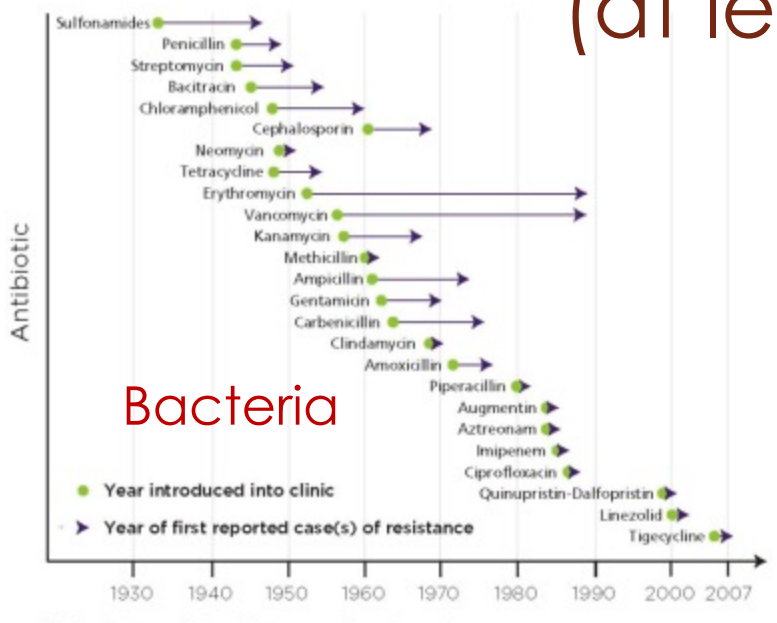
# NWO Groen III – One-Health Aspects of circularity

Monday the 27th of June 2022

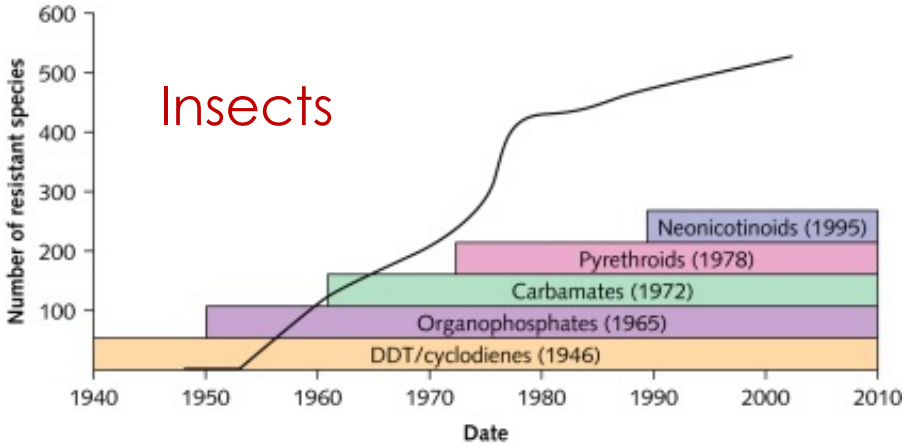
Laboratory of Genetics, Wageningen University & Research
Radboud University Medical Center, Nijmegen
+ PARTNERS!




# Chemical resistance selection; it will happen (at least with current practices)




Bacteria




Insects

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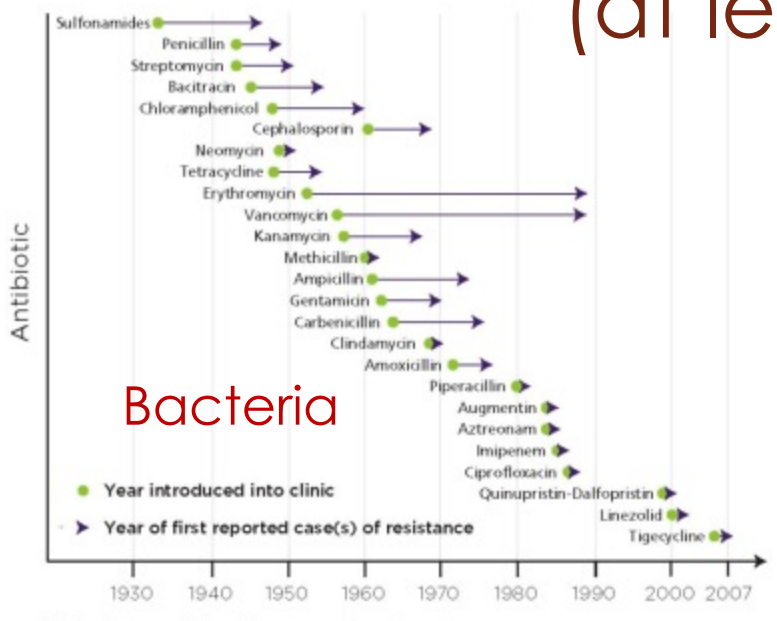


