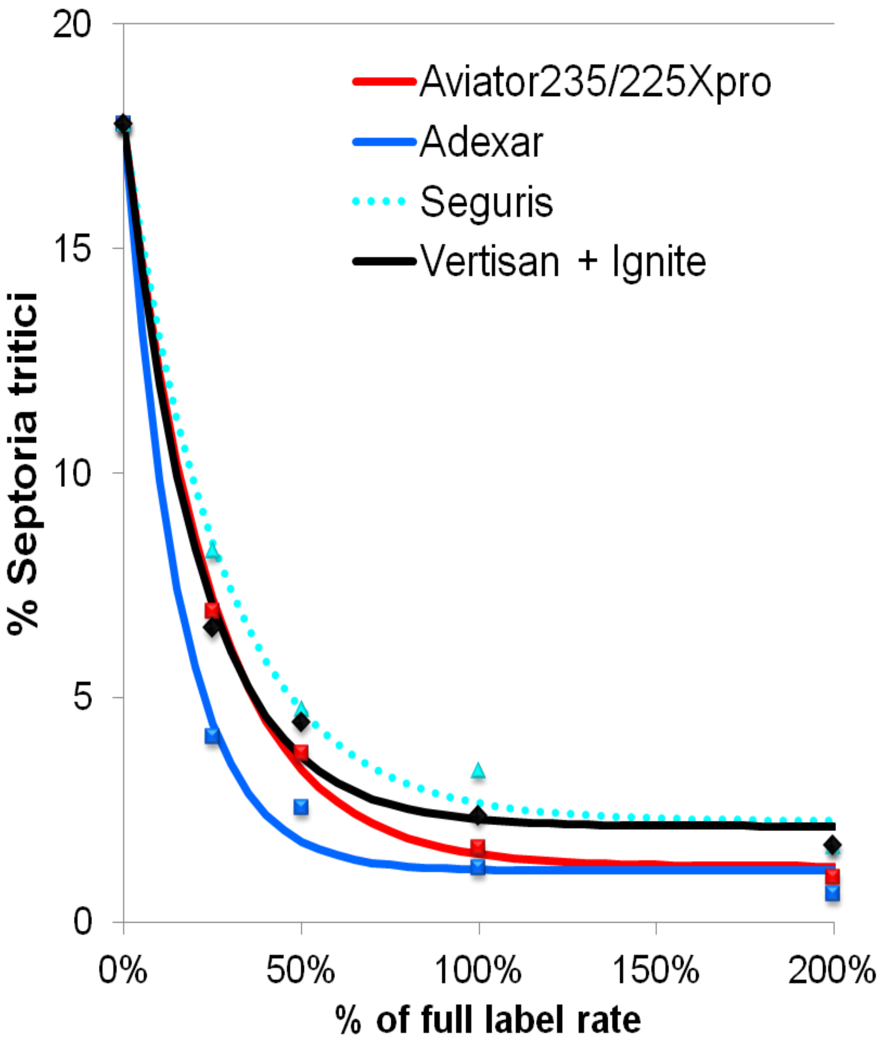


QoI (strobilurin) efficacy



Pyraclostrobin (HGCA data)

