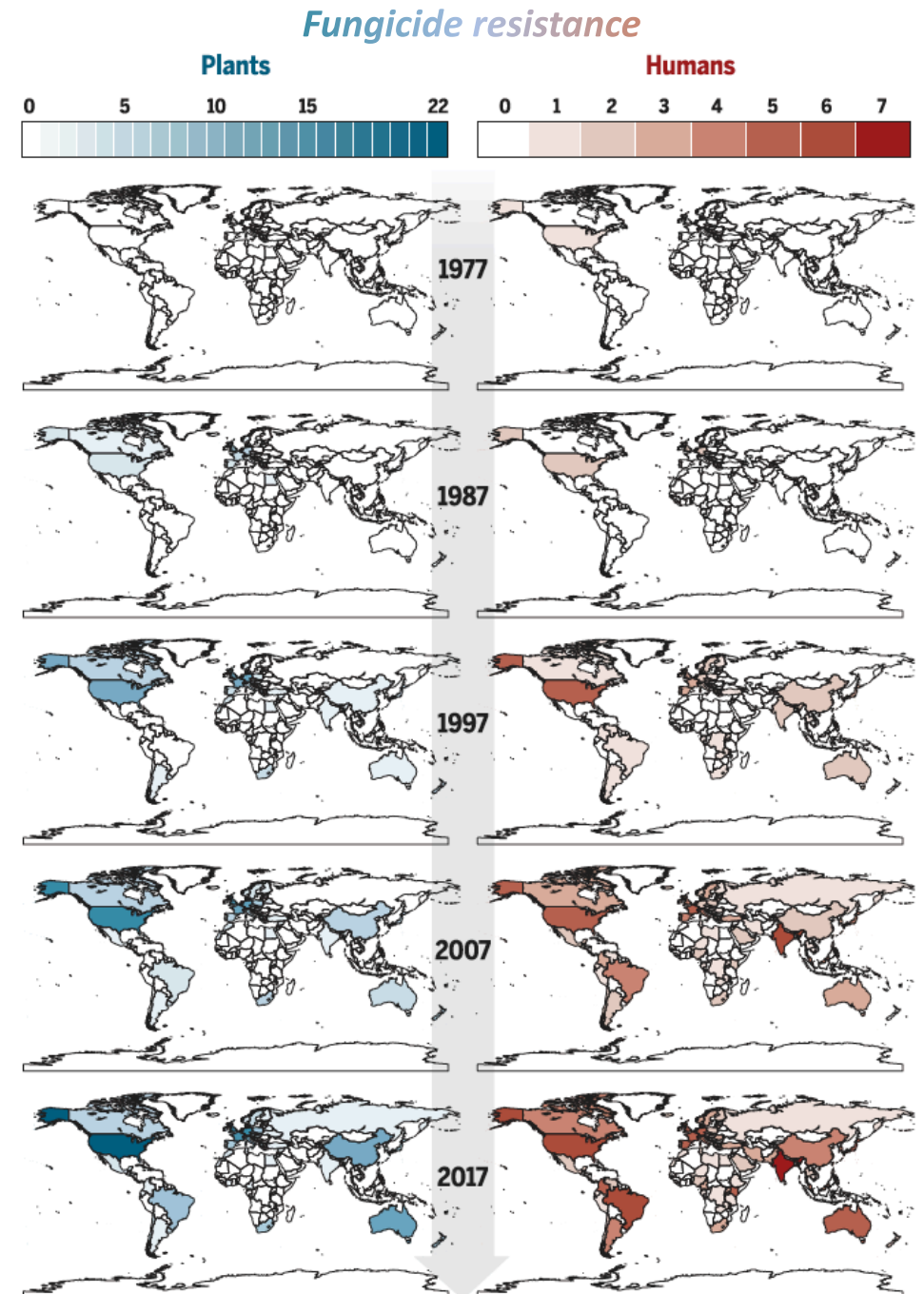


Genetics and mechanisms of fungicide resistance

Professor Mat Fisher

MRC Centre for Global Infectious Disease Analysis

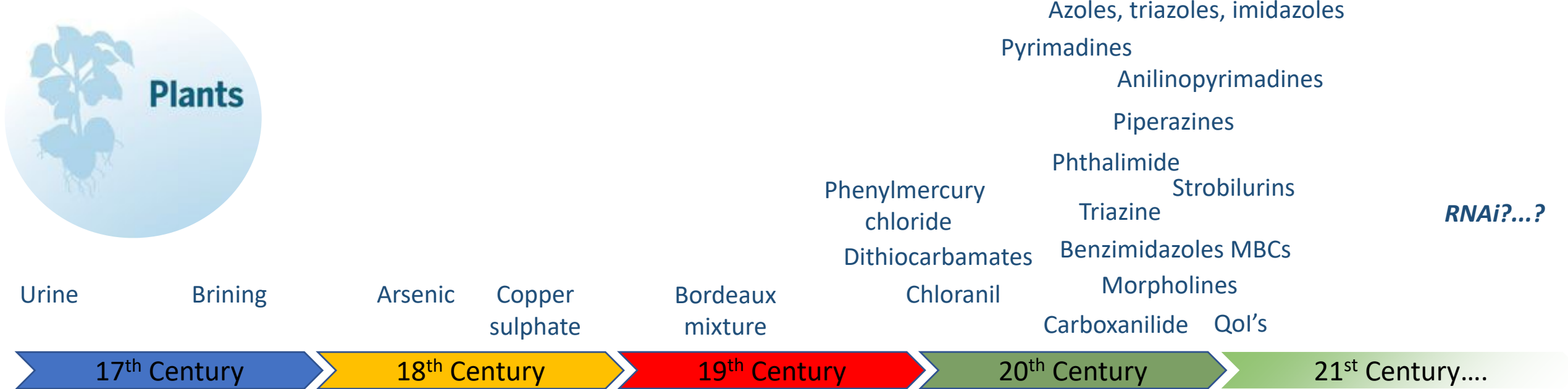
Imperial College School of Public Health



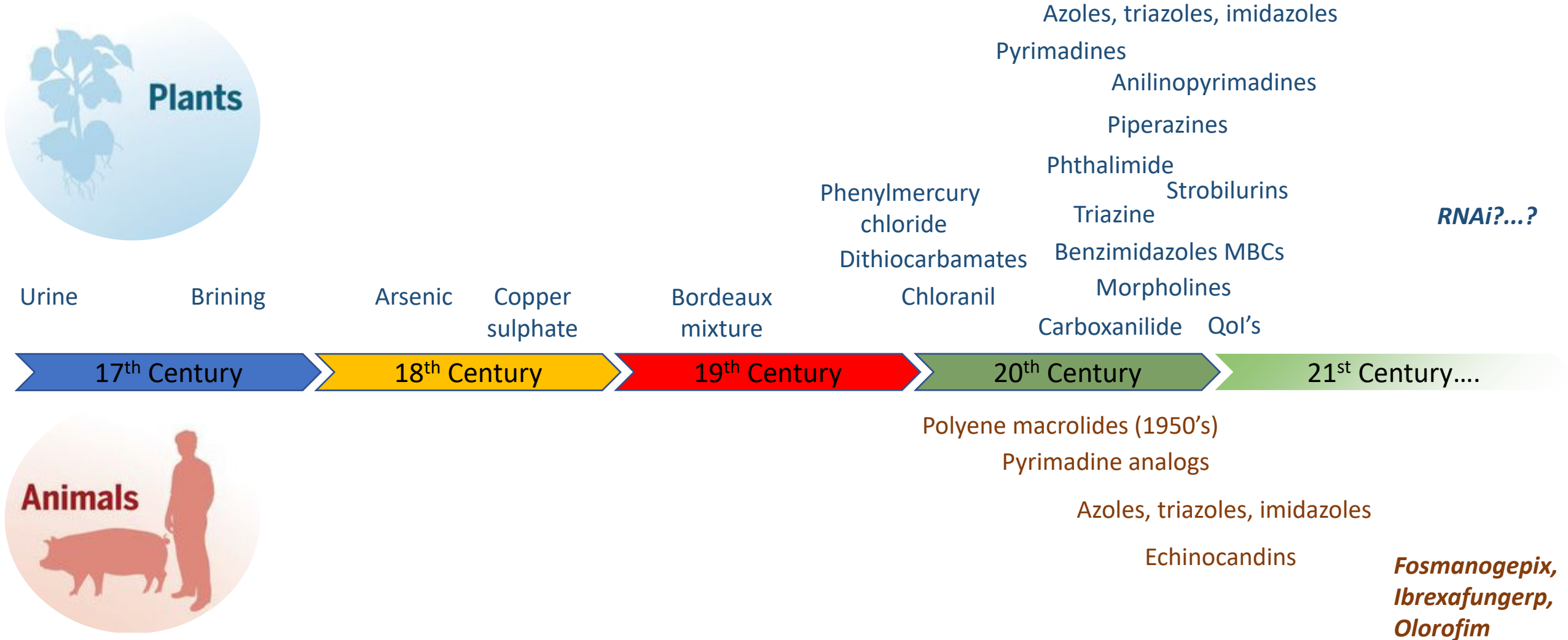
Roadmap to the talk

- The history of antifungal development
- *...and the history of antifungal resistance*
- The current pandemic of fungal resistance
- *...the coming pandemic of fungal resistance?*