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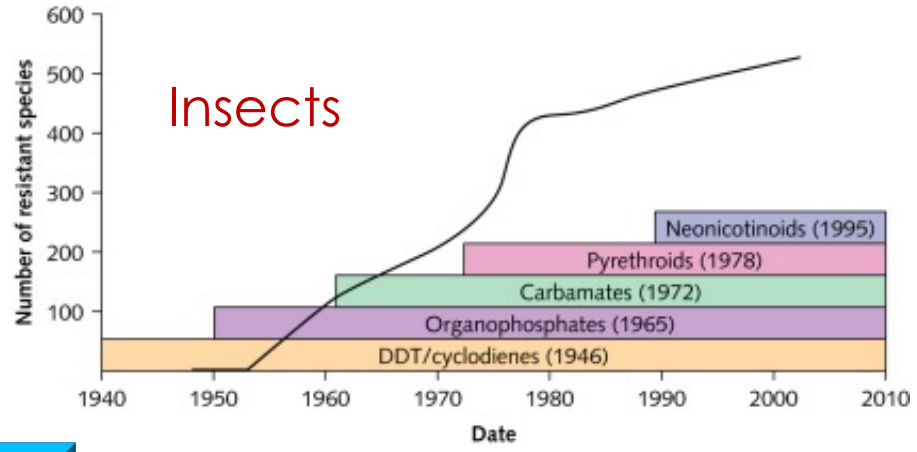
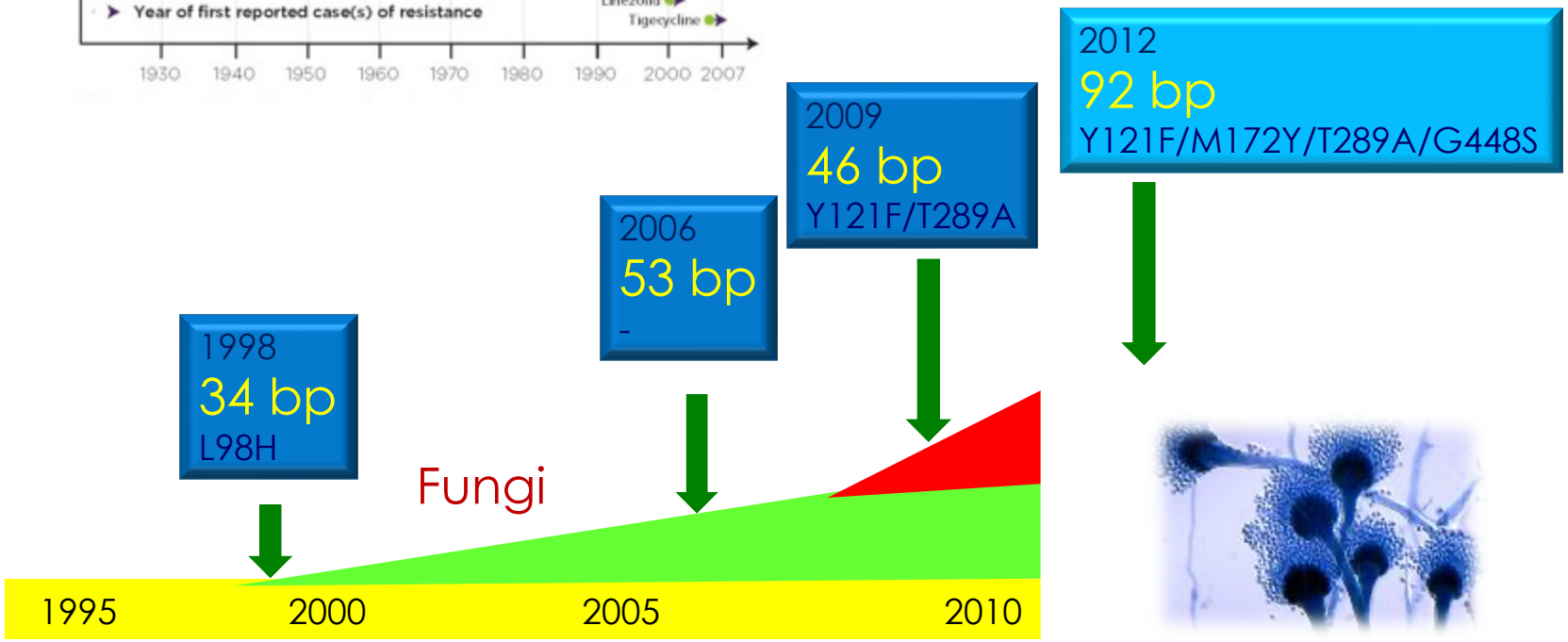