SDHI efficacy



		IC50 (nM)							
		Carboxin		**Isopyrazam**		**Fluopyram**		**Boscalid**	
		in vivo	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*
WT (IPO323)		6470 ±1085	1156 ±102	55 ±2	5 ±0.3	584 ±100	44 ±2.2	455 ±47	60 ±1.8
		Resistance Factors (RFs)							
		Carboxin		**Isopyrazam**		**Fluopyram**		**Boscalid**	
Substitution	Strain	*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*
None	IPO323	1	1	1	1	1	1	1	1
B_S218F	Flu41	5.3	46.4	15.0	18.2	18.0	276.5	6.5	46.3
B_P220T	Flu110	0.1	0.7	3.8	1.8	11.5	84.9	6.1	28.3
B_P220L	Flu75	1.4	4.4	4.8	1.2	5.0	3.3	2.2	2.6
B_N225H	Cbx106	20.2	38.0	13.6	15.7	8.0	15.2	12.2	20.1
B_N225I	Flu7	10.2	6.9	15.0	7.0	17.5	25.0	12.9	18.5
B_R265P	Cbx56	20.1	271.1	7.6	88.3	2.8	63.0	13.3	256.0
B_H267L	Cbx29	14.5	1959.3	152.2	3248.5	22.4	766.1	258.0	9126.6
B_H267N	Cbx96	12.6	232.7	4.5	11.1	9.9	68.3	3.4	34.8
B_H267Q	Bos59	4.4	85.5	12.5	34.6	0.9	11.8	21.7	1034.3
B_H267Y	Cbx28	14.1	65.9	16.1	142.6	0.6	5.1	305.6	473.8
B_I269V	Cbx1	12.4	19.9	5.0	3.6	10.0	18.0	4.2	12.7
B_N271K	Flu33	2.6	1069.2	5.7	89.5	10.0	2481.0	3.4	223.7
C_T79I	Bos64	5.2	62.0	15.8	56.4	2.2	12.3	18.9	145.6
C_S83G	Flu1	10.7	11.7	61.2	38.6	126.8	201.0	564.3	1392.4
C_A84V	Flu4	0.8	1.2	3.5	3.1	19.0	102.7	2.6	3.7
C_A84I	Flu21	1.2	1.8	8.8	8.1	31.9	260.0	2.9	4.4
C_L85P	Cbx12	11.5	265.4	10.0	35.3	5.8	130.1	4.1	34.7
C_N86K	Izm7	23.8	135.9	464.4	849.9	49.9	245.8	78.1	160.2
C_R87C	Flu9	3.2	nd*	3.7	nd*	6.1	nd*	18.8	nd*
C_V88D	Ol50	4.4	67.2	36.5	140.4	5.9	55.4	5.0	59.8
C_H145R	Cbx76	4.4	nd*	27.6	nd*	2.0	nd*	17.3	nd*
C_H152R	Izm1	13.2	45.4	133.9	95.0	11.5	14.3	30.2	58.1
D_D129E	Cbx22	35.9	631.6	6.8	145.3	1.3	10.5	42.8	1220.9
D_D129G	Cbx67	17.2	2030.3	22.9	583.2	5.6	1880.2	37.1	2052.4
D_D129S	Cbx101	39.7	377.7	10.8	82.5	1.9	12.3	15.9	341.2
D_D129T	Flu29	60.4	FR**	33.5	4095.5	11.5	3724.4	78.3	5515.8

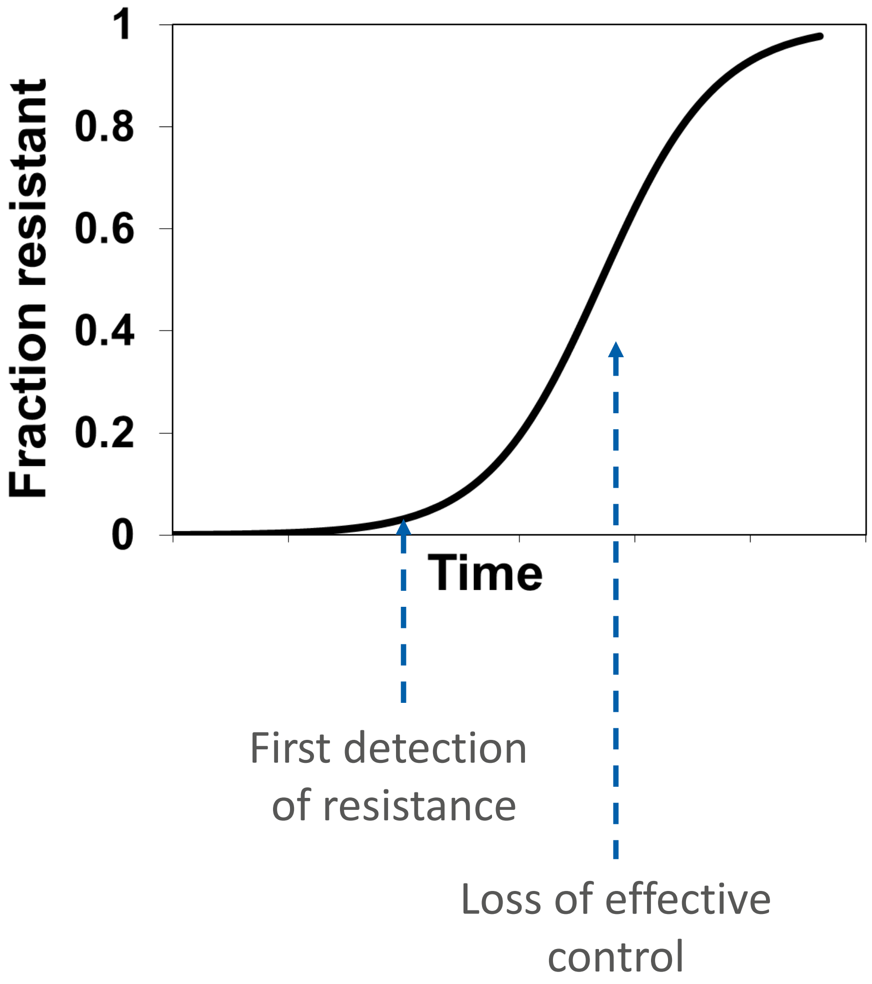
Scalliet G, Bowler J, Luksch T, Kirchhofer-Allan L, Steinhauer D, et al. (2012) Mutagenesis and Functional Studies with Succinate Dehydrogenase Inhibitors in the Wheat Pathogen Mycosphaerella graminicola. PLoS ONE 7(4): e35429. doi:10.1371/journal.pone.0035429
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0035429