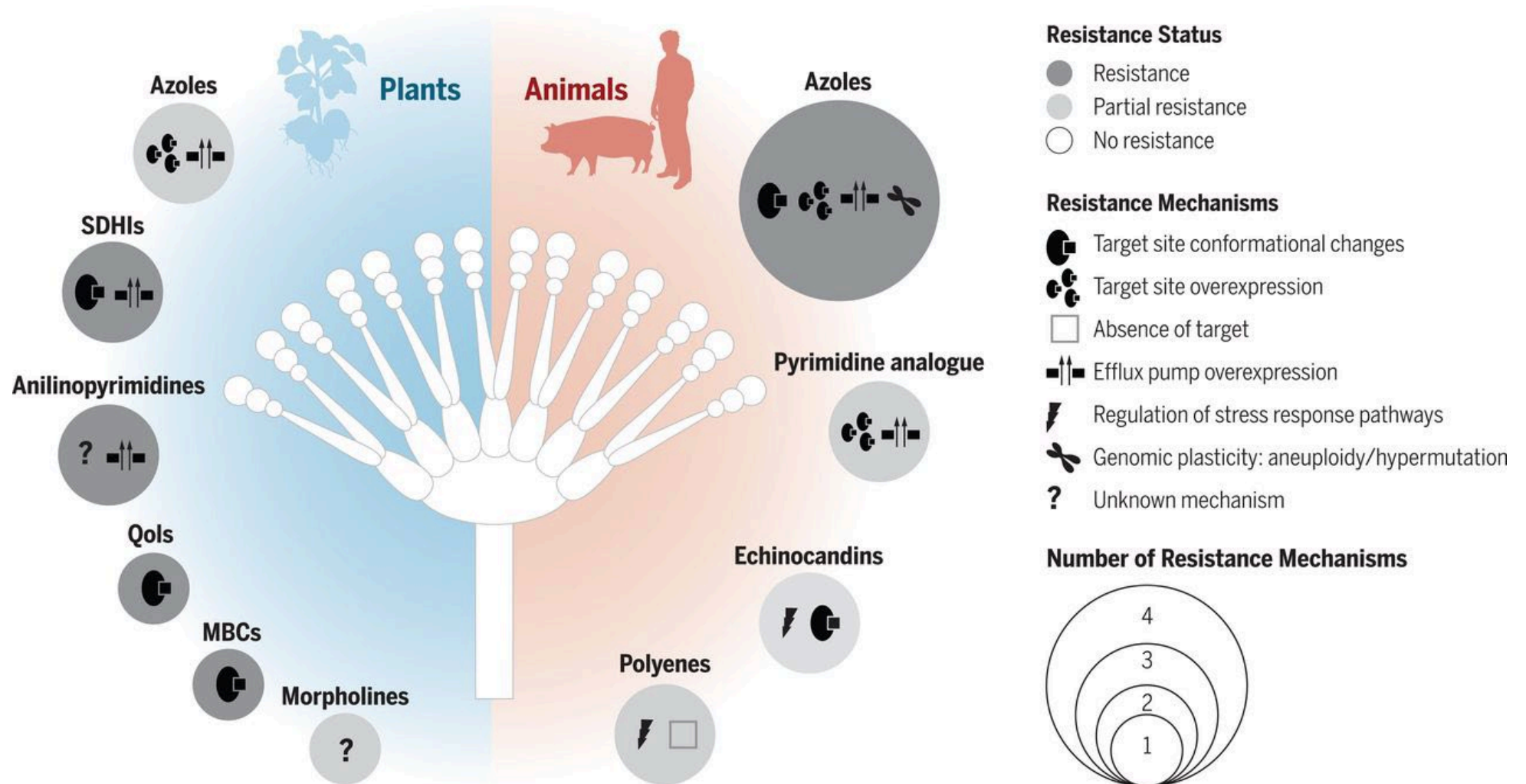
Humanities history of combating fungal disease using antifungal chemicals



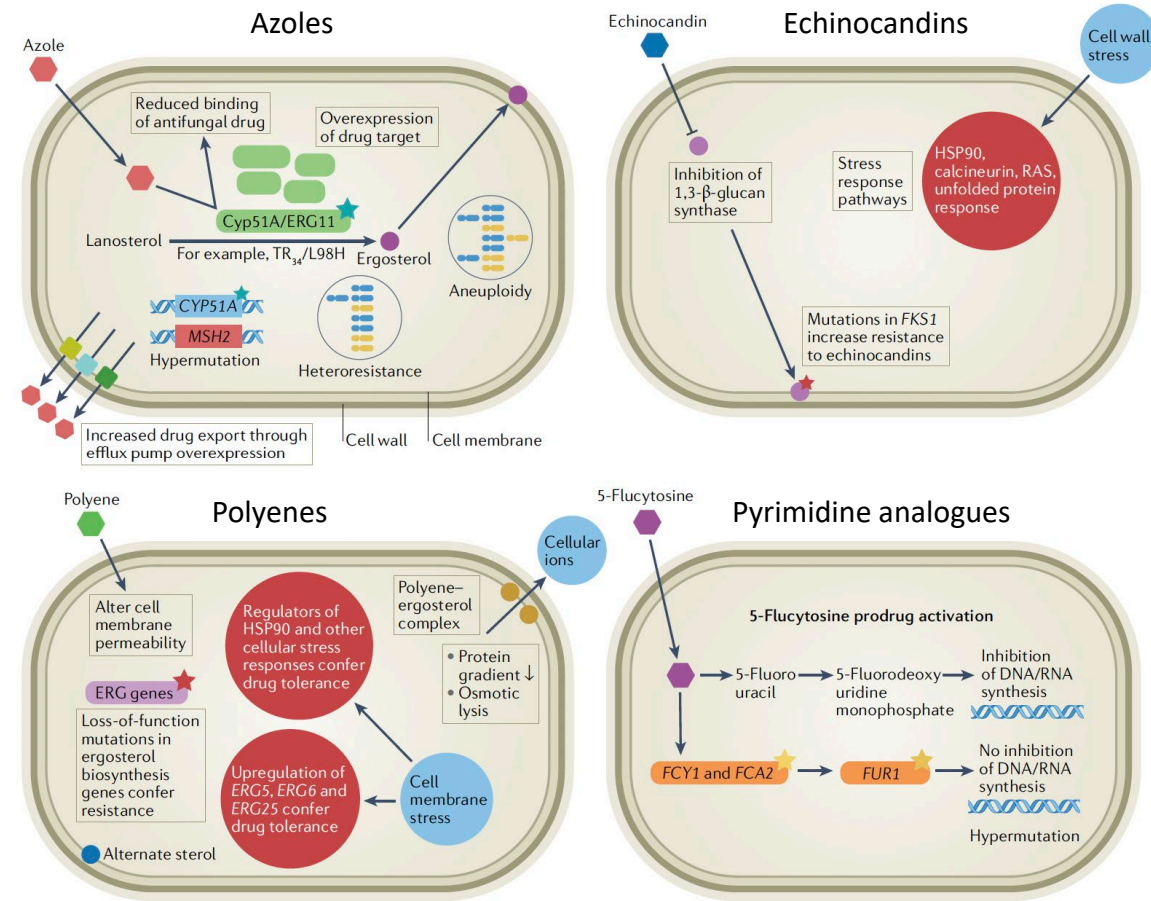
Humanities history of combating fungal disease using antifungal chemicals



Resistance occurs to *all* classes of drugs used against plant and animal fungal infections