Insects

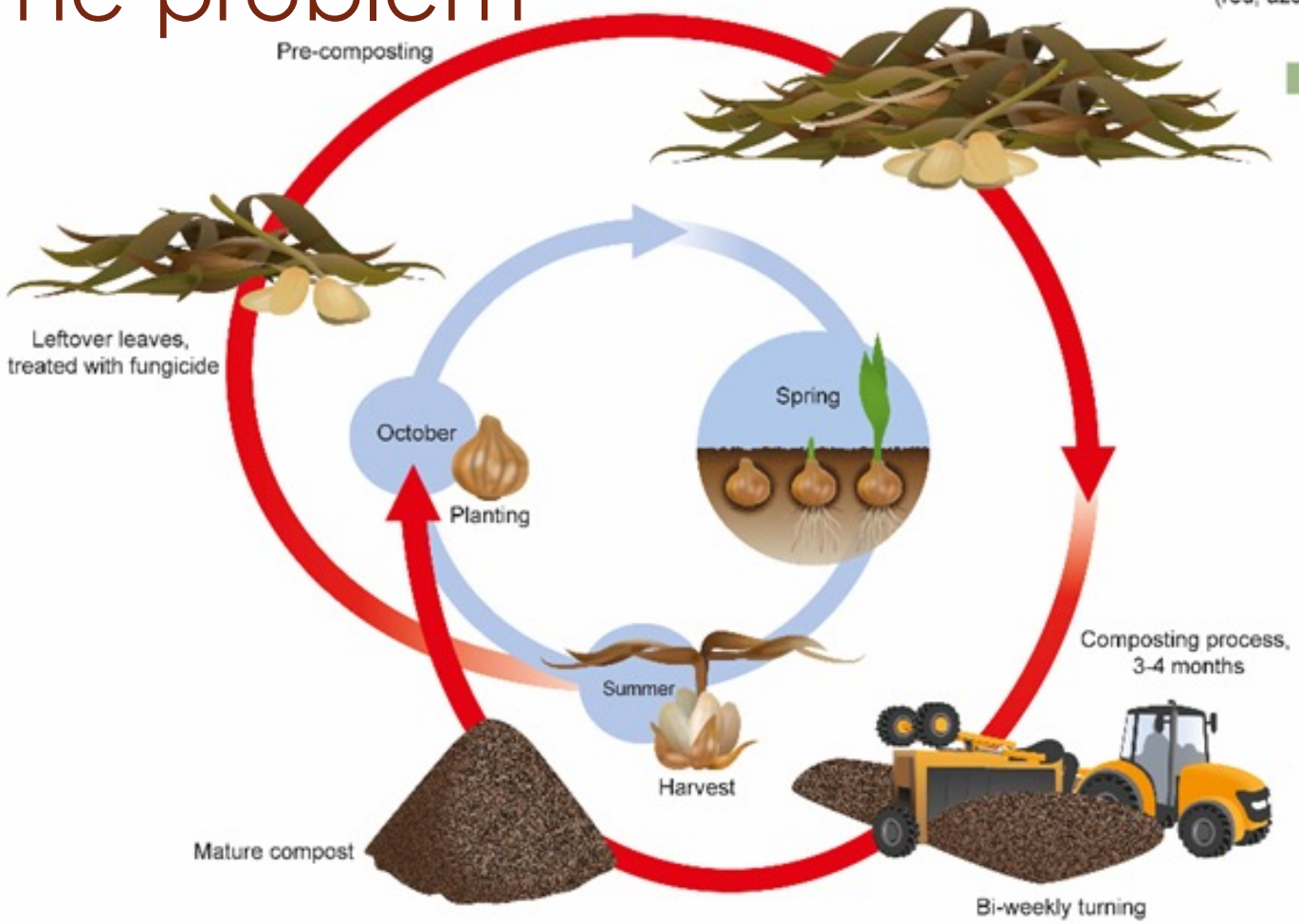


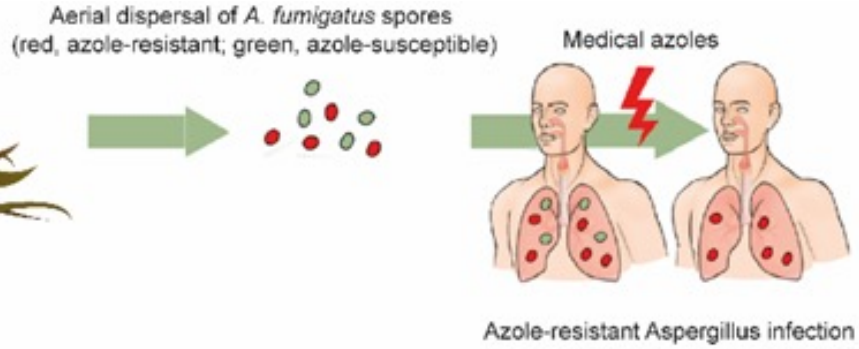



*Aspergillus fumigatus*

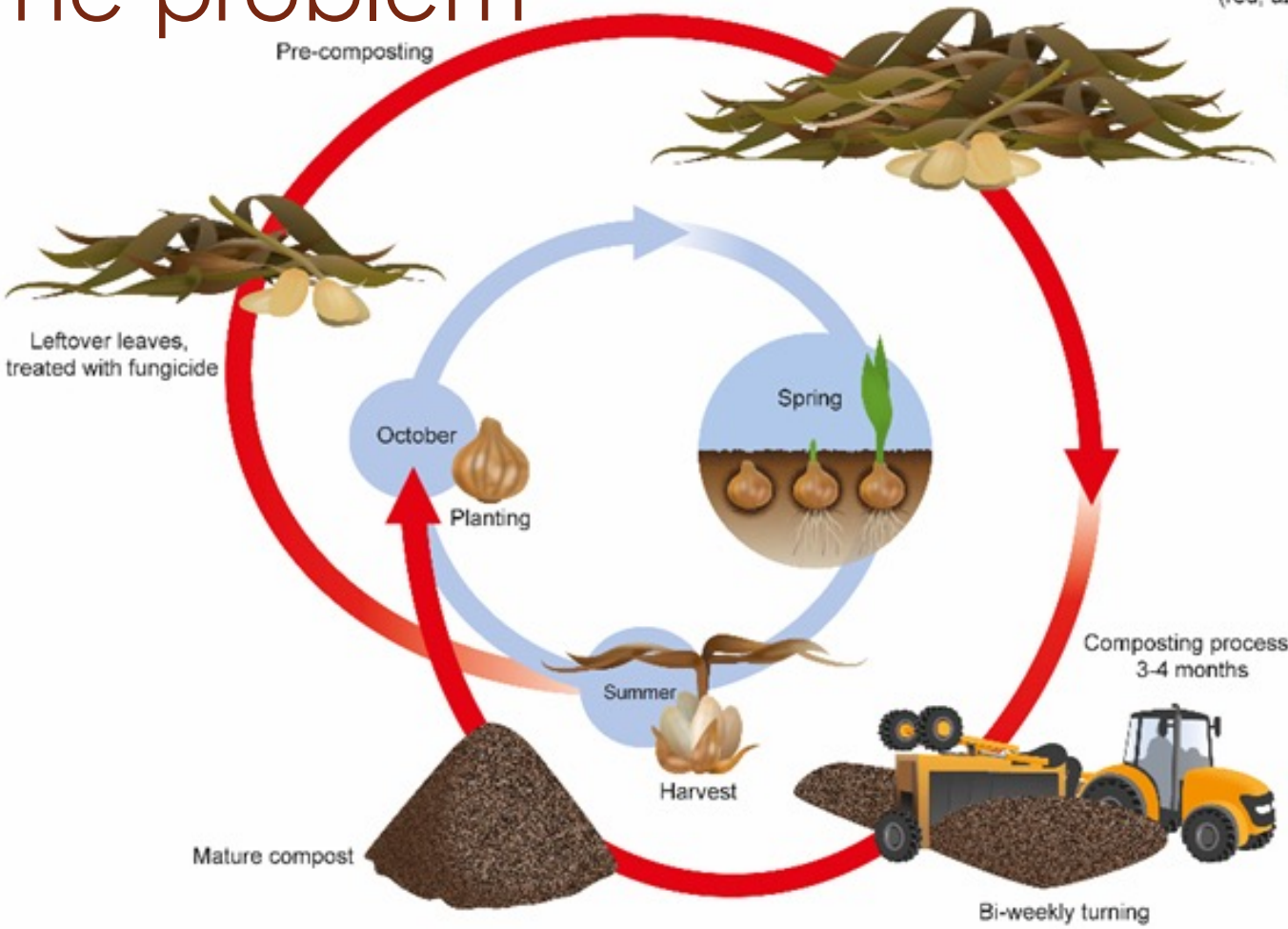
Resistance of the fungus *Aspergillus fumigatus* against fungicide of the azole-class