What should we **do**?

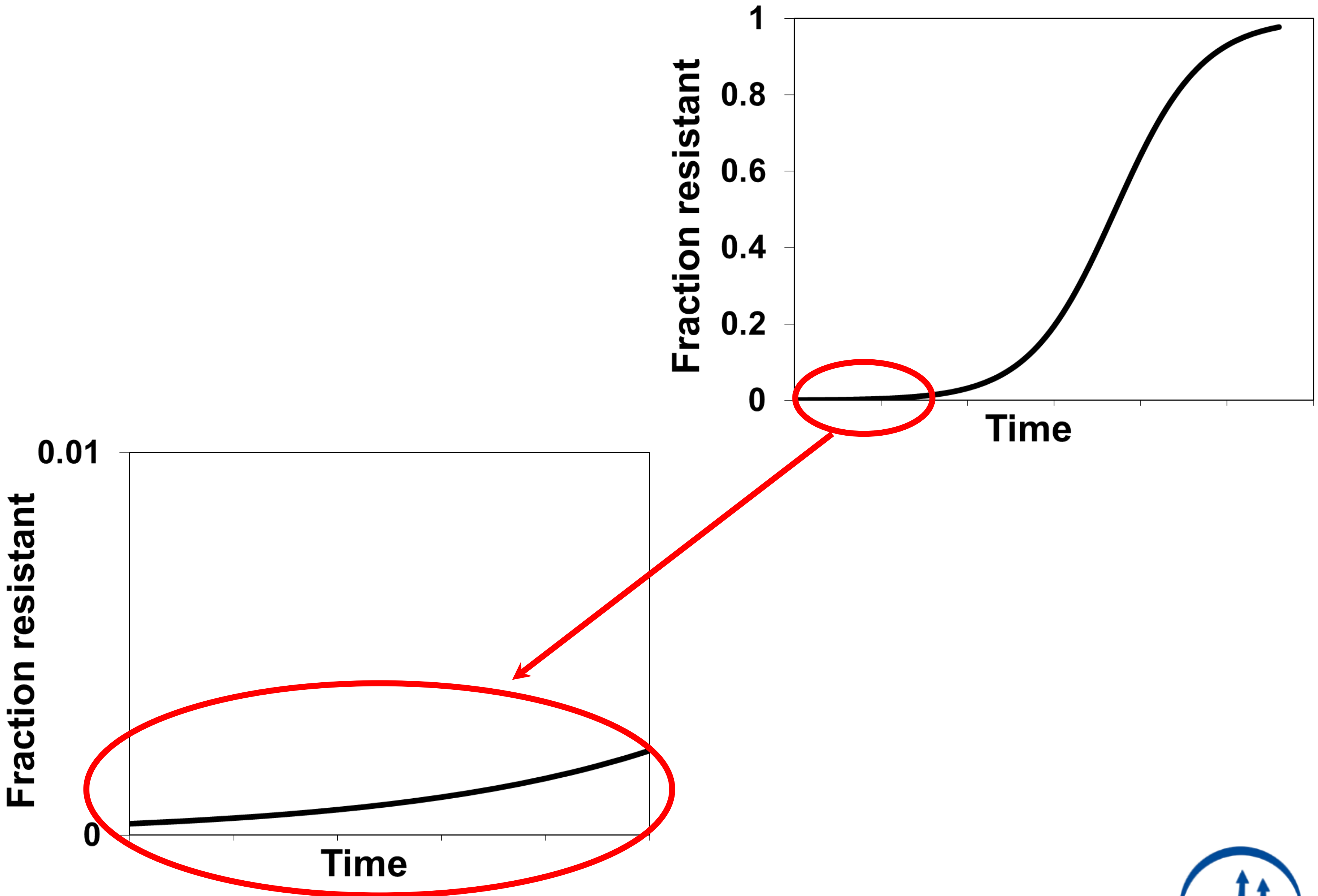
Azole insensitive isolates (data courtesy Bart Fraaije, Rothamsted)