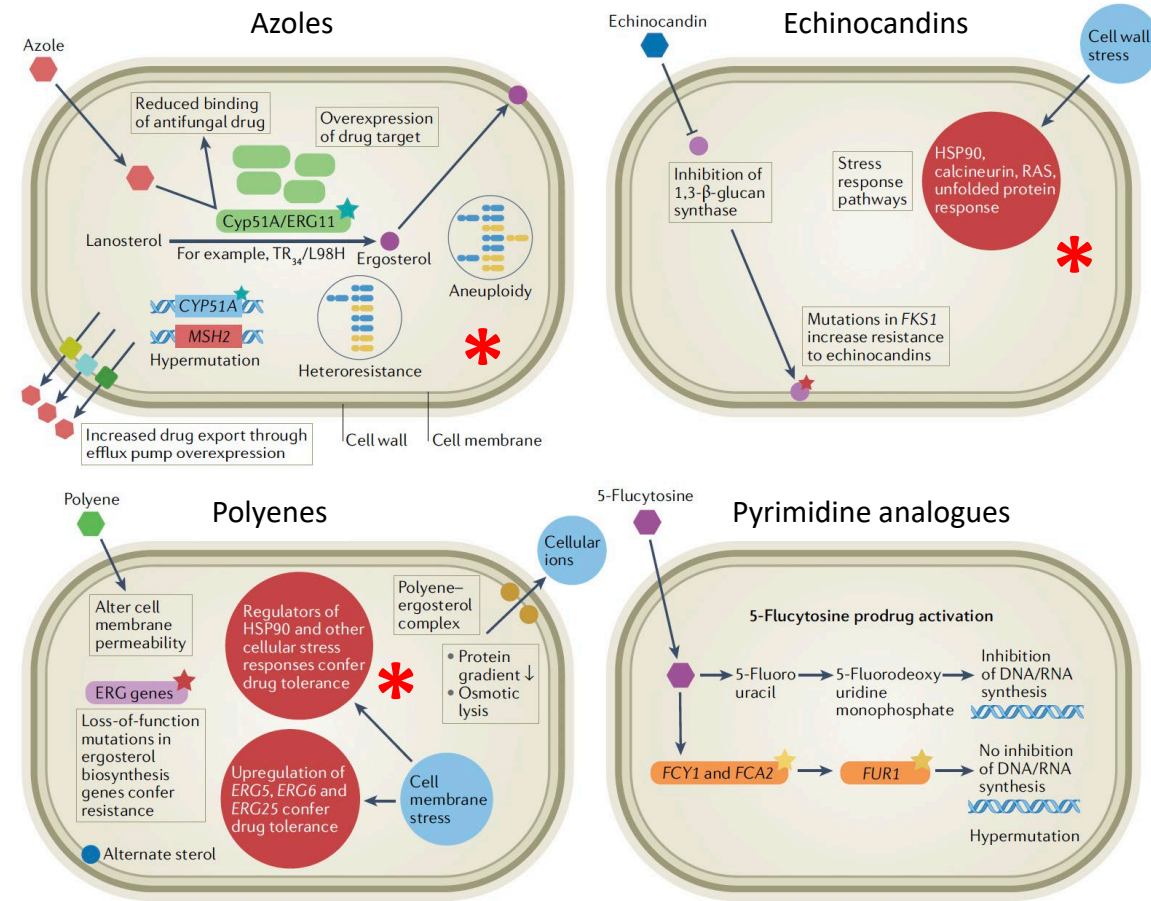
Resistance $\neq$ Tolerance



Resistance

Owes to changes that directly or indirectly affect the drug-target interaction

Resistance ≠ Tolerance



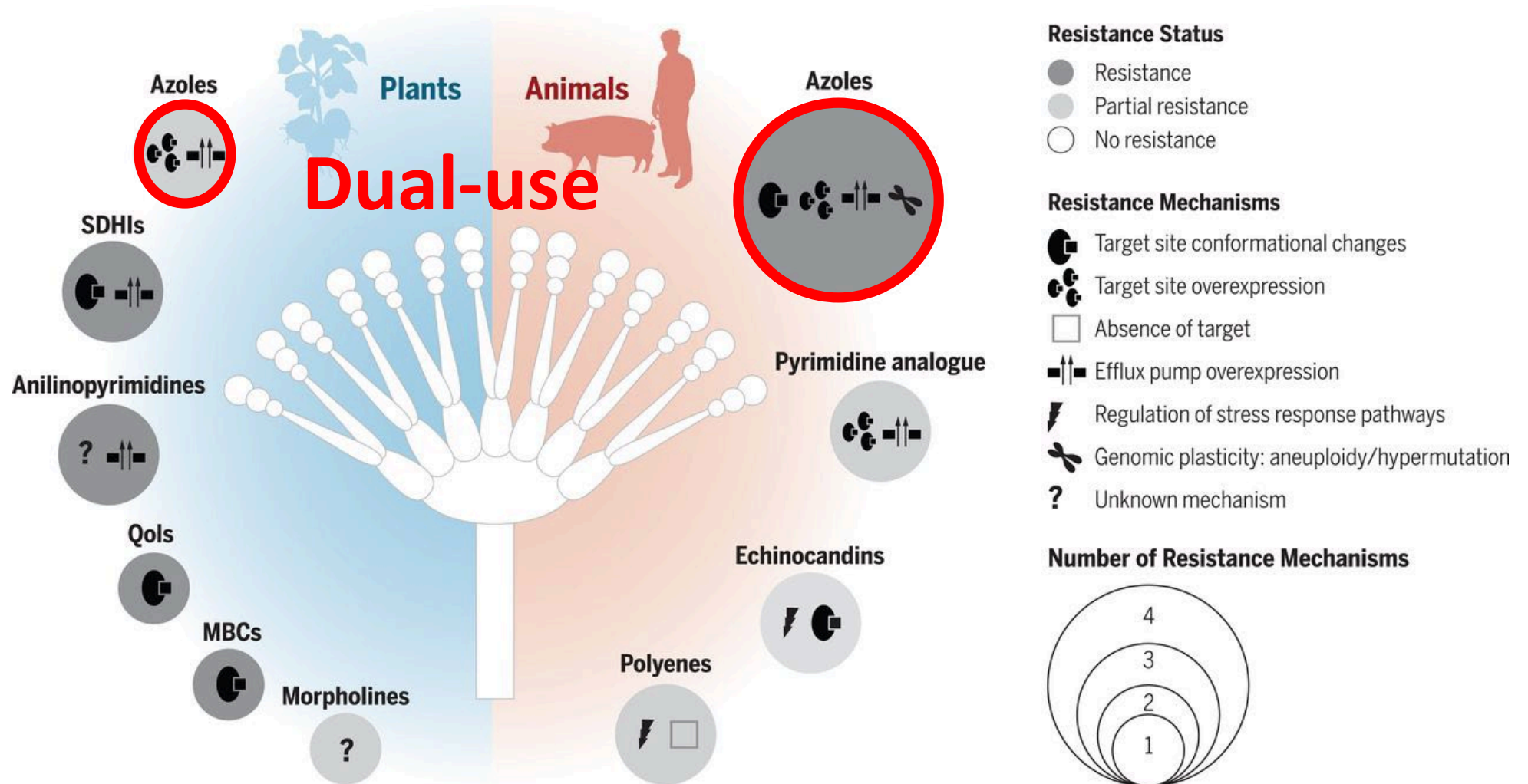
Resistance

Owes to changes that directly or indirectly affect the drug-target interaction

* Tolerance

Growth at drug concentrations *above the minimum inhibitory concentration (MIC)* and involving a wide range of general stress response and/ or epigenetic pathways

Resistance occurs to *all* classes of drugs used against plant and animal fungal infections

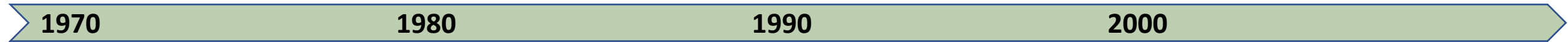


Azoles



Plants

1970: triadimenol, triadimefon
1980: flutriafol, propiaconazole
1990: difenoconazole, myclobutanil, cyproconazole, tebuconazole, hexaconazole, bromuconazole, fenbuconazole, tetraconazole, fluquinconazole, metconazole
2000: prothioconazole

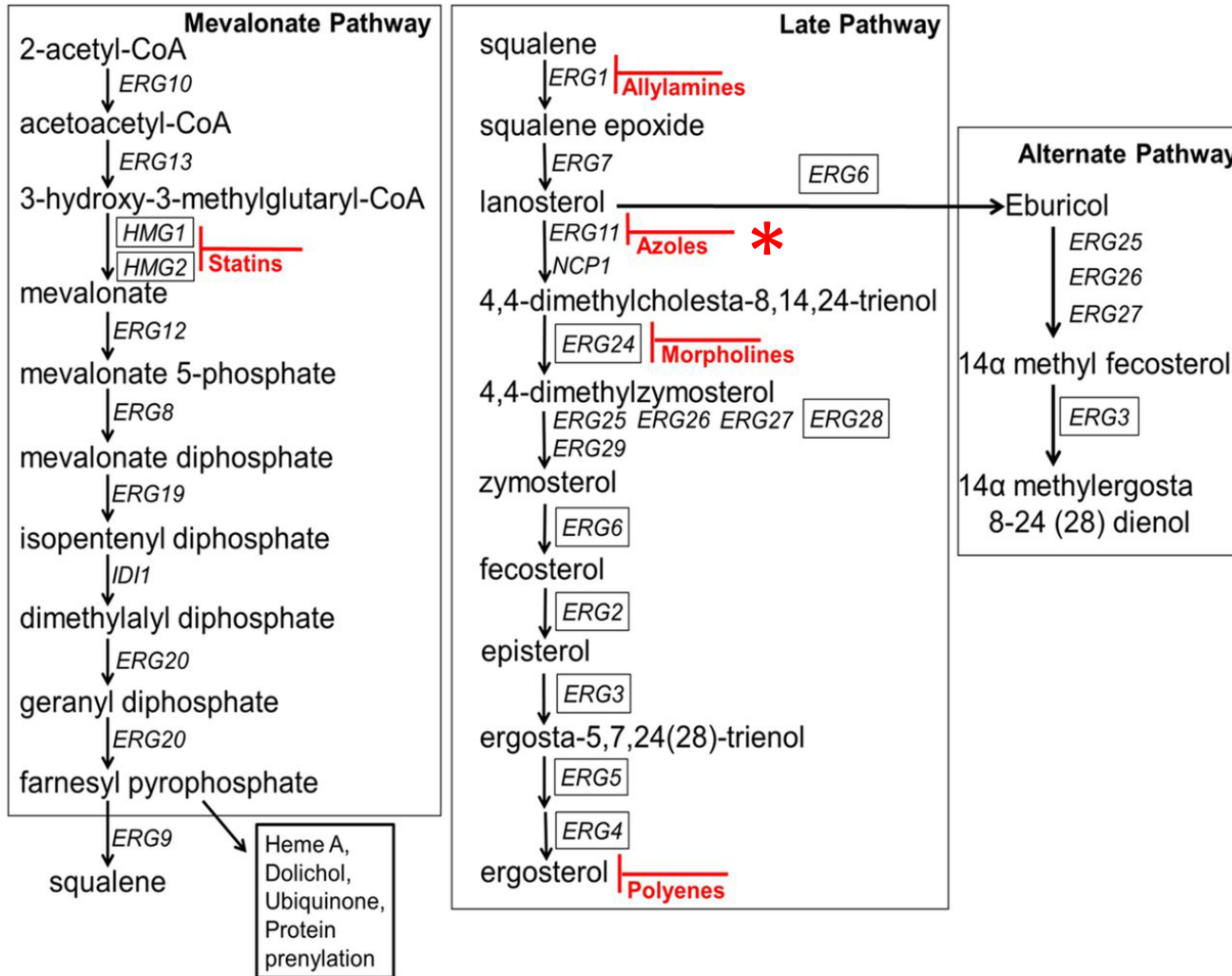


Animals

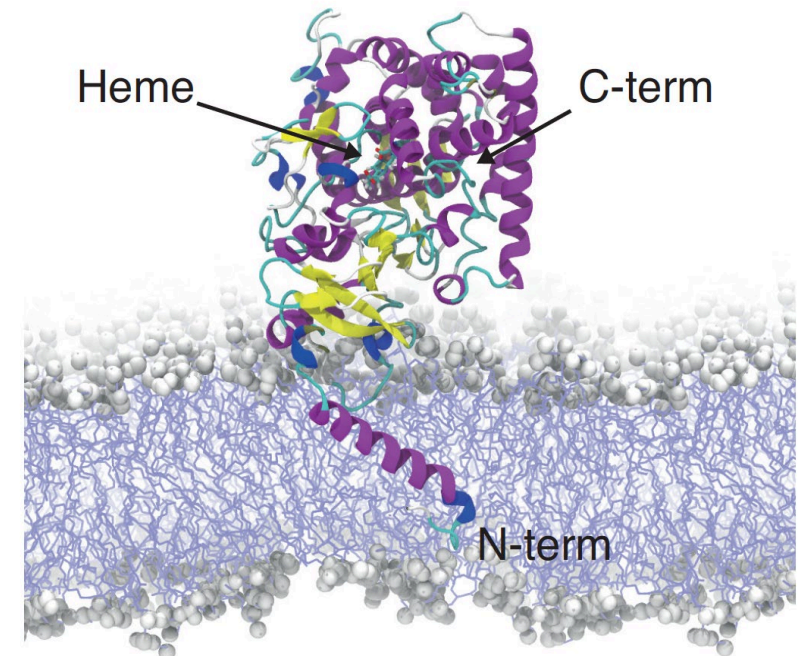
1988: fluconazole
1990: itraconazole
1992: voriconazole
2006: posaconazole
2015: isavuconazole
pulmazole
opelconazole



