

Hot spot –cold spot

Azole resistance in *Aspergillus fumigatus*

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2012-2022@Wageningen University, The Netherlands---Azole resistance in *A. fumigatus*

2022-@National Institute for Health and Environment (RIVM), The Netherlands—Public health related fungal infection (Aspergillus and Candida)

Contents

1. What we know about the hotspot
2. What strategies can we apply to change the hotspots into a cold spot?

A Novel Environmental Azole Resistance Mutation in *Aspergillus fumigatus* and a Possible Role of Sexual Reproduction in Its Emergence

Authors: Jianhua Zhang, Eveline Snelders, Bas J. Zwaan, Sijmen E. Schoustra, Jacques F. Meis , Karin van Dijk, Ferry Hagen, [SHOW ALL \(13](#)

[AUTHORS\)](#) , [Alfons J. M. Debets](#) | [AUTHORS INFO & AFFILIATIONS](#)

DOI: <https://doi.org/10.1128/mBio.00791-17> •  Check for updates

Azole-containing



Hillegom

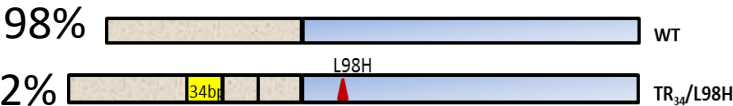
52° 17' 30" N,
4° 34' 46" E



Azole-free



Droeendaal ,
Wageningen
51° 58' 0" N,
5° 40' 0" E



Azole-containing compost is a hot spot for the development and maintenance of azole resistance

Environmental Hotspots of *A. fumigatus*

- **Flower bulb compost**
- **wood chippings**
- **Green waste**
 - Fruit waste (apples, pears, strawberries): “dipped”/sprayed fruits
 - Silage feed for cows
 - Manure/straw of horses
 - Wood waste
 - Grain storage
 - Manure/straw of chicken
 - Manure/straw of cows

Zhang, Jianhua, et al. "Dynamics of *Aspergillus fumigatus* in azole fungicide-containing plant waste in the Netherlands (2016–2017)." *Applied and environmental microbiology* 87.2 (2021): e02295-20.

RIVM report

Schoustra, S. E., Zhang, J., Zwaan, B. J., Debets, A. J. F., Verweij, P., Buijtenhuijs, D., & Rietveld, A. G. (2019). *New insights in the development of azole-resistance in *Aspergillus fumigatus** (No. 2018-0131). National Institute for Public Health and the Environment.

Schoustra, S. E., Debets, A. J., Rijs, A. J., Zhang, J., Snelders, E., Leendertse, P. C., ... & Verweij, P. E. (2019). Environmental hotspots for azole resistance selection of *Aspergillus fumigatus*, the Netherlands. *Emerging Infectious Diseases*, 25(7), 1347.



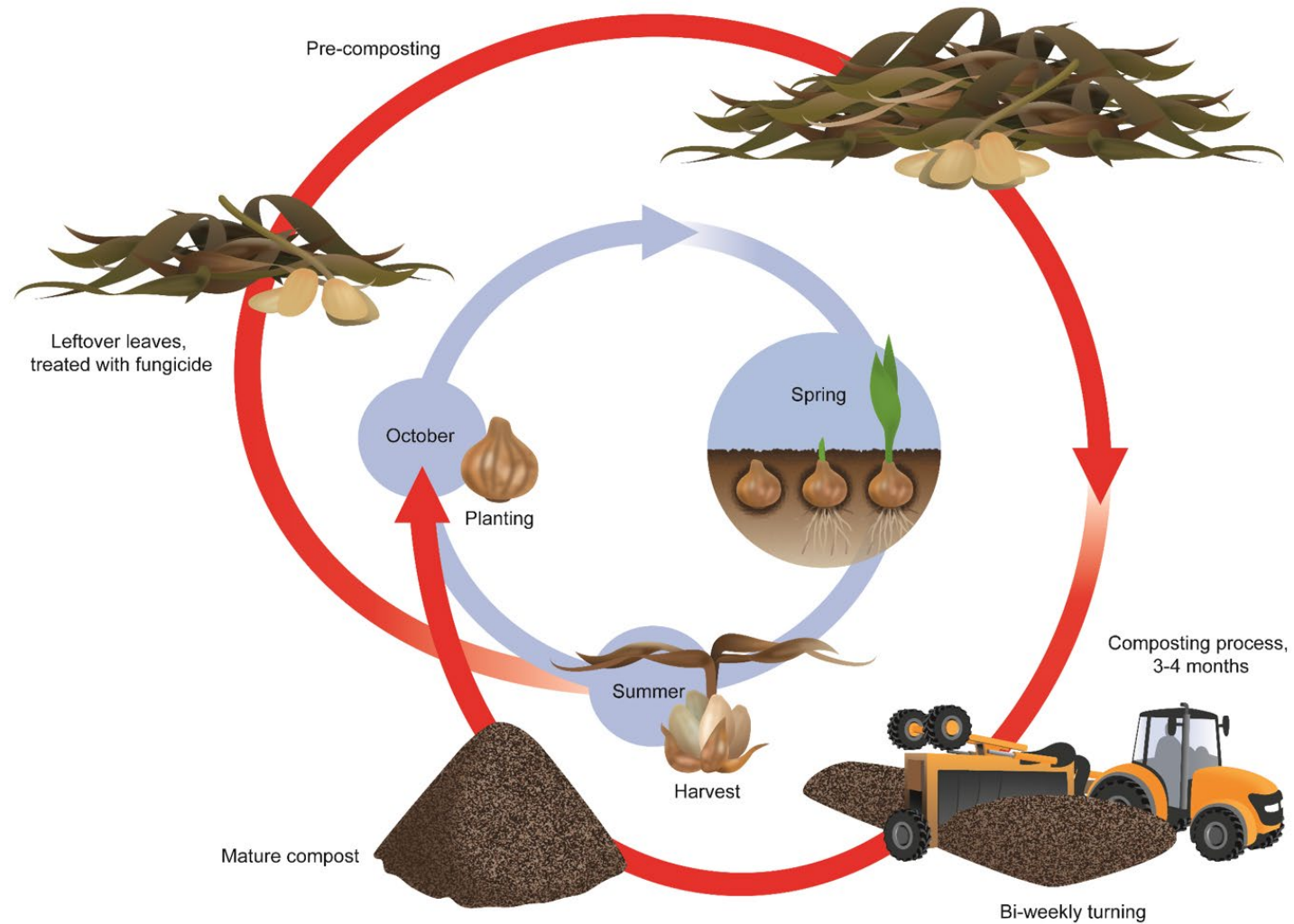
Dynamics of *Aspergillus fumigatus* in Azole Fungicide-Containing Plant Waste in the Netherlands (2016–2017)

Jianhua Zhang,^{Ma} Lidia Lopez Jimenez,^a Eveline Snelders,^a Alfons J. M. Debets,^a Anton G. Rietveld,^b Bas J. Zwaan,^a Paul E. Verweij,^{b,c,d} and Sijmen E. Schoustra^a