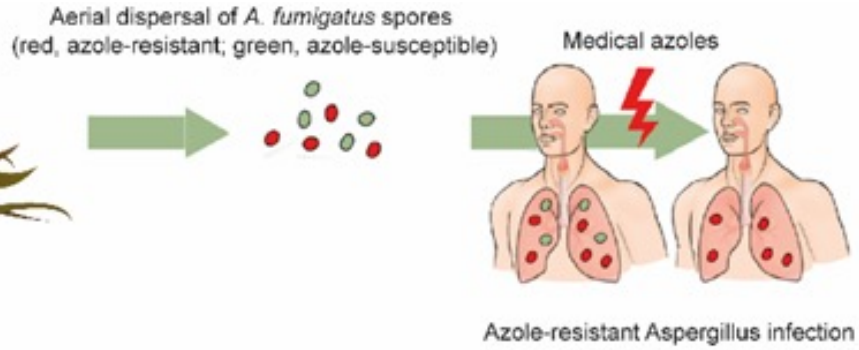
# The problem


Pre-composting
Leftover leaves,
treated with fungicide
October
Planting
Spring
Summer
Harvest
Mature compost
Composting process,
3-4 months
Bi-weekly turning


Aerial dispersal of A. fumigatus spores
(red, azole-resistant; green, azole-susceptible)
Medical azoles
Azole-resistant Aspergillus infection

# The problem




*(i)* <u>*Azoles*</u> are used against plant pathogens

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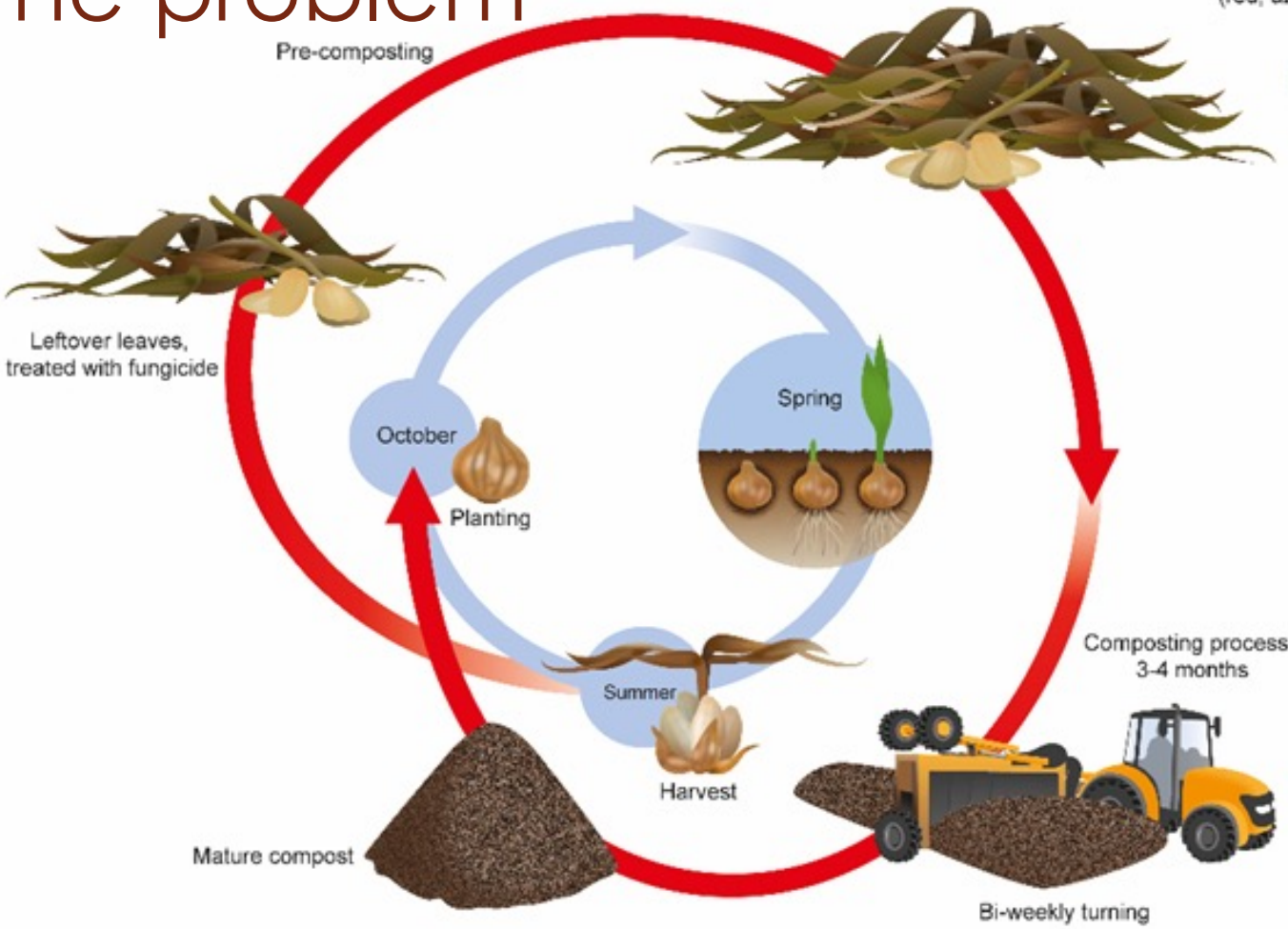