Widespread azole (*CYP51*) resistance occurs in plant fungal pathogens



* *CYP51/ERG11* encoding sterol 14 α -demethylases



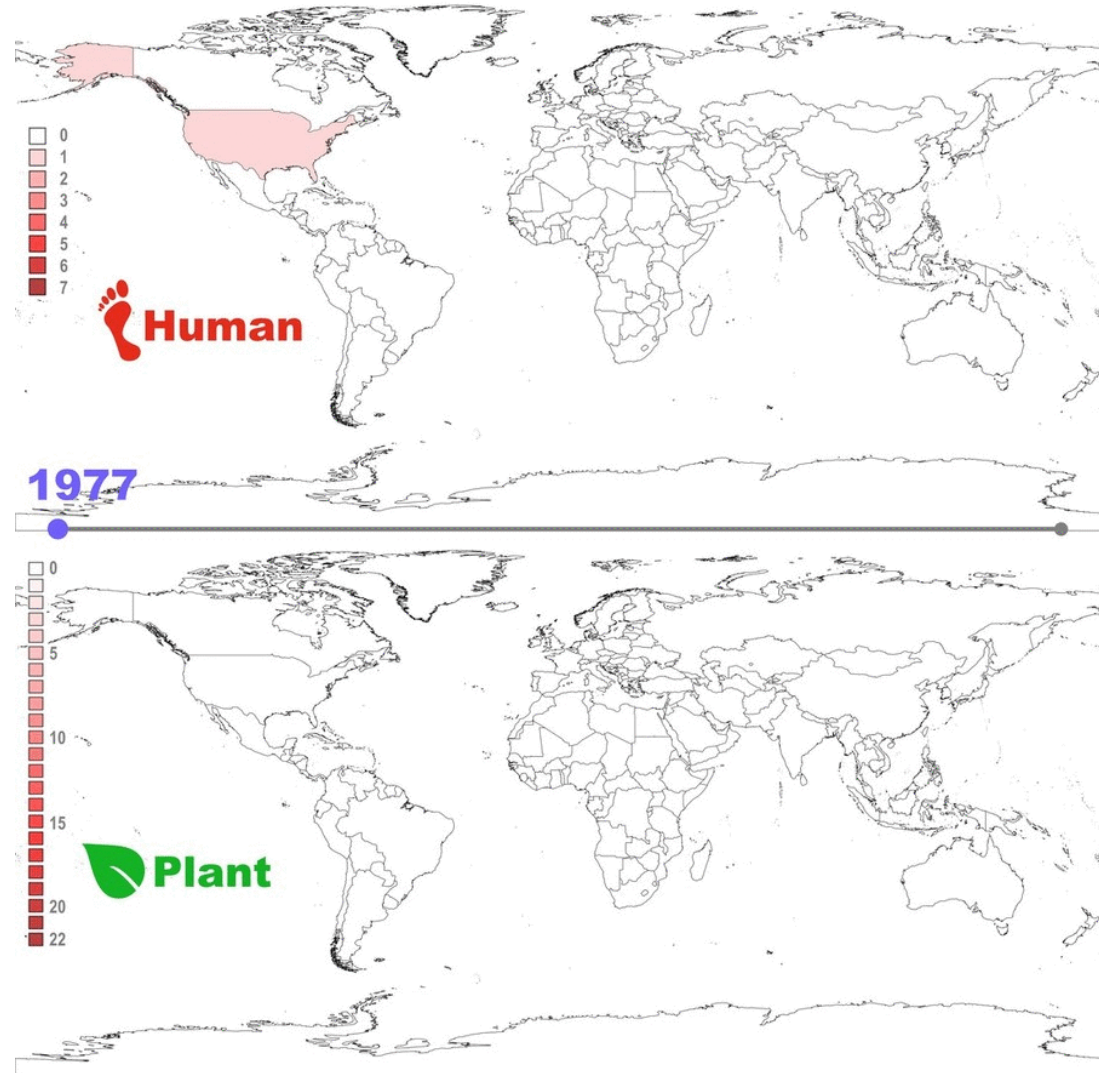


Widespread azole (*CYP51*) resistance occurs in plant fungal pathogens

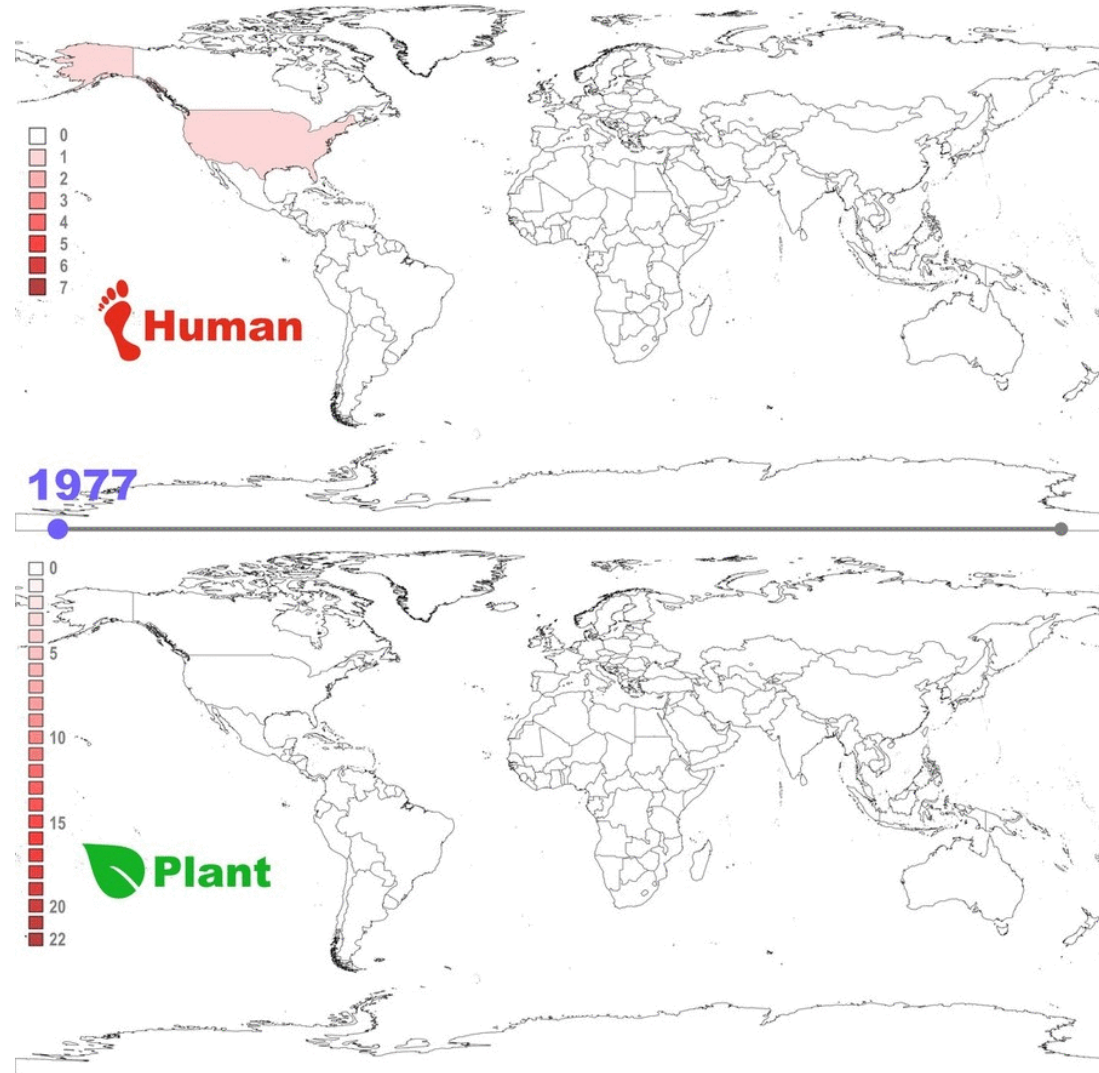
Table 1. Overview of mutations identified in the CYP51 of field isolates

Organism	Crop affected	Alterations in the amino acid sequence	Reference
<i>Zymoseptoria tritici</i>	Wheat	L50S, D107V, D134G, V136A, V136C, V136G, Y137F, M145L, N178S, S188N, S208T, N284H, H303Y, A311G, G312A, A379G, I381V, A410T, G412A, Y459C, Y459D, Y459N, Y459P, Y459S, G460D, Y461D, Y461H, Y461S, ΔY459 or ΔG460, ΔY459/G460, V490L, G510C, N513K, S524T	12
<i>Blumeria graminis</i> f. sp. <i>tritici</i>	Wheat	Y136F	13
<i>Blumeria graminis</i> f. sp. <i>hordei</i>	Barley	Y136F, K147Q	14
<i>Erysiphe necator</i>	Grape	Y136F	15
<i>Mycosphaerella fijiensis</i>	Banana plants and plantain	Y136F, A313G, Y461D, Y463D, Y463H, Y463N	16
<i>Venturia nasicola</i>	Japanese pear	Y133	17
<i>Pyrenopeziza brassicae</i>	Oilseed rape	G460S, S508T	18
<i>Puccinia triticina</i>	Wheat	Y134F	19
<i>Penicillium digitatum</i>	Citrus fruit	V55A, Y136H, M144T, K253E, Q309H, E331A, T432, I440V, K449R, G459S, R462H, F506I, S507P, K508R, G511S	20
<i>Oculimacula yallundae</i>	Wheat	S35T, Q43H, D78Y, E106K, N244S, S505Q	21
<i>Oculimacula acuformis</i>	Wheat	A29P, V37A, Q167H, Y486H, S505Q	21