Isolate	CYP51 variant	Epoxi	Prochl	Tebu	Prot-des
Reference		EC_{50} values in µg/ml			
n=4	Wild-type	0.0029	0.0164	0.072	0.0014
1994-2008					
n=3	Y137F	2.8	0.9	1.2	0.6
n=2	Y137F & S524T	12	5.8	4.7	1.8
n=11	L50S, V136A & Y461H	55	25	4.8	8.8
n=35	L50S, I381V & Y461H	81	3.5	36	39
n=4	L50S, S188N, I381V, Δ & N513K	86	3.2	28	15
n=47	L50S, S188N, A379G, I381V, Δ & N513K	149	1.2	82	21
2009-2014					
n=19	L50S, S188N, I381V, Δ & N513K + CYP51⬆	389	17	235	92
n=6	L50S, V136A, Y461S & S524T	206	62	5.4	46
n=2	V136C, I381V, Y461H & S524T	1111	15	110	93
n=1	L50S, D134G, V136A, Y461S & S524T	209	12	6.5	112
n=35	L50S, D134G, V136A, I381V & Y461H	196	11	5.0	102
n=7	L50S, V136A, I381V, Y461H & S524T	529	19	20	181
n=3	L50S, V136C, S188N, I381V, Y461H, S524T	733	7.9	69	85
n=1	L50S, S188N, A379G, I381V, Δ, N513K & S524T	477	4.5	77	81
n=1	L50S, S188N, H303Y, A379G, I381V, Δ & N513K	376	2.3	62	109
n=6	L50S, D134G, V136A, I381V, Y461H & S524T	809	18	11	336
n=6	L50S, V136A, S188N, A379G, I381V, Δ & S524T	999	12	21	418
n=4	L50S, V136C, S188N, A379G, I381V, Δ & S524T	1486	3.3	242	162
n=4	L50S, V136A, S188N, A379G, I381V, Δ, N513K & S524T	923	7.7	22	623
n=1	L50S, S188N, I381V, Δ & N513K + CYP51⬆ + efflux⬆	1428	66	303	207

Low frequency of most 'resistant' isolates in field populations (fitness penalties?)



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


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

van den Bosch et al. 2014 *Annual Review Phytopathology*

	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*

ROTHAMSTED RESEARCH Curtin University GRDC Grains Research & Development Corporation HSE defra Department for Environment Food and Rural Affairs BBSRC bioscience for the future ADAS

Practical trade-offs:

Reducing dose or limiting number of treatments reduces efficacy.

Limiting number of treatments constrains use of mixtures.