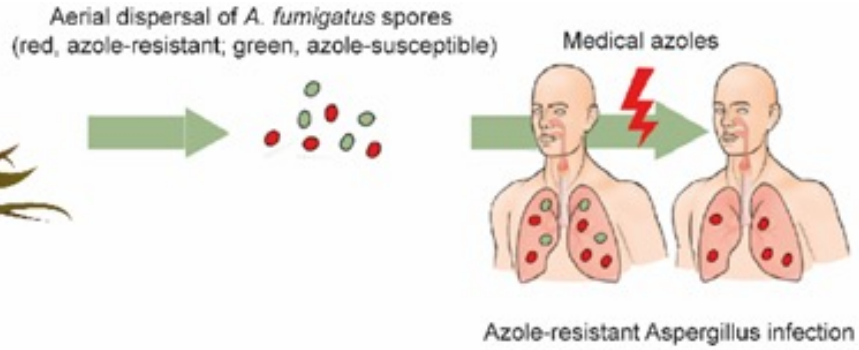


*(i)* <u>*Azoles*</u> are used against plant pathogens

*(ii)* <u>*Azoles*</u> accumulate in organic waste

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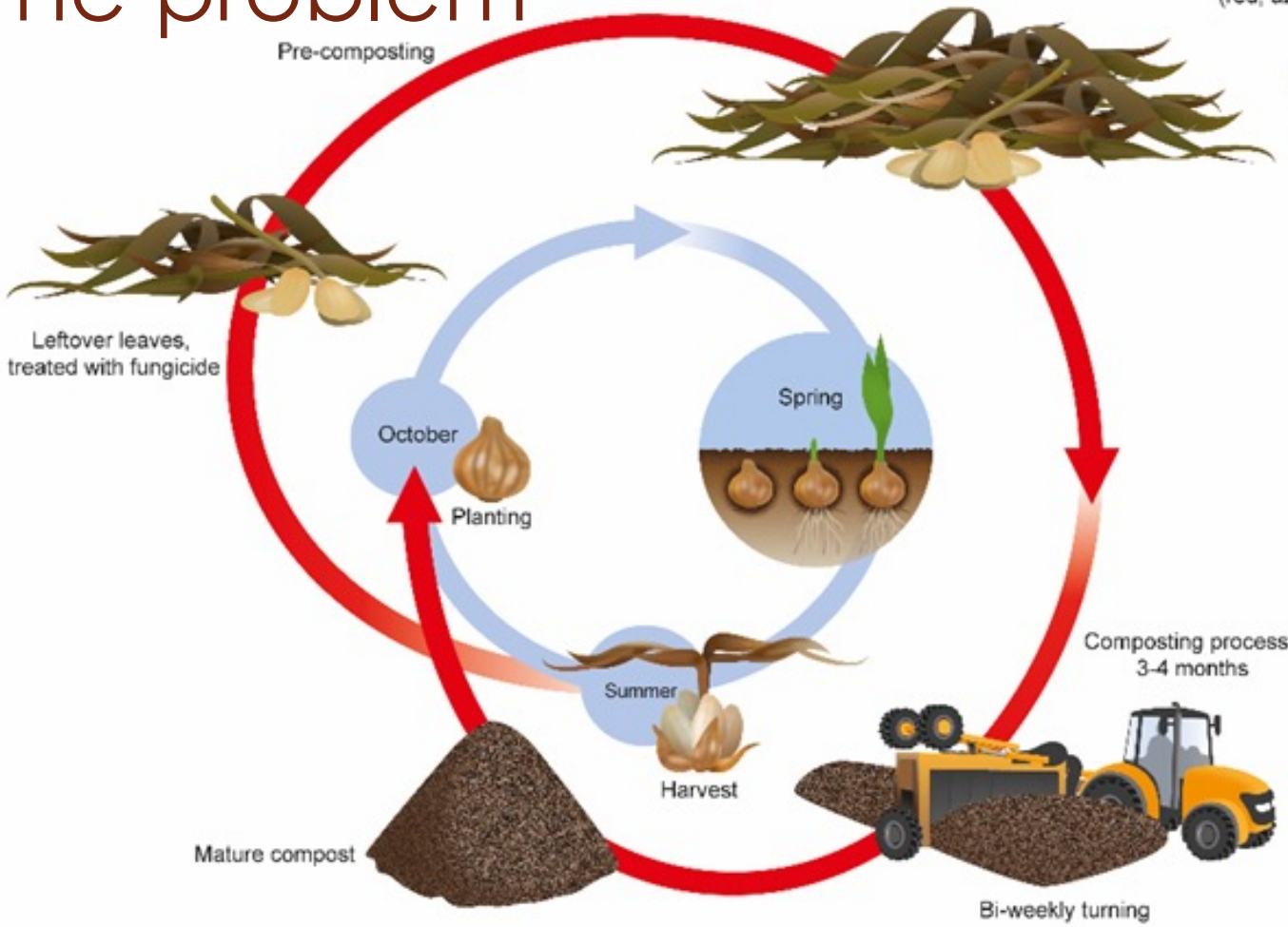


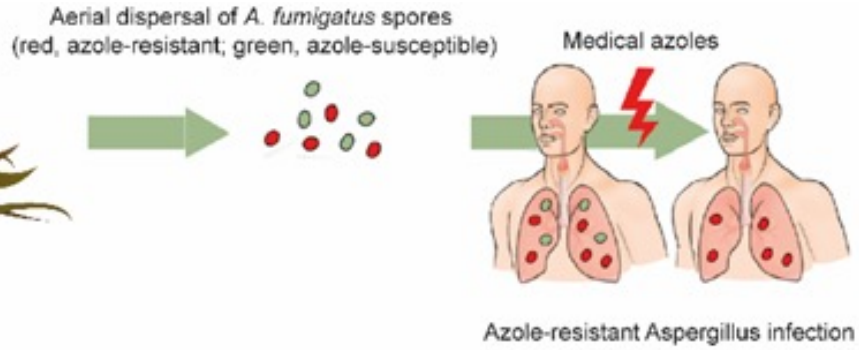


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*(iii)* <u>*Aspergillus fumigatus*</u> is key for composting but to grow acquires resistance to azoles