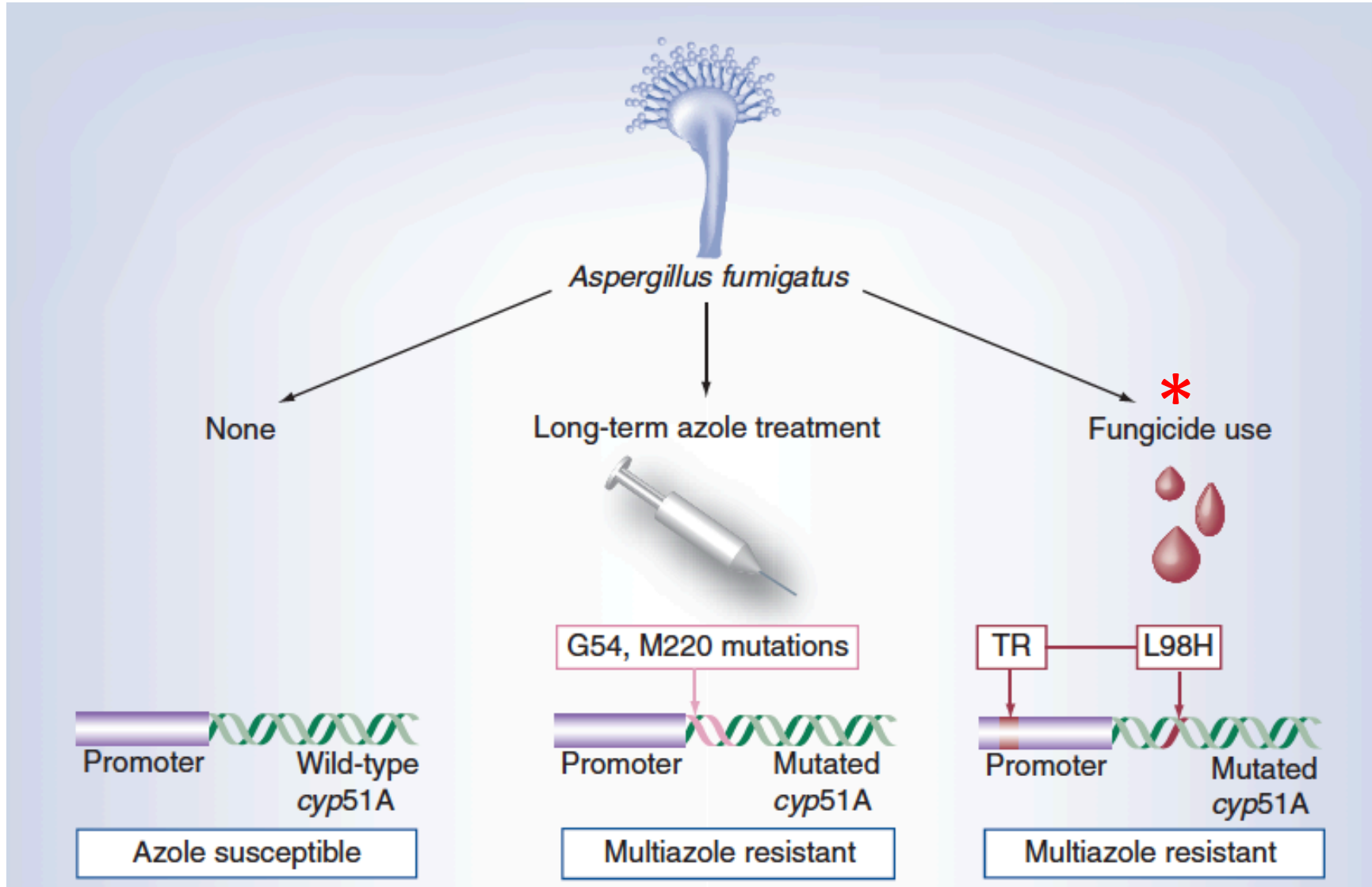
Resistance to azoles is increasing through time



Resistance to azoles is increasing through time



Evolution of clinical azole-resistance *in situ* versus *ex situ* for *Aspergillus fumigatus* (Snelders *Future Medicine* 2011)



CBC transcription factor binding (Gsaller 10.1371/journal.ppat.1005775)

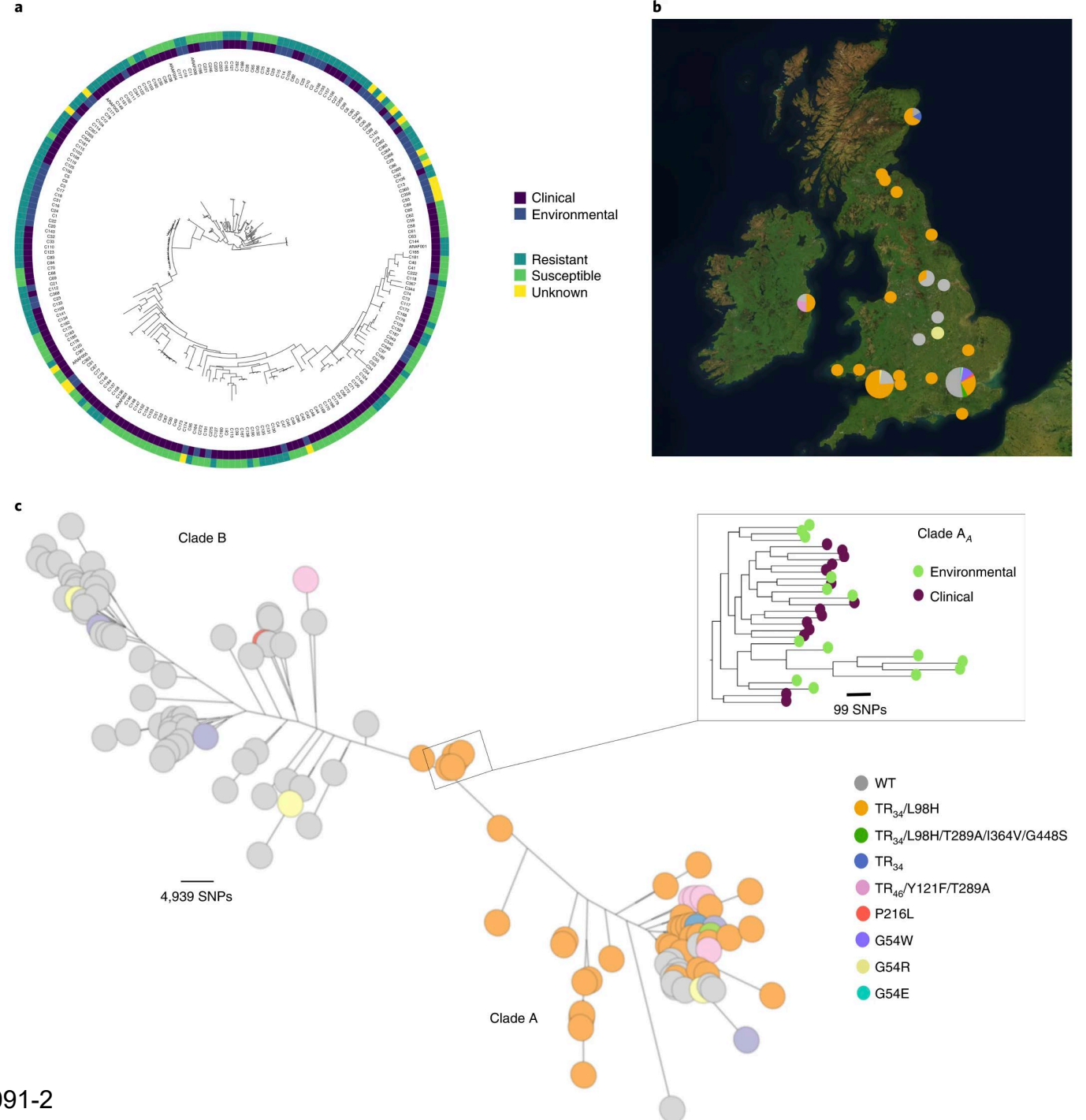
Protein conformational change

* TR₃₄ L98H

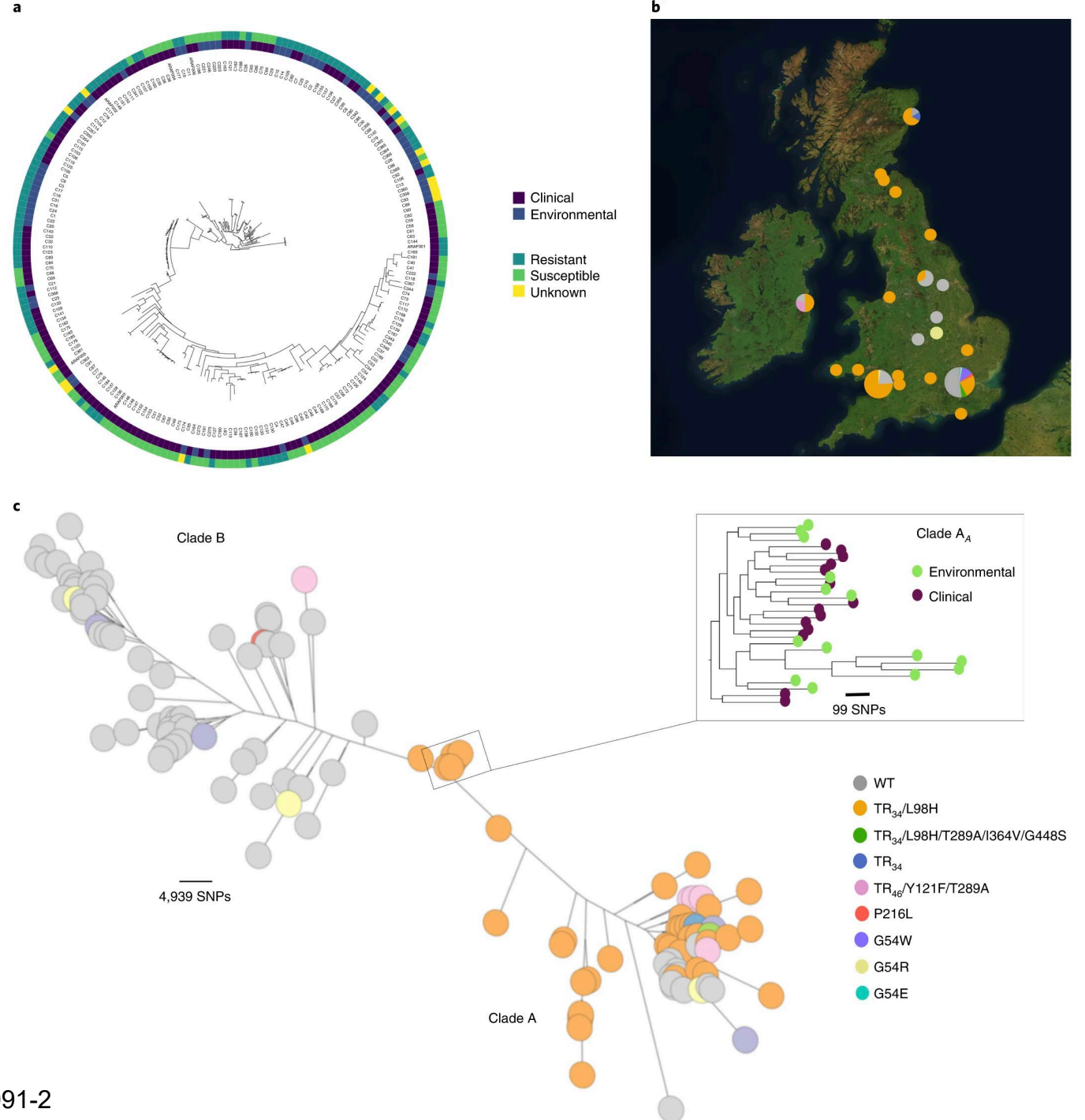
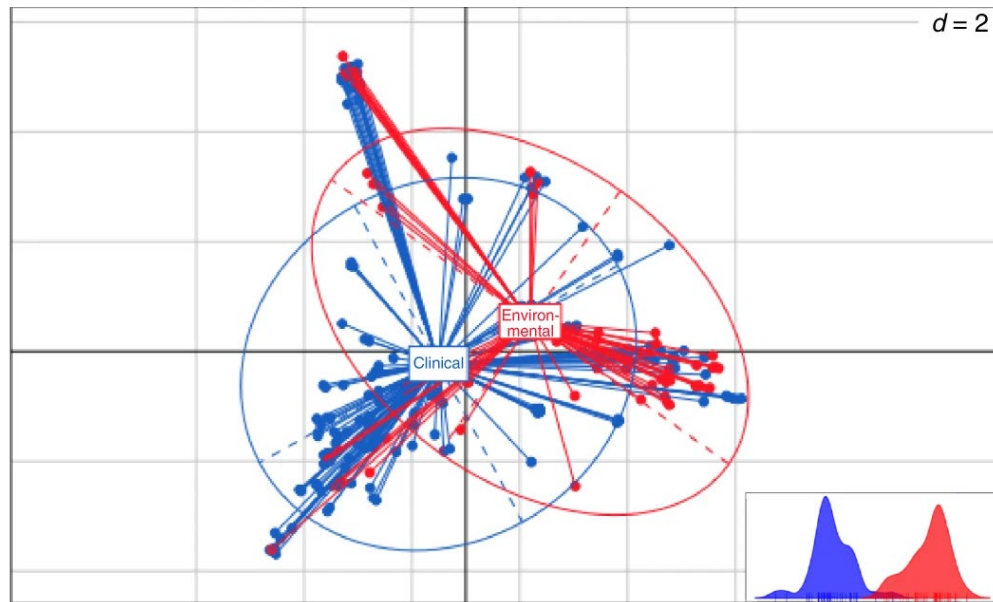
* TR₄₆ Y121F/T289A