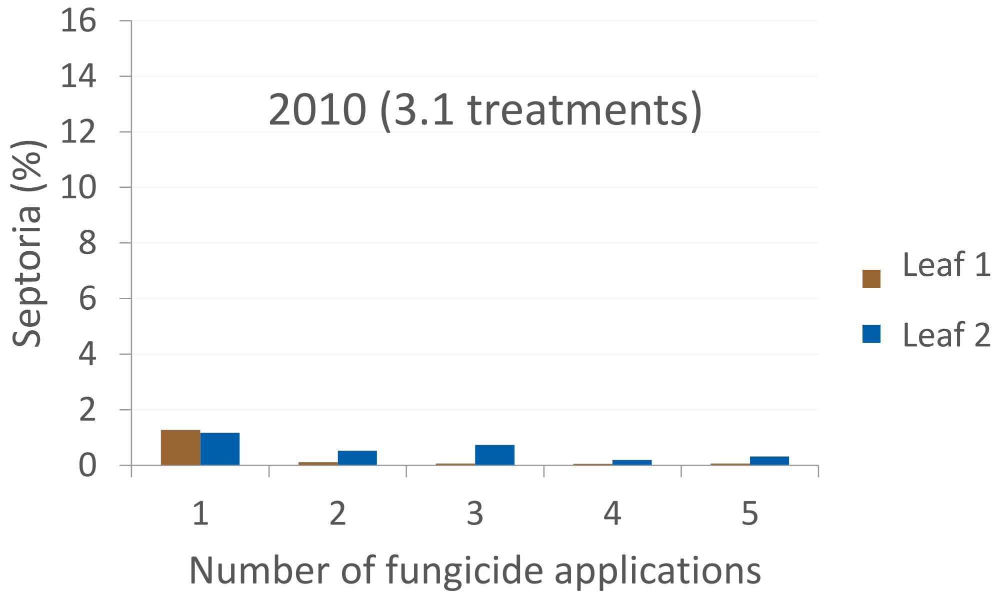
Need another effective MOA to mix or alternate with.

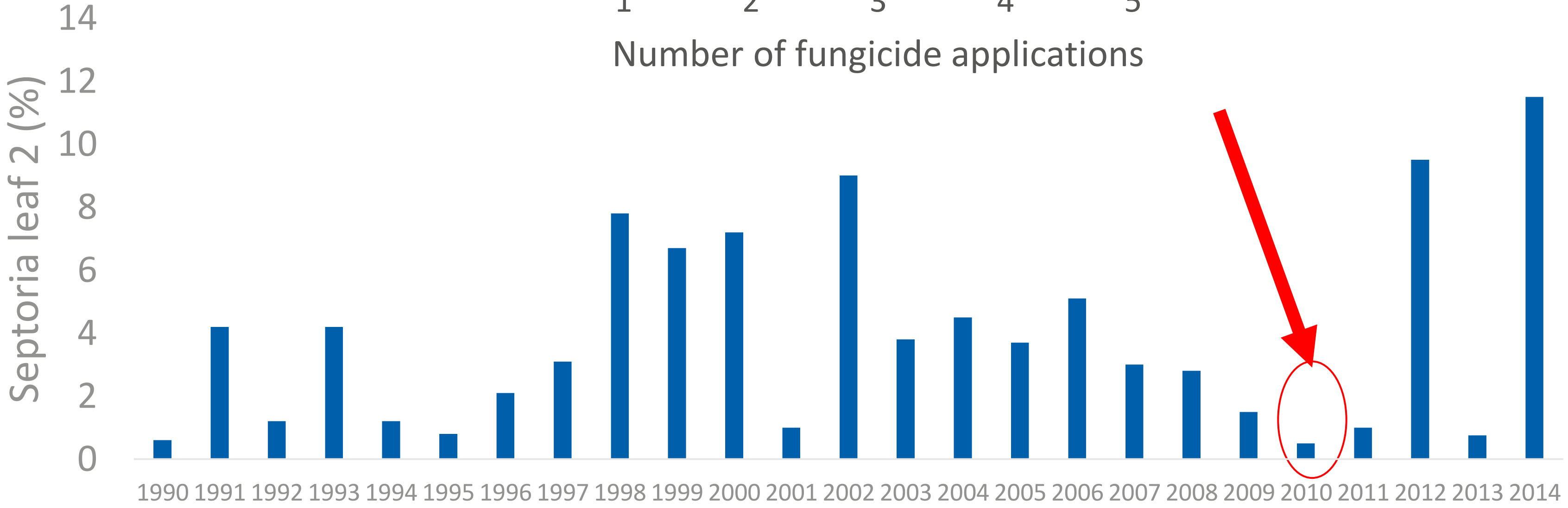
Knowledge gaps:

Mixtures vs. alternation when there is concurrent selection for resistance against two MOA?