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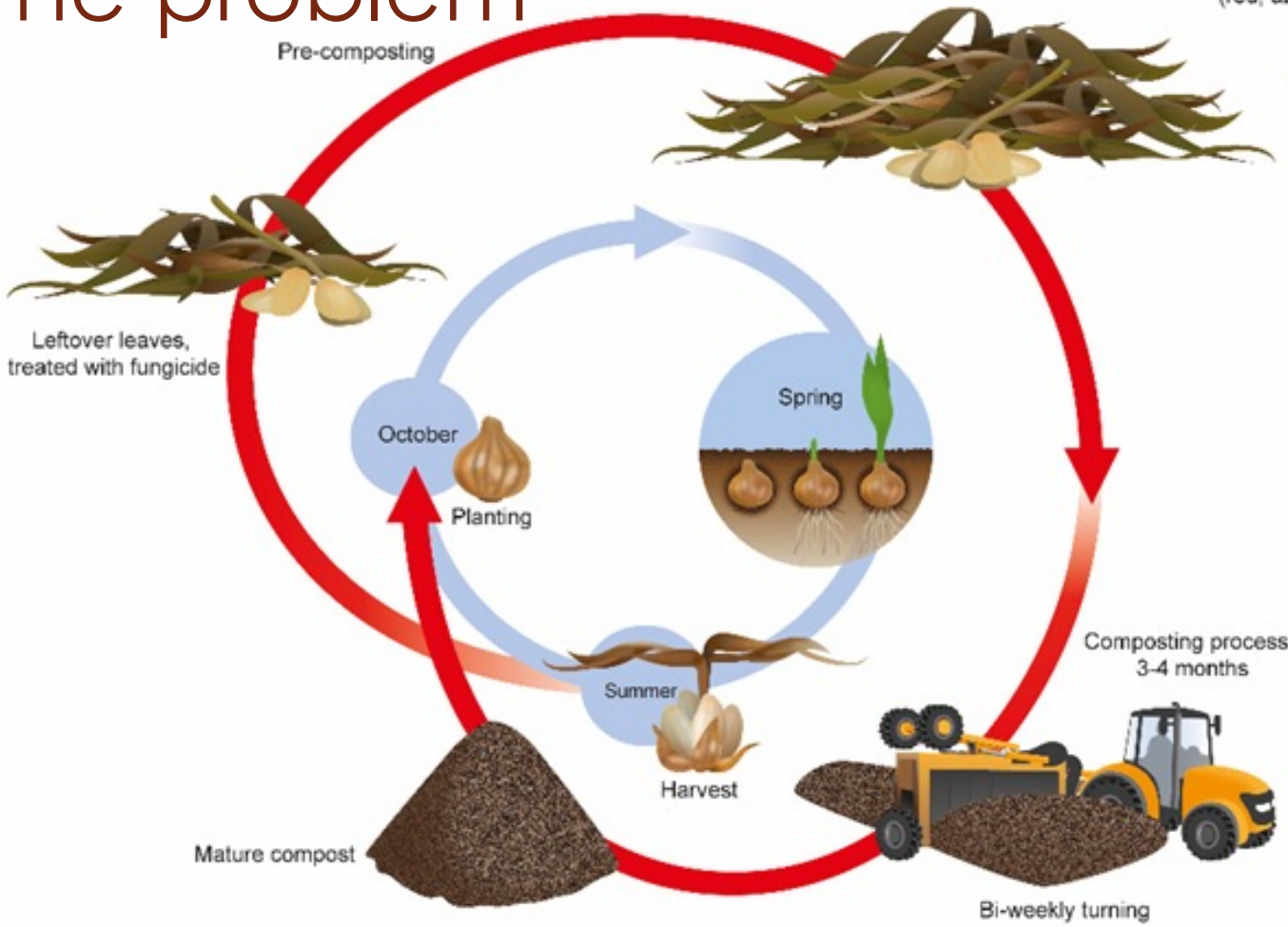


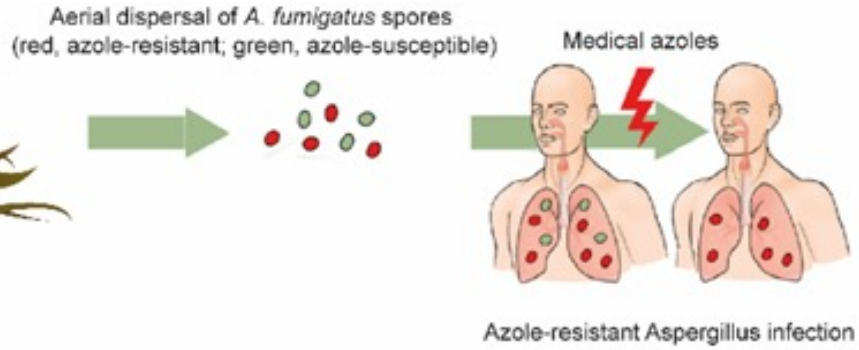


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(ii) Azoles accumulate in organic waste

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(iv) *A. fumigatus* is a super spore-spreader which are inhaled by humans

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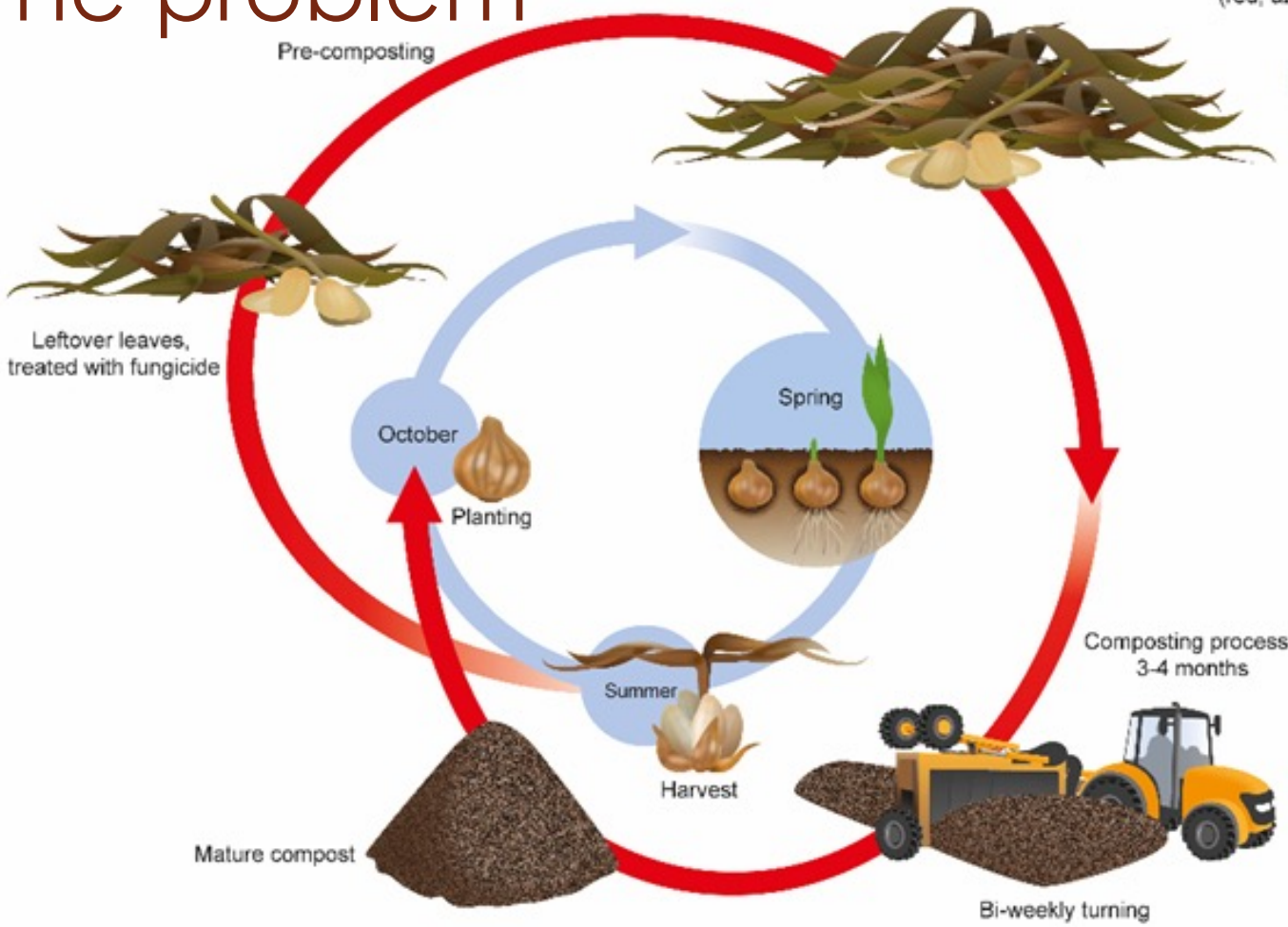