Genome sequencing of 218 UK *Aspergillus fumigatus* isolates (153 Clinical; 65 environmental) confirms acquisition of drug resistant infections from the environment



Genome sequencing of 218 UK *Aspergillus fumigatus* isolates (153 Clinical; 65 environmental) confirms acquisition of drug resistant infections from the environment



+

+

SCIENCE SOLSTICE

THURSDAY 21ST JUNE 2018

**** BE A CITIZEN SCIENTIST FOR THE DAY! ****

Join fellow citizen scientists across the U.K. in sampling your local air for fungal spores, to monitor for resistance to azole fungicide drugs



 @jenmghe
#scienceolstice

Celebrate Summer Solstice by collecting outdoor air samples from your home & workplace!
Why is this research important? To find out visit: www.fishlab.co.uk/2018/science-solstice

A pack containing everything you need will be posted to you and a reminder text will be sent the day before.
On Summer Solstice leave air samplers on ground floor windowsills at your two chosen locations for 8 hours.
Return your samples in the Frequent envelope and follow a link to an online interactive map to see your data points!
(Your personal data will be kept confidential and online data points anonymised.)

To participate enter your details in the Google form: www.imperial.ac.uk/scienceolstice

Imperial College
London



Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

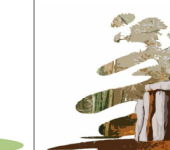
NERC
NATURAL ENVIRONMENT RESEARCH COUNCIL

SUMMER SOLSTICE

Monday 24th September 2018

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AUTUMN AIR-INOXO

Monday 24th September 2018

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 @jenmghe
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WINTER SOLSTICE

Mon 17th-Fri 21st December 2018

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Join fellow citizen scientists across the U.K. in sampling your local air for fungal spores, to monitor for resistance to azole fungicide drugs



 @jenmghe
#scienceolstice

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SPRING AIR-INOXO

Wednesday 20th March 2019

**** BE A CITIZEN SCIENTIST FOR THE DAY! ****

Join fellow citizen scientists across the U.K. in sampling your local air for fungal spores, to monitor for resistance to azole fungicide drugs



 @jenmghe
#scienceolstice

Celebrate Spring Air-INOXO by collecting outdoor air samples from your home & workplace!
Why is this research important? Visit our website to find out: www.fishlab.co.uk

A pack containing everything you need will be posted to you and a reminder text will be sent the day before.
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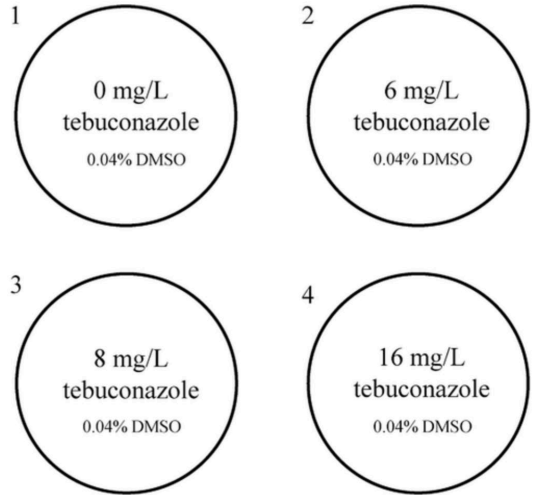
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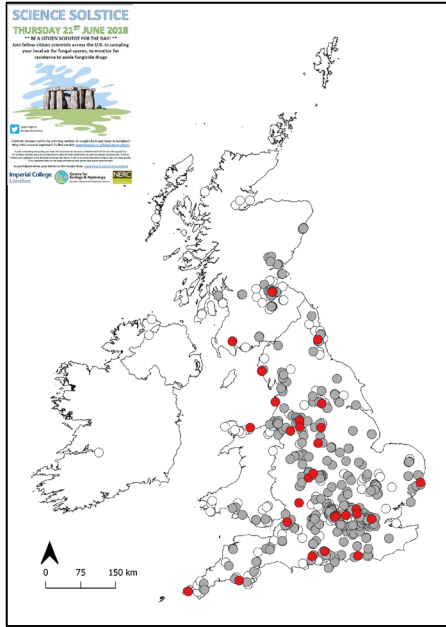


UK experiences a landscape-scale exposure to multidrug-resistant *Aspergillus fumigatus* bioaerosols

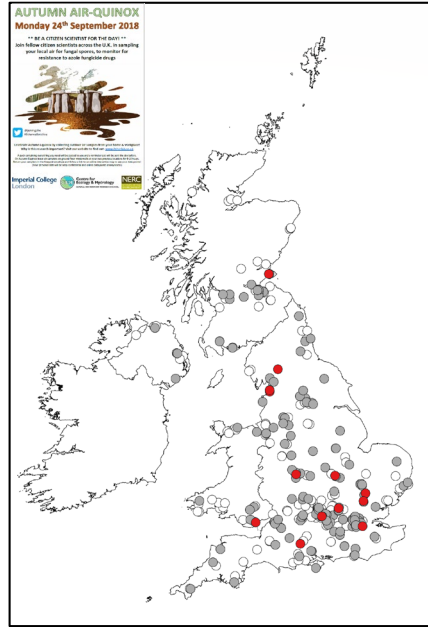


Jen Shelton

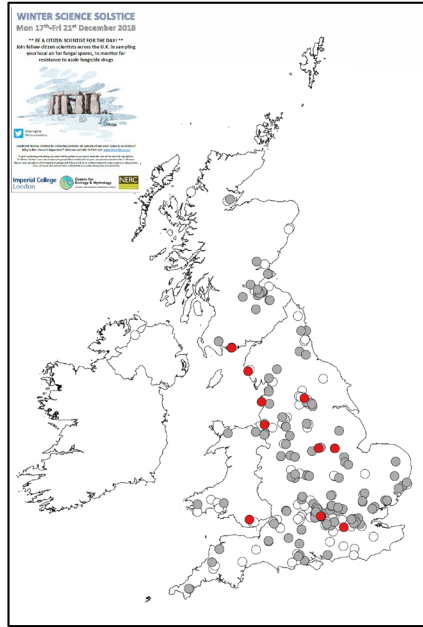
Solstice 2018



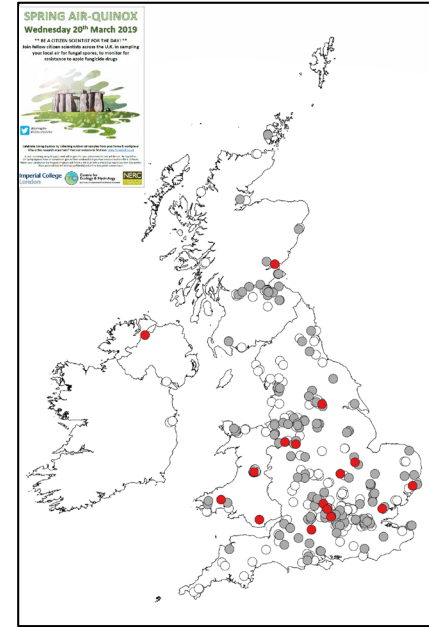
Equinox 2018



Solstice 2019



Equinox 2019



- Resistant *Af*
- Sensitive *Af*

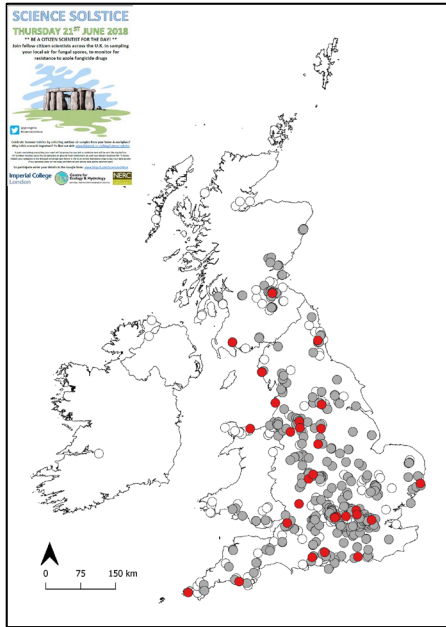
1,894 sample-periods collected 2,366 aerosolised *A. fumigatus*