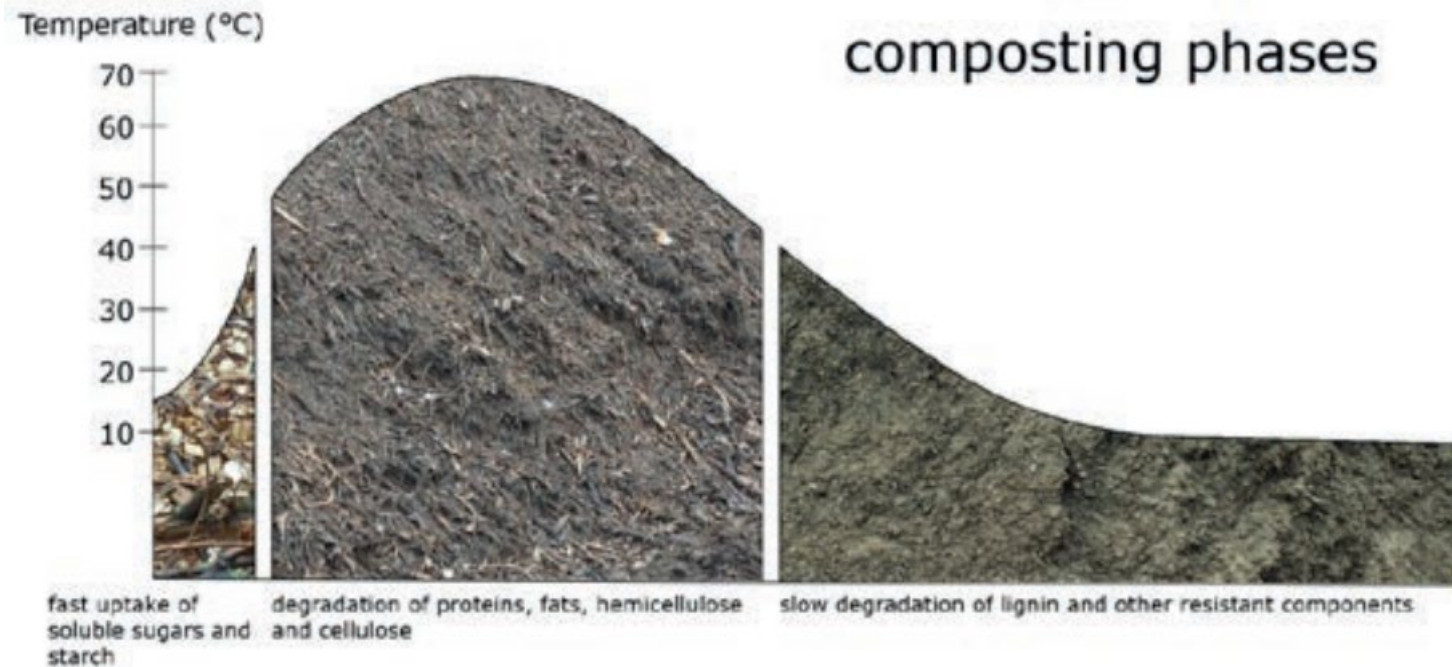
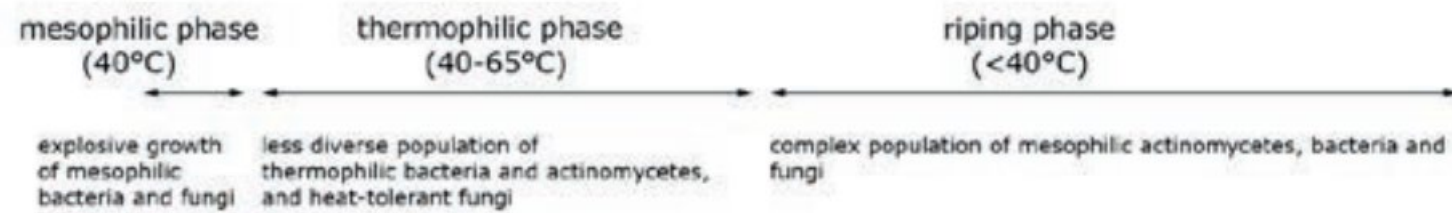
A