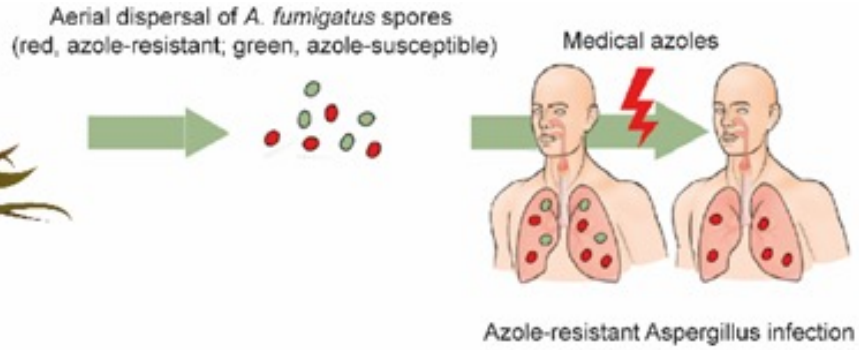


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(v) *A. fumigatus* can be a human pathogen especially in immunocompromised patients

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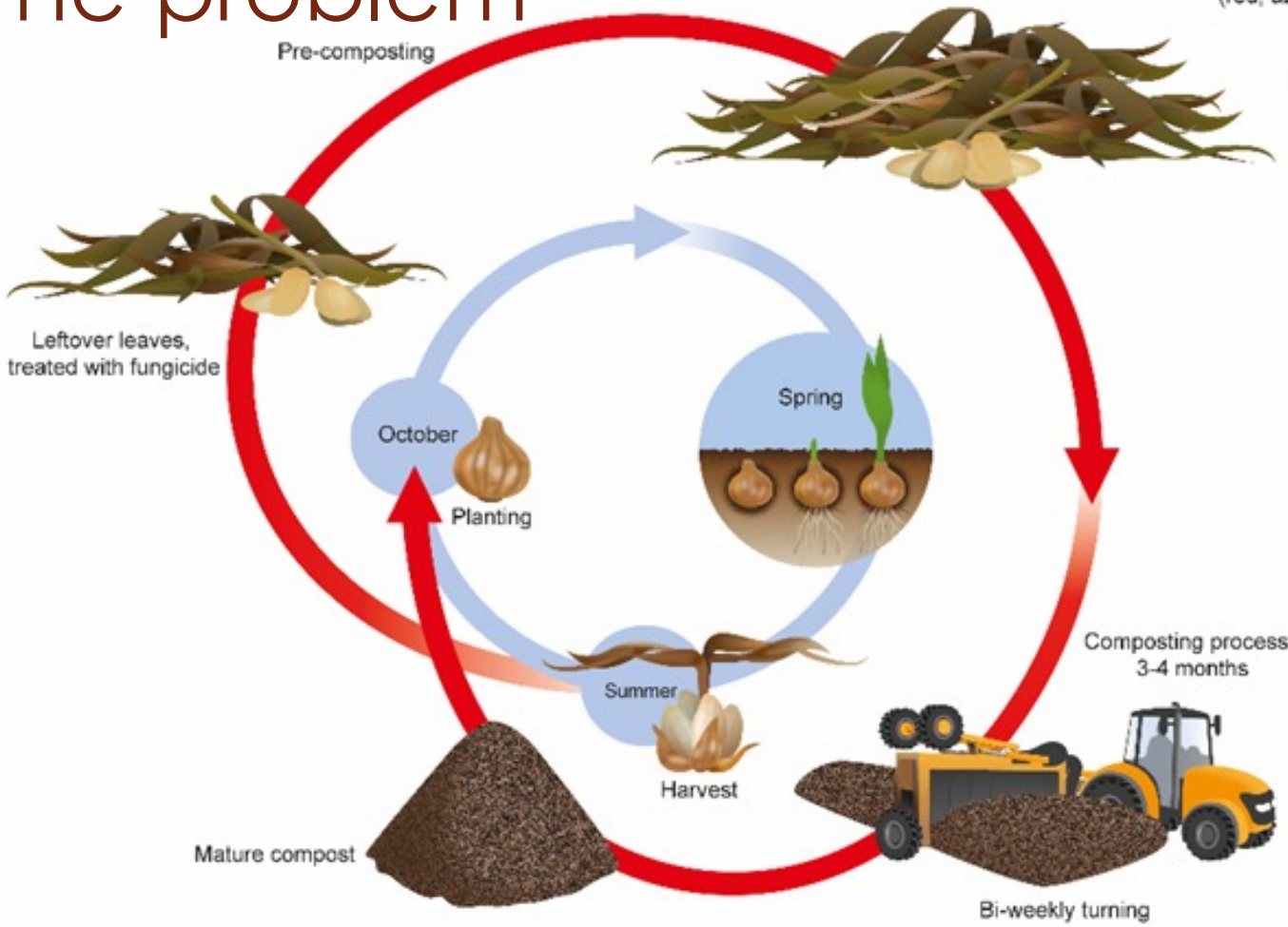