UK experiences a landscape-scale exposure to multidrug-resistant *Aspergillus fumigatus* bioaerosols

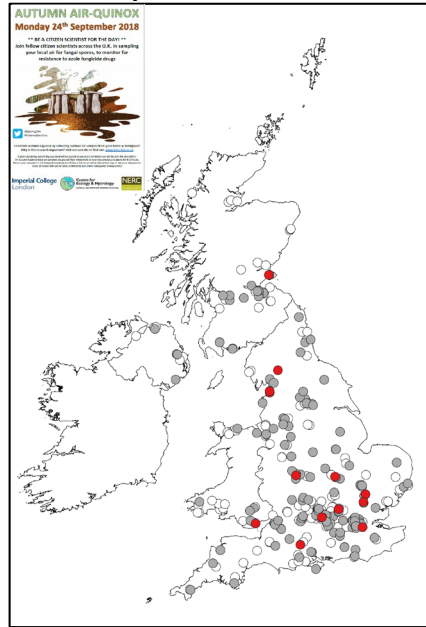


Jen Shelton

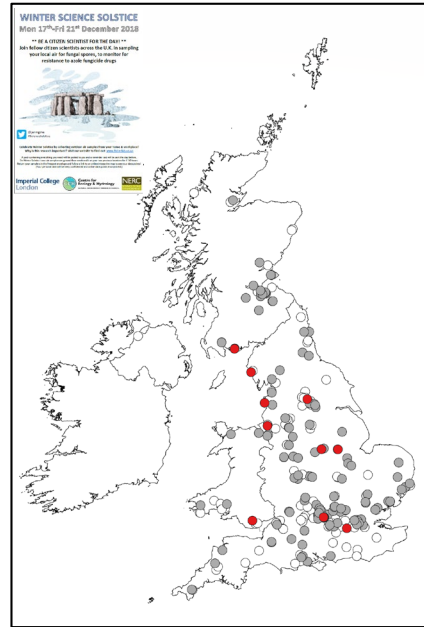
Solstice 2018



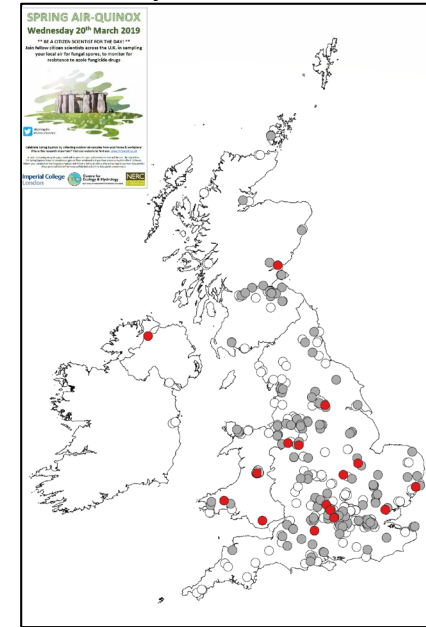
Equinox 2018



Solstice 2019



Equinox 2019



- Resistant *Af*
- Sensitive *Af*

1,894 sample-periods collected 2,366 aerosolised *A. fumigatus* of which:

1: 20 were resistant to tebuconazole

1:25 were resistant to itraconazole; **1:40** to voriconazole; **1:25** to isavuconazole and

1:150 were resistant to all tested medical azoles

UK experiences a landscape-scale exposure to multidrug-resistant *Aspergillus fumigatus* bioaerosols

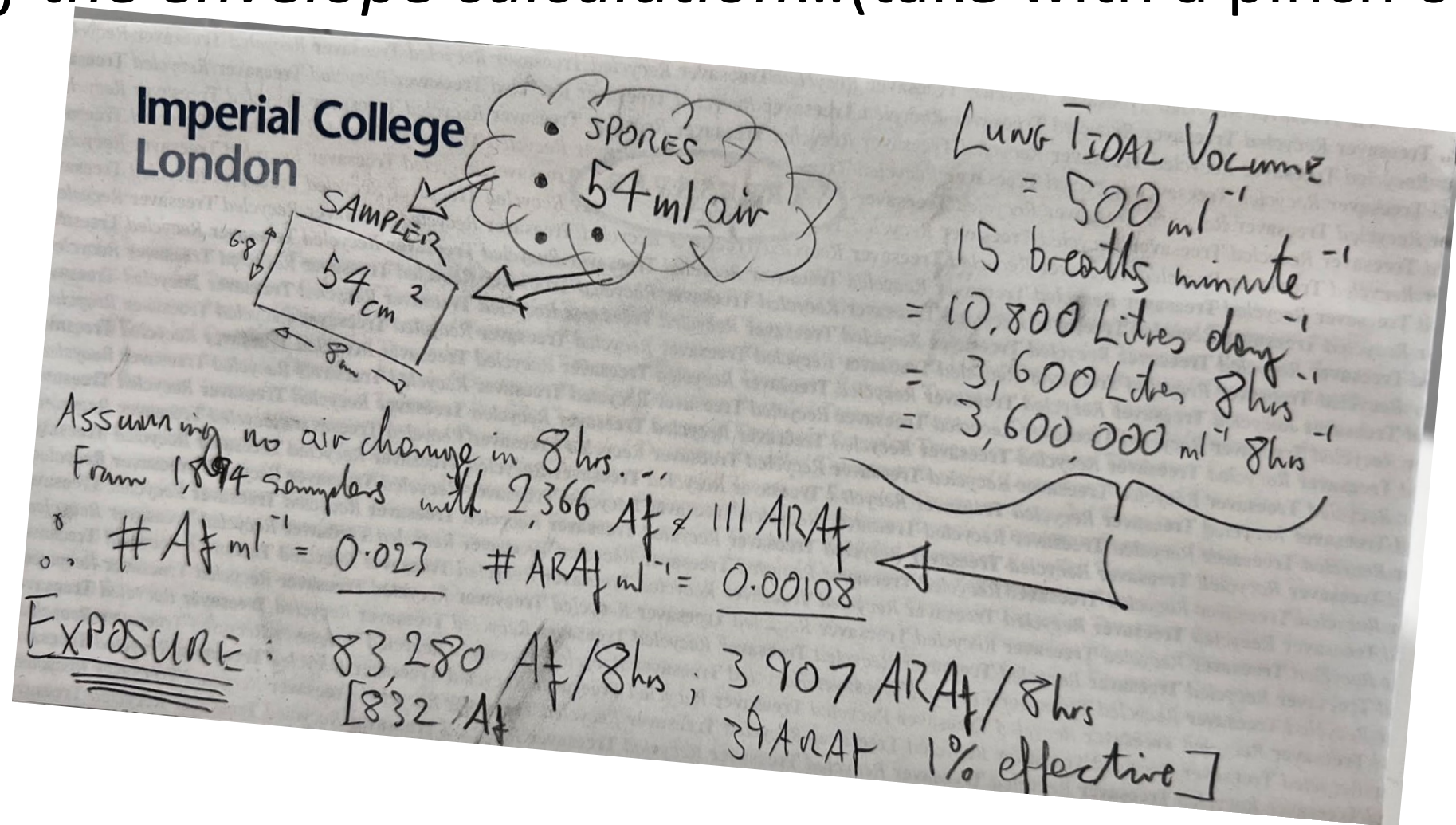


Jen Shelton

The majority of this exposure is associated with the resistance allele TR₃₄ / L98H

<i>cyp51A</i> polymorphisms	No. of isolates (n = 111)	Proportion of isolates
Not <i>A. fumigatus</i>	12 (9 x <i>A. lentulus</i> , 2 x <i>A. nidulans</i>)	11%
TR34	1	1%
TR34/L98H	58	52%
TR46/Y121F/T289A	6	5%
Amino acid subs only	4	4%
No polymorphisms	30	3%

A back of the envelope calculation...(take with a pinch of salt...)



Calculating from a 54cm² capture surface, we inhale 3,907 azole-resistant *A. f* spores in 8 hrs (or 39 resistant spores assuming only 1% efficiency)

A way out: Future upgrading of the armamentarium of clinical antifungals with novel modes-of-action

i.

Mitochondria respiratory chain

- *ATI-2307 (PI)*

Acetyl CoA synthetase inhibitors

- *AR-12 (No longer in development)*

Isocitrate lyase inhibitors

- *Mohangamides A & B (pre-clinical)*

GPI-anchor biosynthesis

- *Fosmanogepix (PIII)*

HOG pathway inhibitors

- *e.g. Ambruticins (pre-clinical)*

Calcineurin inhibitors

- *Tacrolimus (not currently in development)*

Fungus-specific transporters

- *Sertraline (no recent progress)*
- *Tamoxifen (no recent progress)*
- *VL-2397 (discontinued – but maybe revived)*

ii.

Cell wall

- *Chitin synthase inhibitors e.g., Nikkomycin Z (no recent progress)*
- *Farnesylation or prenylation inhibitors (pre-clinical)*
- *Glucan synthase inhibitors e.g. Ibrexafungerp (Now approved) Rezafungin (PIII)*

Cell membrane

- *Modified inhaled azoles e.g., Pulmazole (PIIB), Opelconazole (PIII)*
- *Tetrazole inhibitors VT-1598 (PI), Oteseconazole (NDA filing)*
- *Polyene MAT2203 (PII)*