B







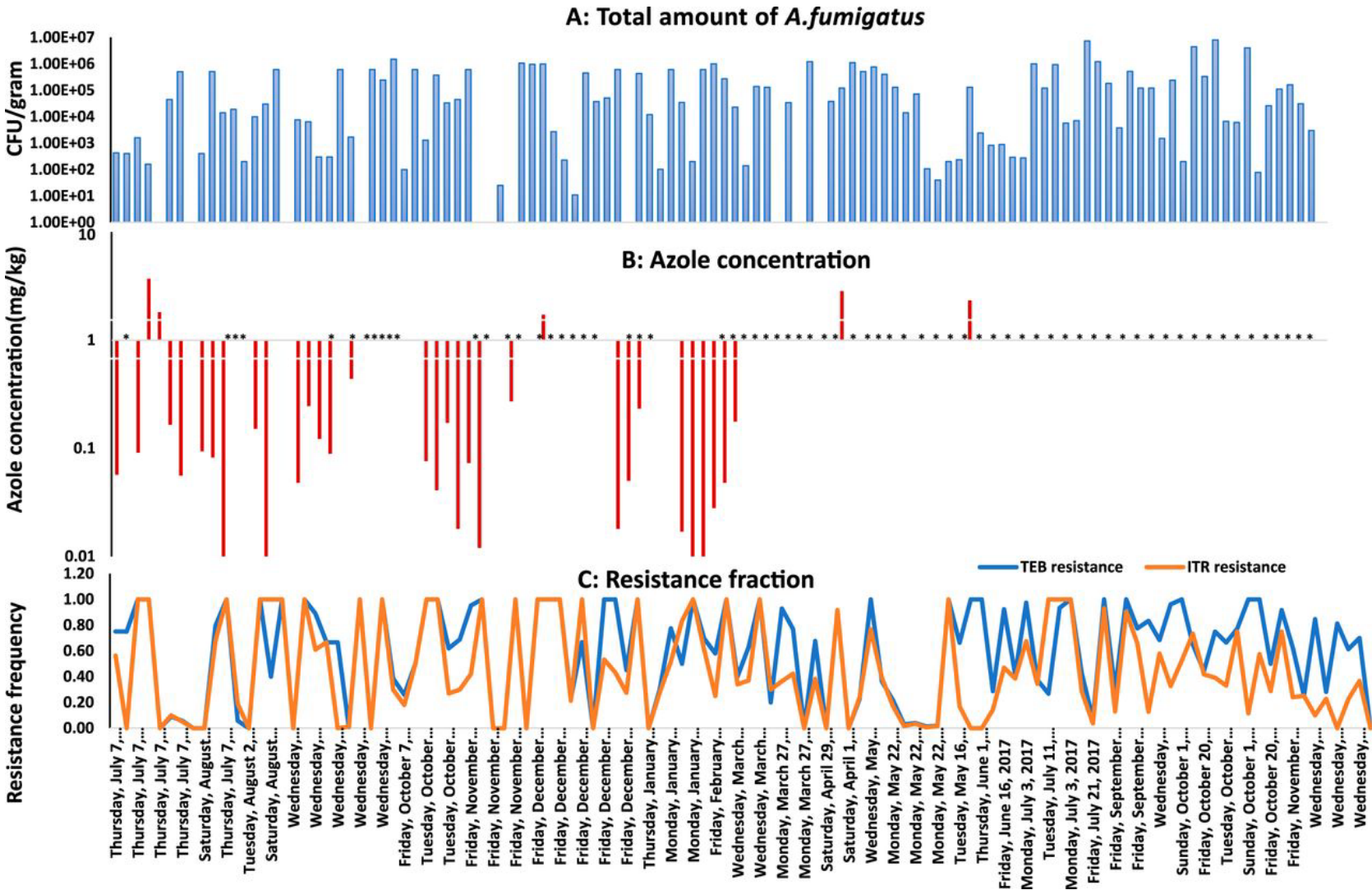
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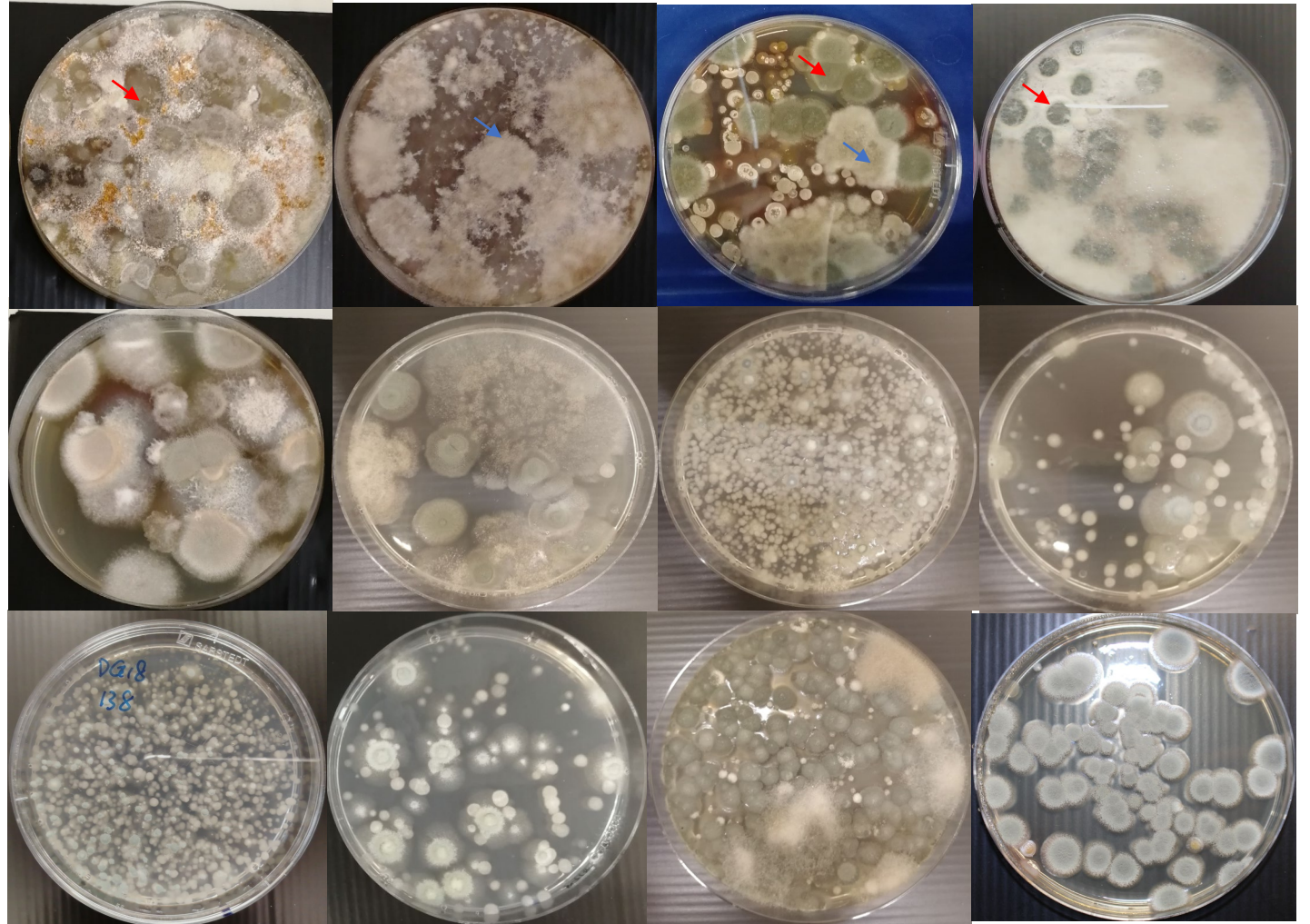
High number of *A. fumigatus*

All contain fungicides

High fraction of resistance



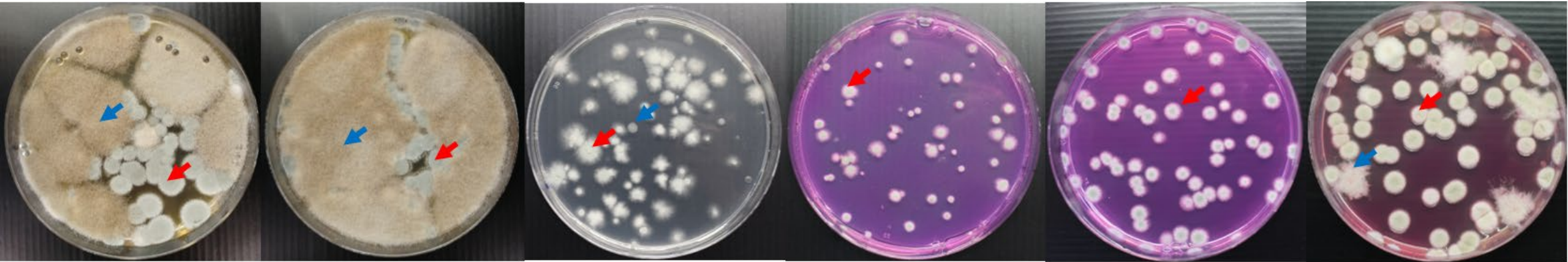
Difficulties to culture *Aspergillus fumigatus* from compost samples



Selective Flamingo Medium for the Isolation of *Aspergillus fumigatus*

Jianhua Zhang ¹, Alfons J M Debets ¹, Paul E Verweij ^{2 3 4}, Sijmen E Schoustra ¹

Affiliations + expand
PMID: 34072240 PMCID: PMC8228204 DOI: 10.3390/microorganisms9061155
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MEA+C+S