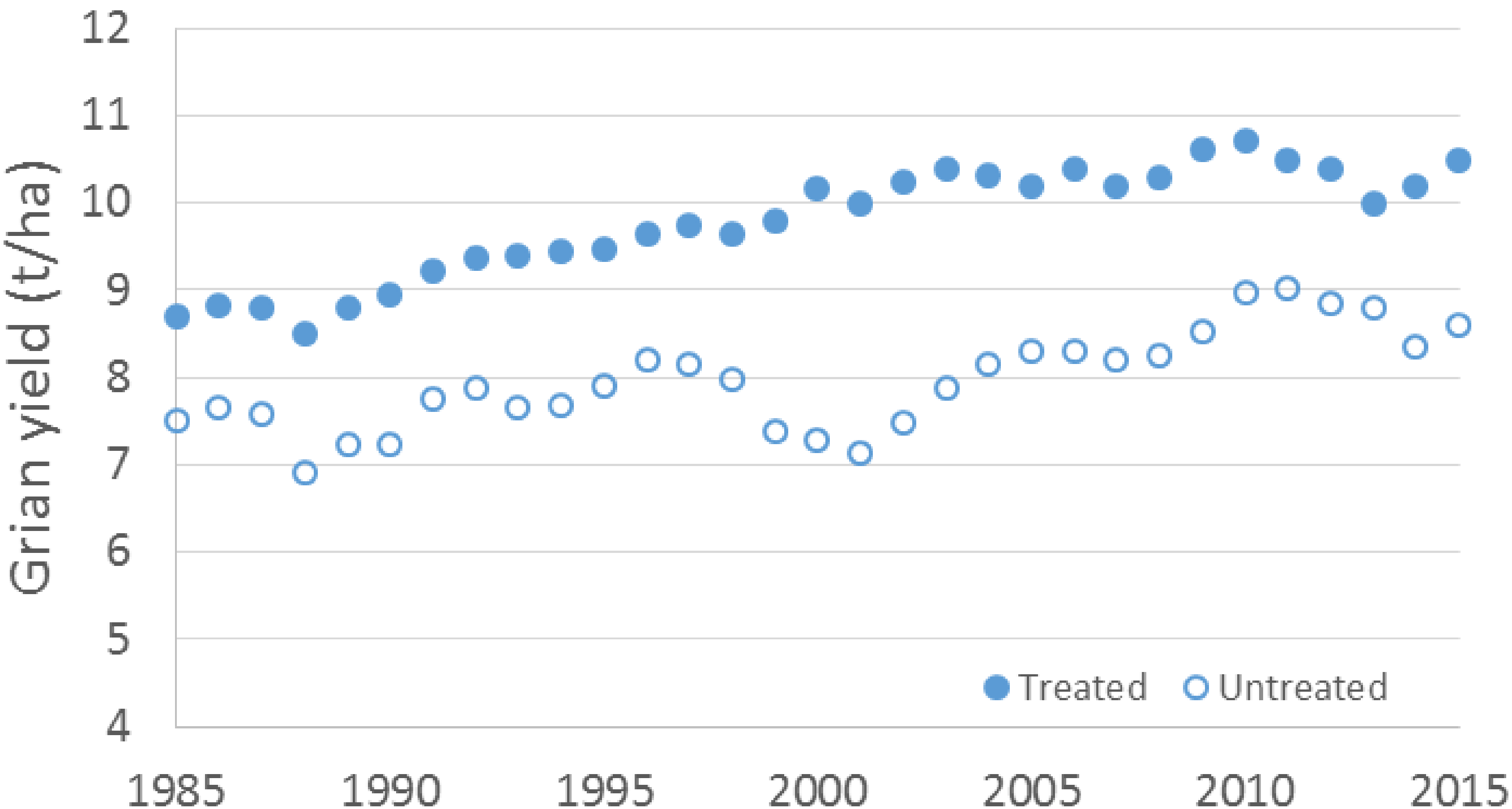
Which resistance management tactics work for monocyclic pathogens?

Septoria in treated crops





Yield response to fungicide treatment (long-term RL rolling average)



‘Mixtures’ for effective control and slow pathogen evolution

Fungicide MOA + Fungicide MOA

Host resistance gene + Host resistance gene

Host resistance + Fungicide

+

What should we **do**?

- Resistance is not Schrodinger's cat – it does not change when we observe it
  - don't change strategy on detection of resistance
- Argue for pesticide regulation based on risk not hazard
- Implement evidence-based resistance management
- Fill evidence gaps (monocyclic diseases, mixtures vs. alternation with concurrent selection)
- Forecast disease to minimise selection of resistant strains
- Integrate chemical and genetic control - more sustainable than either alone
- Enable high-yielding, disease resistant varieties
  - RL criteria, tolerance, yield drag, durability