Pyrimidine biosynthesis

- *Olorofim (PIIB)*

RNA synthesis inhibitor

- *Rifampin (pre-clinical research)*

Isoleucyl-tRNA synthetase inhibitors

- *Icofungipen (discontinued)*

EF-2 / ribosome complex

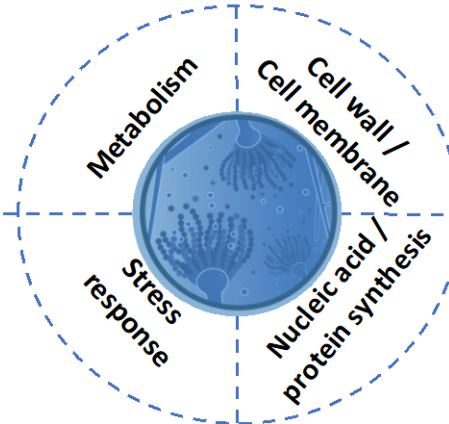
- *Sordarins (pre-clinical)*

Hsp90 inhibitors

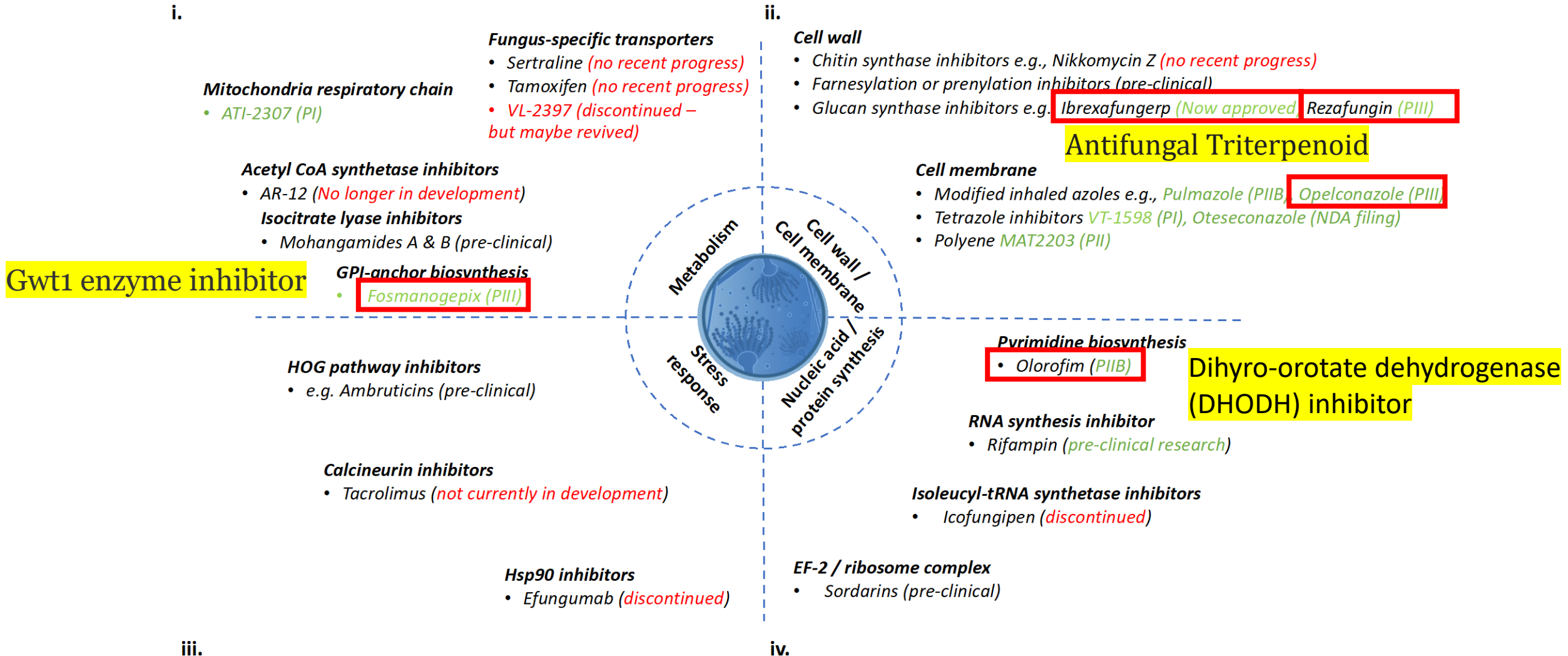
- *Efungumab (discontinued)*

iii.

iv.



A way out: Future upgrading of the armamentarium of clinical antifungals with novel modes-of-action



A way out: Future upgrading of the armamentarium of clinical antifungals with novel modes-of-action



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Pesticides Home

A-Z Index

Bed Bugs

Antimicrobial Pesticides

EPA Proposes Registration of the New Fungicide Ipflufenquin

For Release: June 25, 2021

- HOG pathway inhibitors**
 - e.g. Ambruticins (pre-clinical)
- Calcineurin inhibitors**
 - Tacrolimus (not currently in development)
- Hsp90 inhibitors**
 - Efungumab (discontinued)

MOA	TARGET SITE AND CODE	GROUP NAME	CHEMICAL OR BIOLOGICAL GROUP	COMMON NAME	COMMENTS	FRAC CODE
A: nucleic acids metabolism	A1 RNA polymerase I	PA – fungicides (PhenylAmides)	acylalanines	benalaxyl benalaxyl-M (=kiralaxyl) furalaxyl metalaxyl metalaxyl-M (=mefenoxam)	Resistance and cross resistance well known in various Oomycetes but mechanism unknown. High risk. See FRAC Phenylamide Guidelines for resistance management	4
			oxazolidinones	oxadixyl		
			butyrolactones	ofurace		
	A2 adenosin-deaminase	hydroxy-(2-amino-) pyrimidines	hydroxy-(2-amino-) pyrimidines	bupirimate dimethirimol ethirimol	Medium risk. Resistance and cross resistance known in powdery mildews. Resistance management required.	8
	A3 DNA/RNA synthesis (proposed)	heteroaromatics	isoxazoles	hymexazole	Resistance not known.	32
			isothiazolones	octhilinone		
	A4 DNA topoisomerase type II (gyrase)	carboxylic acids	carboxylic acids	oxolinic acid	Bactericide. Resistance known. Risk in fungi unknown. Resistance management required.	31
	A5 inhibition of dihydroorotate dehydrogenase within de novo pyrimidine biosynthesis	DHODH-fungicides	phenyl-propand	ipflufenquin	Medium to high risk.	52

inhibitors e.g., Nikkomycin Z (no recent progress)

prenylation inhibitors (pre-clinical)

inhibitors e.g. **Ibrexafungerp (Now approved)** **Rezafungin (PIII)**

membrane

Modified inhaled azoles e.g., Pulmazole (PIIB), **Opelconazole (PIII)**,
etrazole inhibitors VT-1598 (PI), Oteseconazole (NDA filing)
polyene MAT2203 (PII)

sis

Dihydro-orotate dehydrogenase (DHODH) inhibitor

(research)

ibitors

https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2022--final.pdf?sfvrsn=b6024e9a_2

?A way out: Future upgrading of the armamentarium of clinical antifungals with novel modes-of-action?



United States
Environmental Protection
Agency

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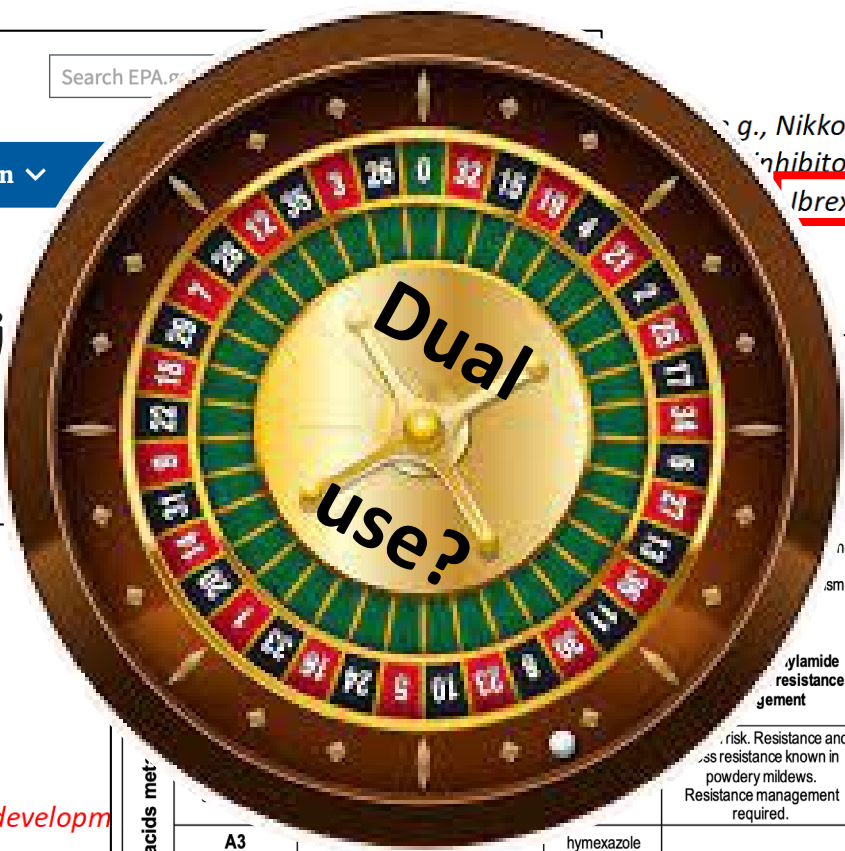
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Pesticides

[Pesticides Home](#)
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[Antimicrobial Pesticides](#)

EPA Proposes Regi New Fungicide Ip

For Release: June 25, 20



- HOG pathway inhibitors**
 - e.g. Ambruticins (*pre-clinical*)
- Calcineurin inhibitors**
 - Tacrolimus (*not currently in development*)
- Hsp90 inhibitors**
 - Efungumab (*clinical*)

g., Nikkomycin Z (*no recent progress*)
inhibitors (*pre-clinical*)
Ibrexafungerp (*Now approved*) Rezafungin (*PIII*)
les e.g., Pulmazole (*PIIB*), **Opelconazole (*PIII*)**,
T-1598 (*PI*), Oteseconazole (*NDA filing*)
I)

Dihydro-orotate dehydrogenase (DHODH) inhibitor

A: nucleic acids metabolism					FRAC CODE
A3 DNA/RNA synthesis (proposed)	heteroaromazoles	isothiazolones	hymexazole	Resistance not known.	4
			octhilineone		8
					32
A4 DNA topoisomerase type II (gyrase)	carboxylic acids	carboxylic acids	oxolinic acid	Bactericide. Resistance known. Risk in fungi unknown. Resistance management required.	31
A5 inhibition of dihydroorotate dehydrogenase within de novo pyrimidine biosynthesis	DHODH -fungicides	phenyl-propand	ipflufenquin	Medium to high risk.	52

https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2022--final.pdf?sfvrsn=b6024e9a_2

And in acknowledgment

Imperial

Jo Rhodes
Jennifer Shelton
Darius Armstrong
Ali Abdolrasouli
Silke Schelenz

St Georges

Tihana Bicanic
Tom Harrison
Matt Beale
Neil Stone

U of Tel Aviv

Judith Berman

NSW Health

Sharon Chen

Sydney Medical Schl.

Tania Sorrell

UCL

Francois Balloux
Adrien Rieux
Matteo Spagnoletti

USDA Forests

Dede Olson
Kathryn Ronnenberg

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