SDA+C

DG18

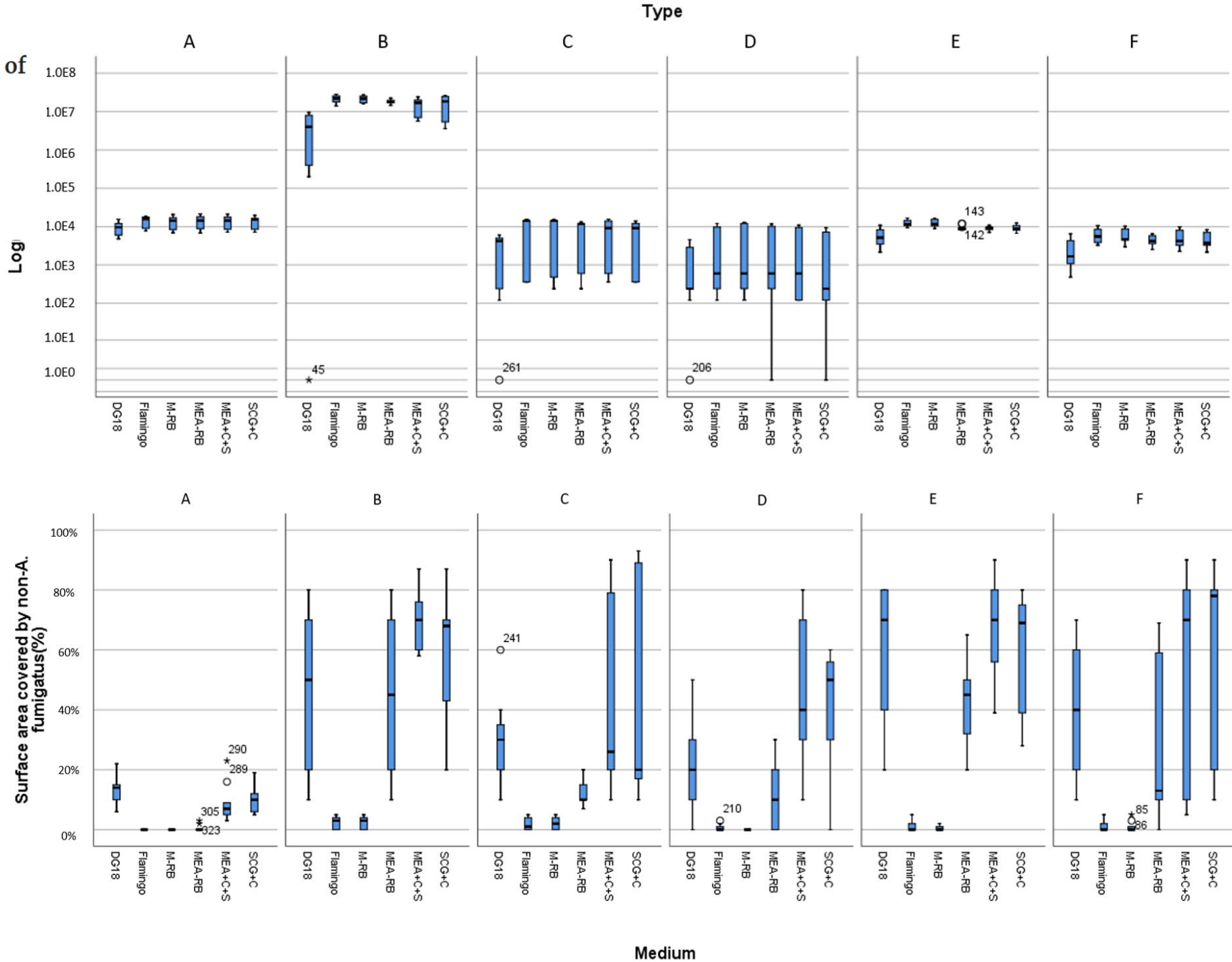
M-RB

Flamingo

MEA-RB

Free PMC article

- A: Air;
- B: Plant waste/compost;
- C: Ditchwater;
- D: Grass/root;
- E: Soil;
- F: Wood



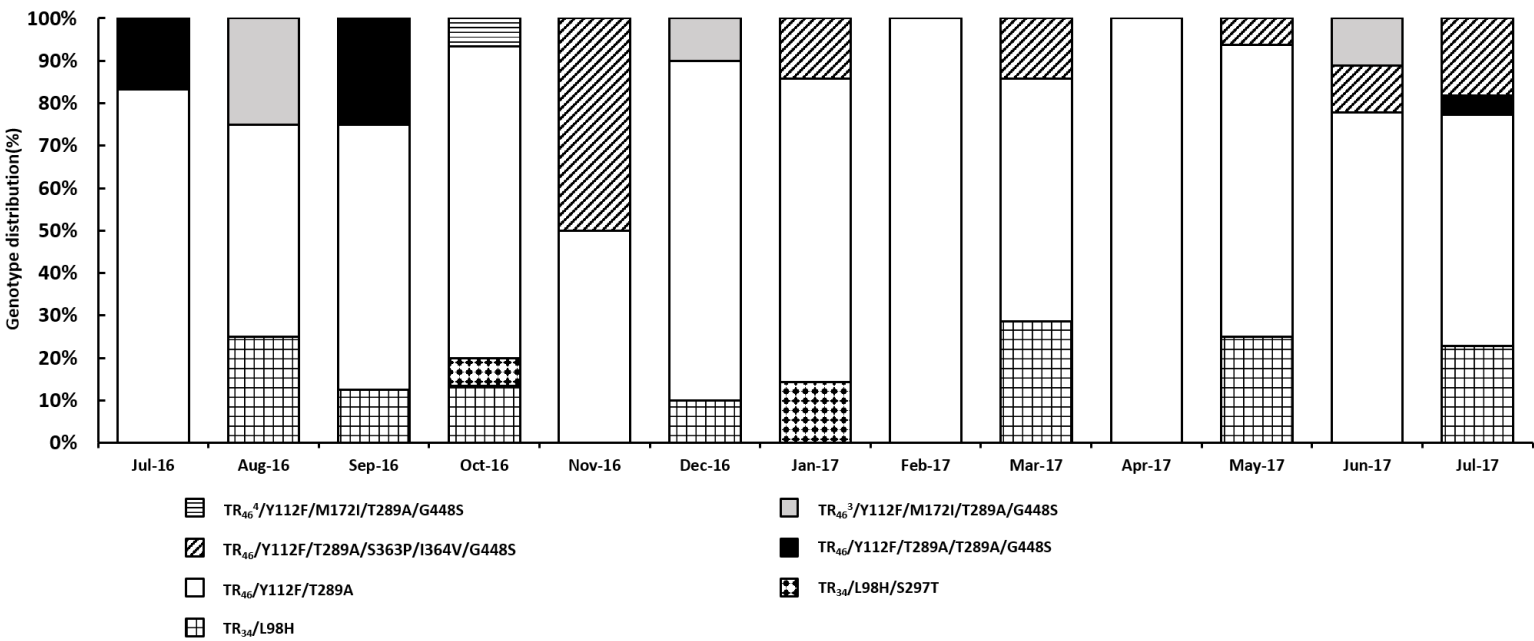
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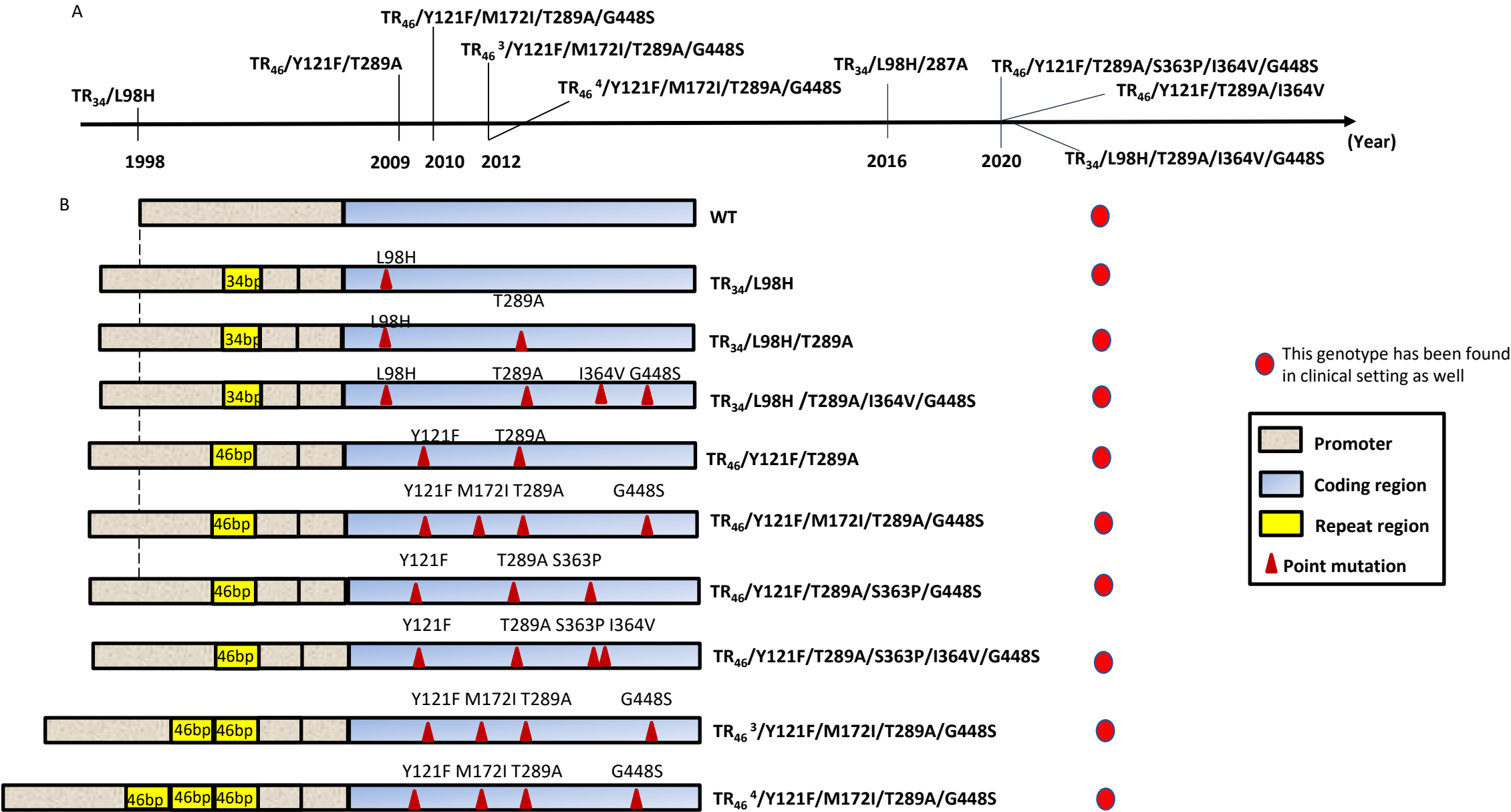
Table 1. Genetic analysis of the *cyp51A* promotor region and structural gene of 117 representative

azole resistant *A. fumigatus* isolates per sampling site (A, B or C).

Tandem repeat (promoter region)	Point mutation(s) (coding gene)	A	B	C	Total
TR ₃₄	L98H	7	2	8	17
TR ₃₄	L98H/S297T	1	1	0	2
TR ₄₆	Y121F/T289A	24	23	33	80
TR ₄₆	Y121F/M172I/T289A/G448S	2	0	2	4
TR ₄₆	Y121F/T289A/S363P/I364V/G448S	1	8	0	9
TR ₄₆ ³	Y121F/M172I/T289A/G448S	0	2	2	4
TR ₄₆ ⁴	Y121F/M172I/T289A/G448S	0	1	0	1
					117



Evolution of resistance on azole targeted gene cyp51A from the heap



Flower Bulb Waste Material is a Natural Niche for the Sexual Cycle in *Aspergillus fumigatus*

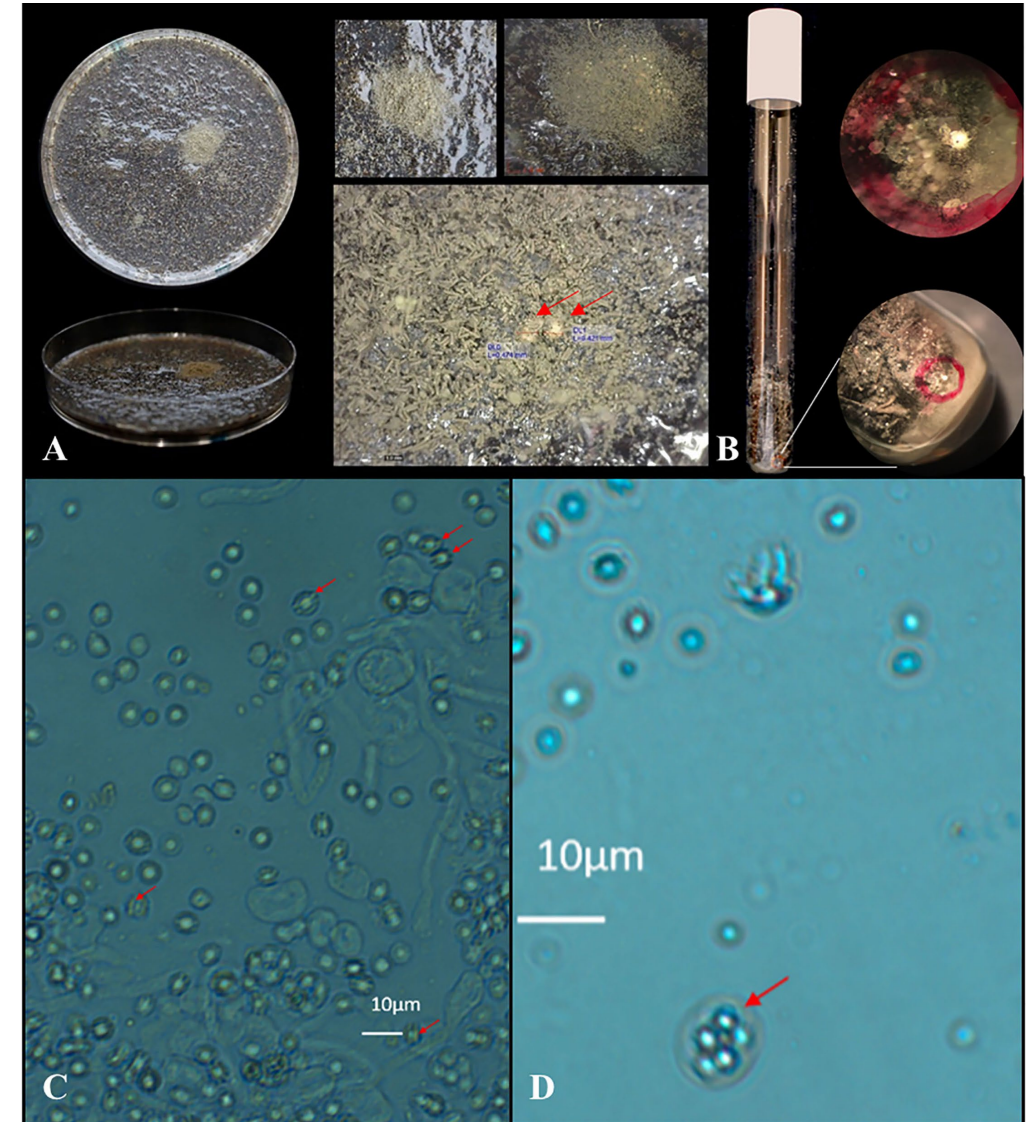
Jianhua Zhang¹, Paul E Verweij^{2 3}, Antonius J M M Rijs^{2 3}, Alfons J M Debets¹,
Eveline Snelders¹

Affiliations + expand

PMID: 35145921 PMCID: PMC8823264 DOI: 10.3389/fcimb.2021.785157


So why are flower bulb waste stockpiles beneficial for *A. fumigatus* to undergo sexual reproduction?

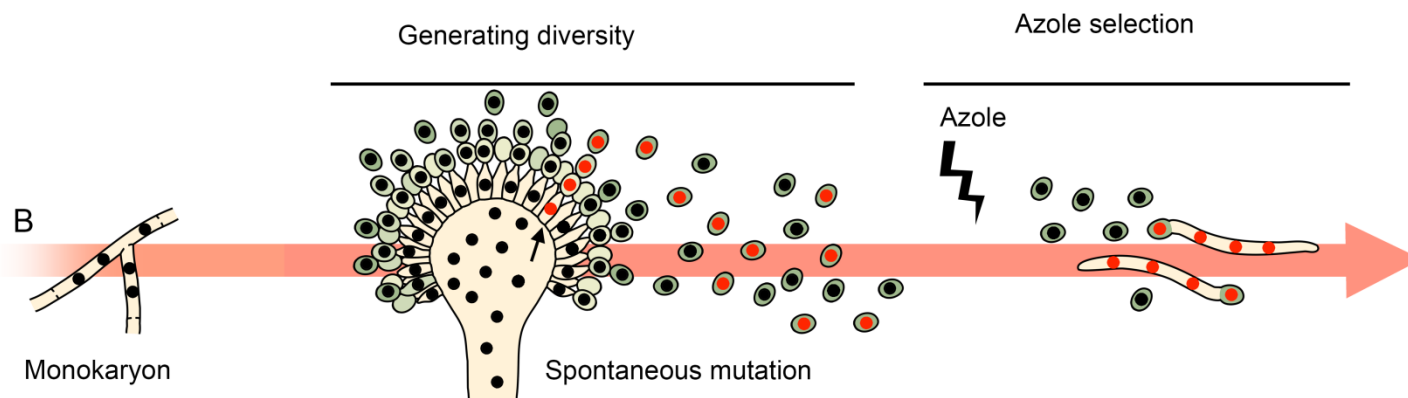
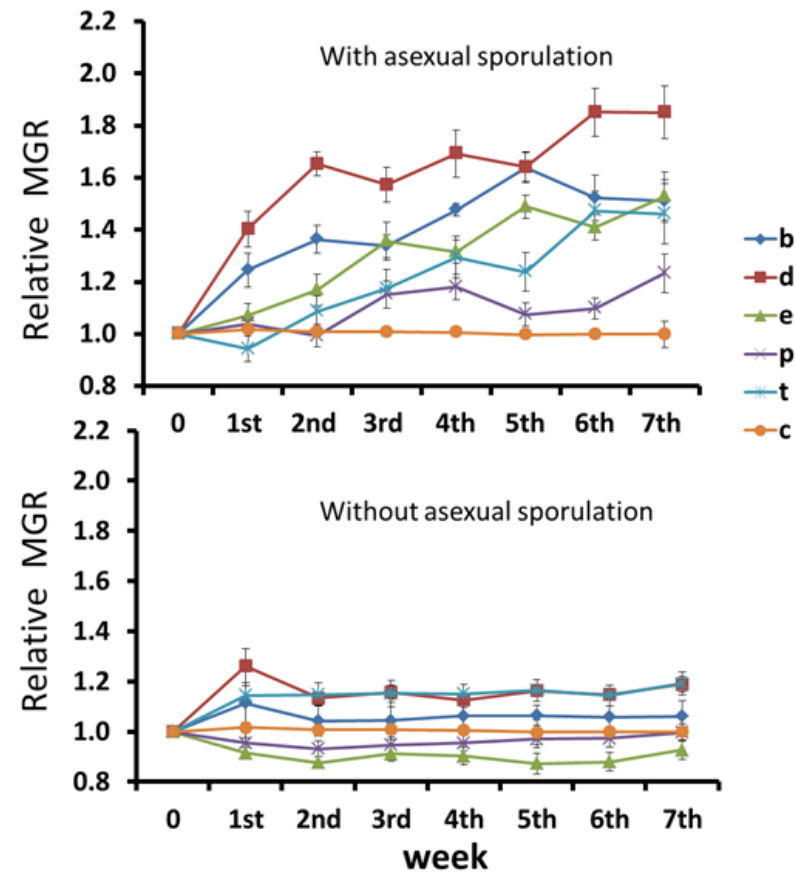
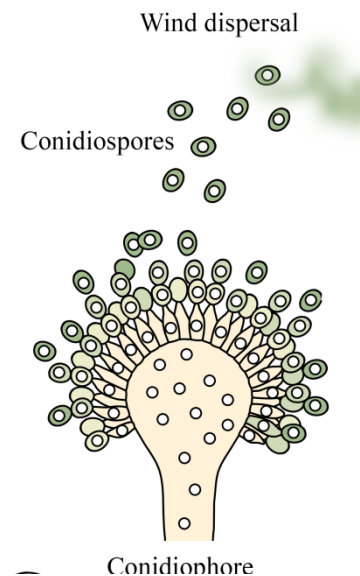
- (1) flower bulb waste provides favorable conditions for sex; nutrition, dark, 30 degree, and different maters.
- (2) Sex could provide benefits for the survival of *A. fumigatus* in the flower bulb waste stockpiles.
 - Sexual ascospores can be resting spores, which can help undergo extreme conditions.
 - Sex can generate the diversity genotypes which can survive different conditions.



ORIGINAL ARTICLE | [Full Access](#)

Asexual sporulation facilitates adaptation: The emergence of azole resistance in *Aspergillus fumigatus*

Jianhua Zhang , Alfons J. M. Debets, Paul E. Verweij, Willem J. G. Melchers, Bas J Zwaan, Sijmen E. Schoustra



Azole-Resistance Development; How the *Aspergillus fumigatus* Lifecycle Defines the Potential for Adaptation

by  Jianhua Zhang¹ ,  Alfons J. M. Debets¹ ,  Paul E. Verweij²  and  Eveline Snelders^{1,*} 

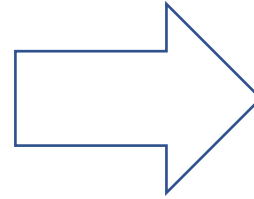


1 gram ~100000 (10^5) *A. fumigatus* spores

A single stockpile of 50*50*10-meter dimension, ~25000000000000000 ($2.5*10^{15}$) spores

Resistant spores: ~12500000000000000 ($1.25*10^{15}$) spores

Various genotypes of *A. fumigatus* as the results of asexual and sexual reproduction and azole selection



~1970 in Europa

Nowadays in the developing countries

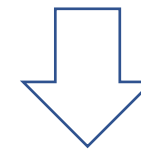
Burn all plant waste



More CO₂ Climate
change/Global warming

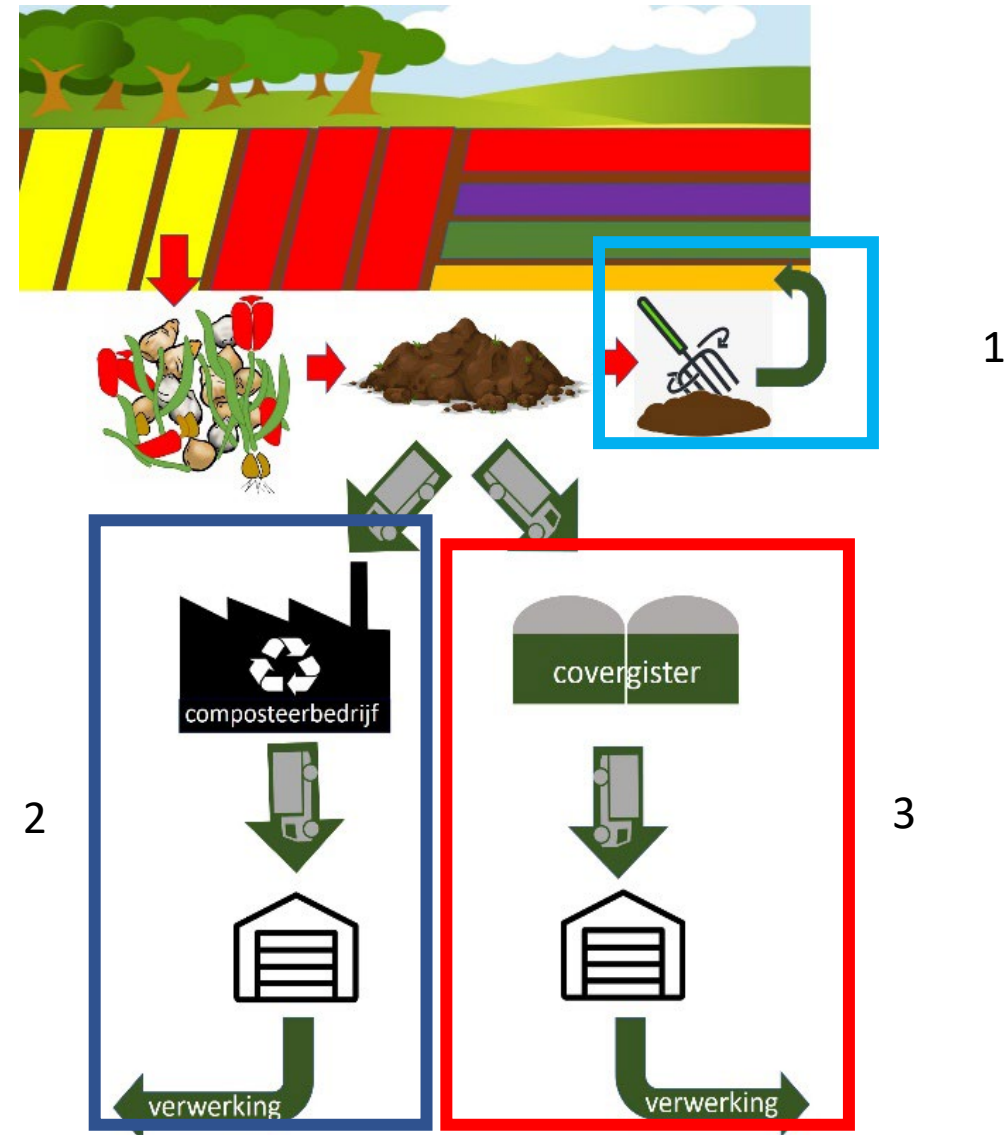
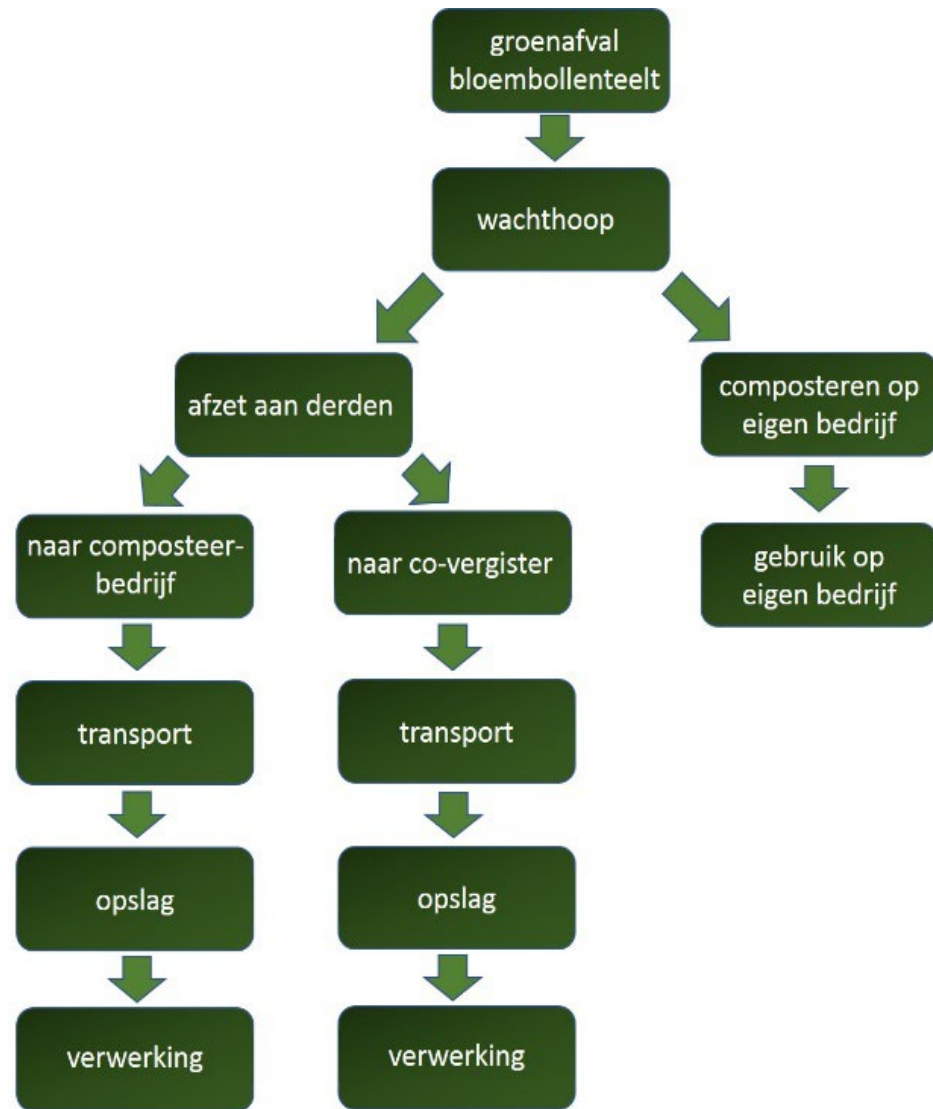
Sustainable development

Recycle ----circularity

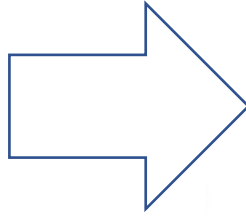
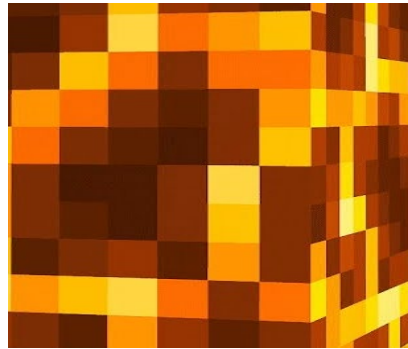


Risk

Dutch waste stream



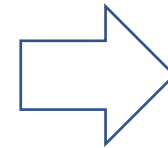
Strategies to change hotspot into cold spot ----ongoing research



Strategies

Under the laboratory /controlled conditions

- ❖ Reducing the total number of *A. fumigatus*
- ❖ Remove the selective pressure by azoles

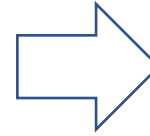


Validation in the farming
system to see whether these
strategies are effective or
not

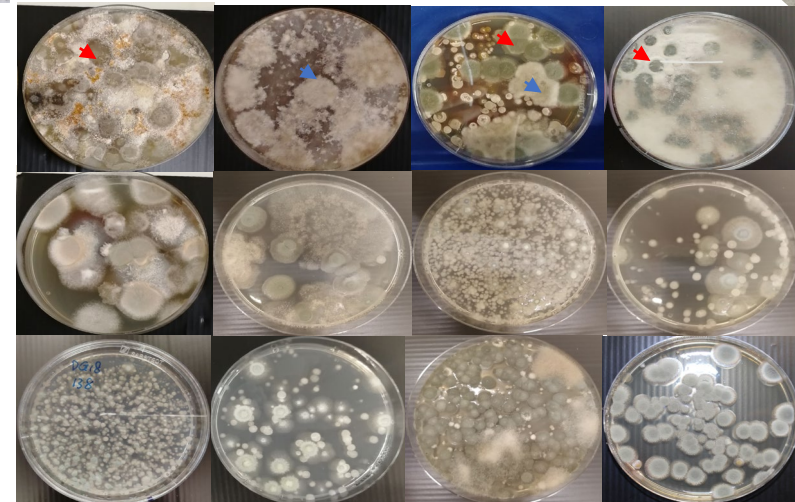
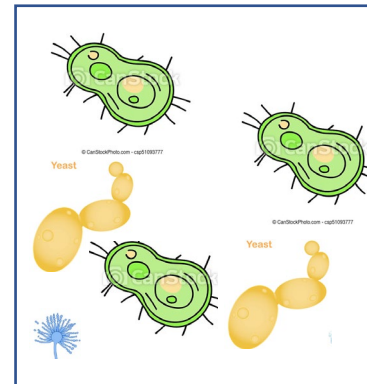
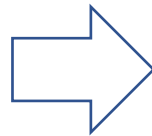
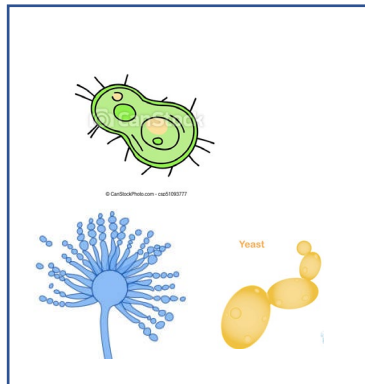
Strategy 1

❖ Reducing the total number of *A. fumigatus*

- Stop/reduce *A. fumigatus* propagating or germinating



- Adjust the ratio of *A. fumigatus* in compost



Strategy 2

❖ Remove the fungicides pressure and selection

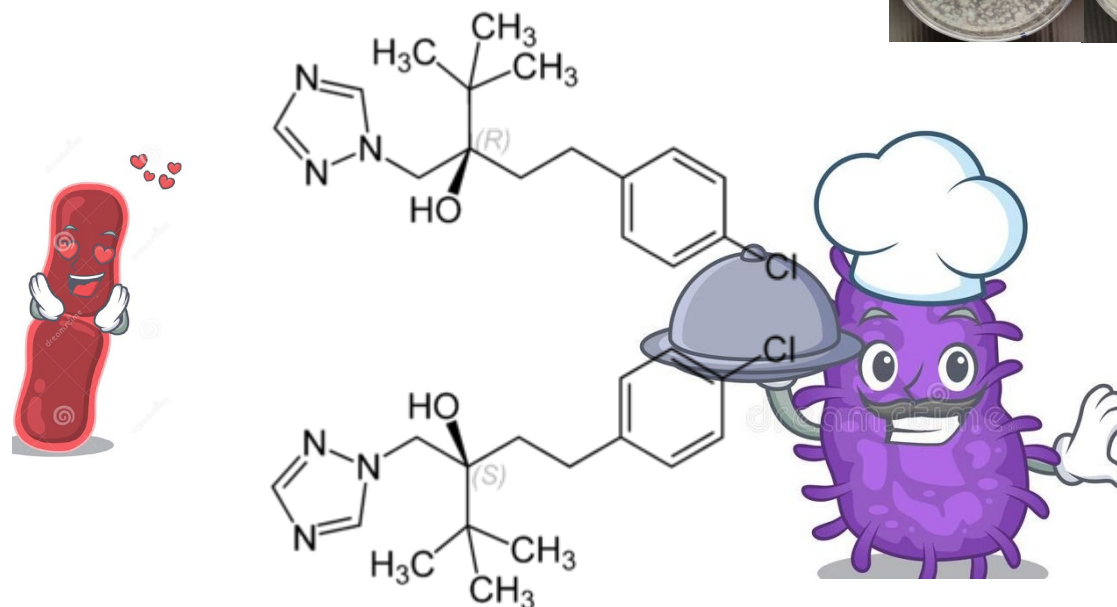
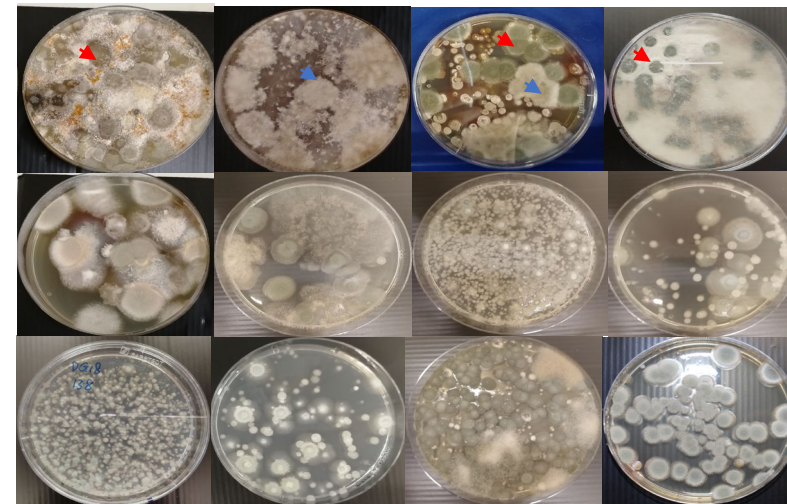
- Search for the fungicides degrading microorganisms

- *Bacillus, Pseudomonas, Klebsiella, Stenotrophomonas, Arthrobacter, Rhodanobacter, Cupriavidus, and Aphanoascus*

effectively utilize pesticide residues as a carbon and nitrogen source.

Biodegradation of the fungicide propiconazole by *Pseudomonas aeruginosa* PS-4 strain isolated from a paddy soil

Praveen Satapute & Basanna Kaliwal



Take home message

- ❖ We know something, but we still don't know what factor drives the emergence of TR genotype
- ❖ We are working on finding effective strategies to cope with azole resistance in *Aspergillus fumigatus*
- ❖ A long way to go ---combat antifungal resistance – together, we can do it

ACKLOWADGEMENT



Tenure track



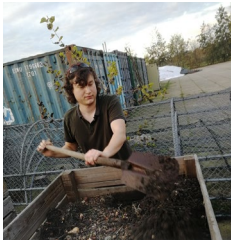
Zwaan Bas



Sijmen Schoustra



Snelders Eveline



Hylke Kortenbosch



Bram de Rooj



Fons Debets



Derks Gerard



Lippe John



Rijksinstituut voor Volksgezondheid
en Milieu
Ministerie van Volksgezondheid,
Welzijn en Sport



Radboudumc
university medical center



Other parties



Ministerie van Landbouw,
Natuur en Voedselkwaliteit



Rijkswaterstaat
Ministerie van Infrastructuur en Waterstaat