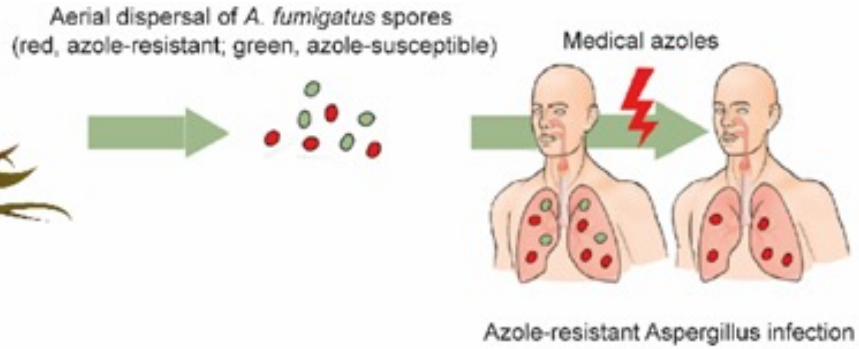


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(vi) <u>*Treatment*</u> with medical azoles is hampered because of azole resistance acquired in the environment

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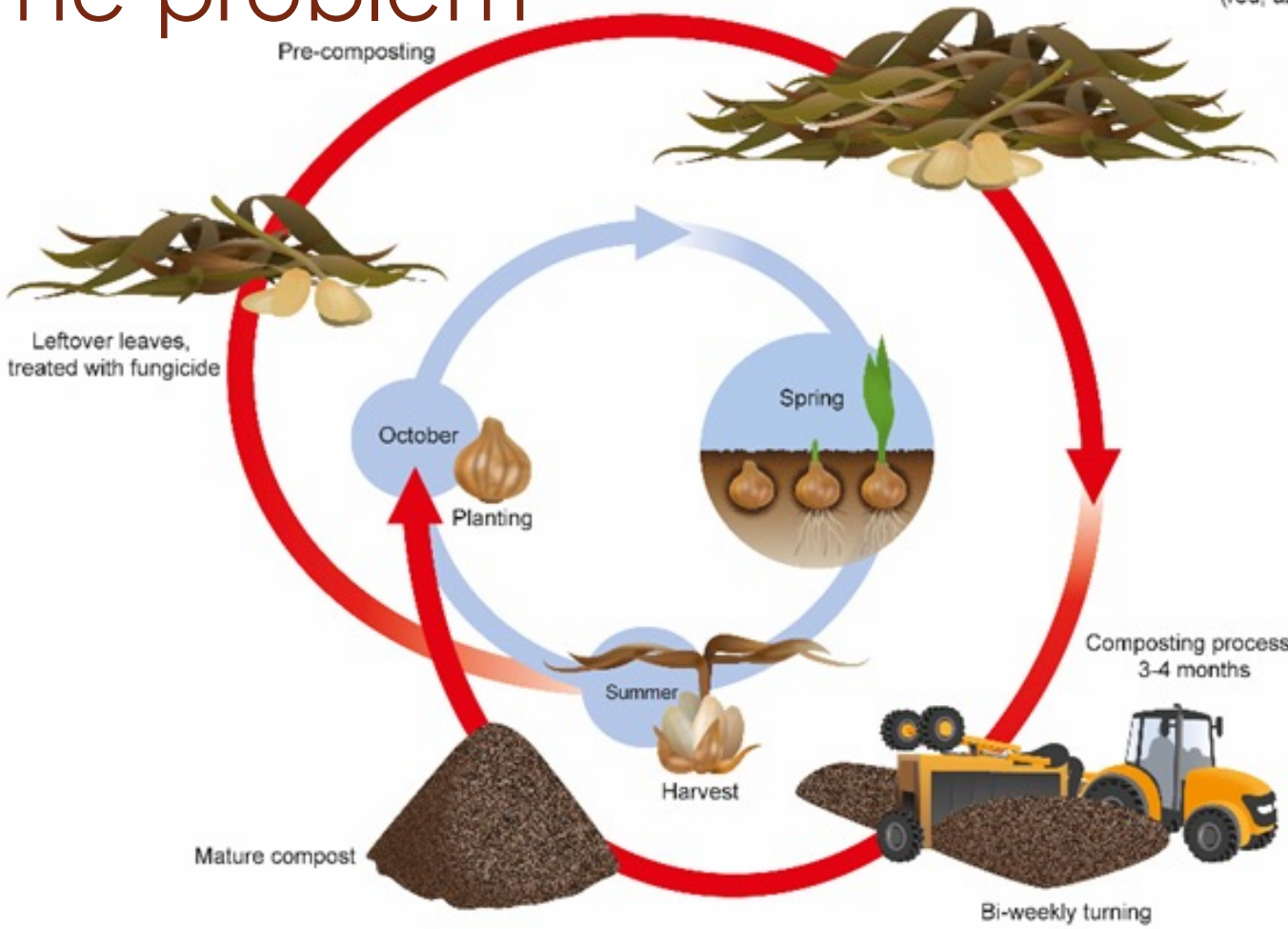


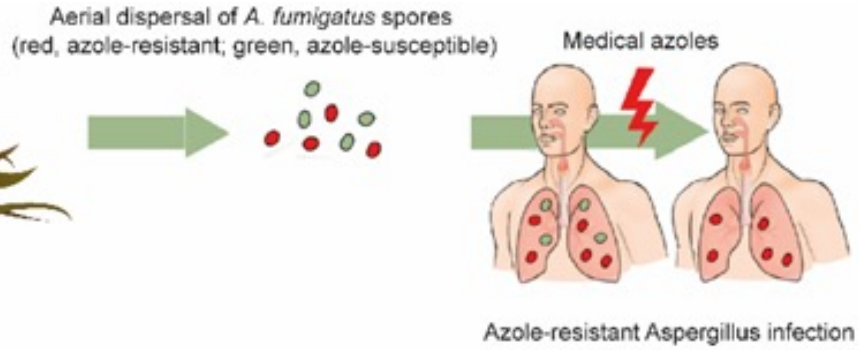


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**When rest-streams, microbes, and chemicals meet**

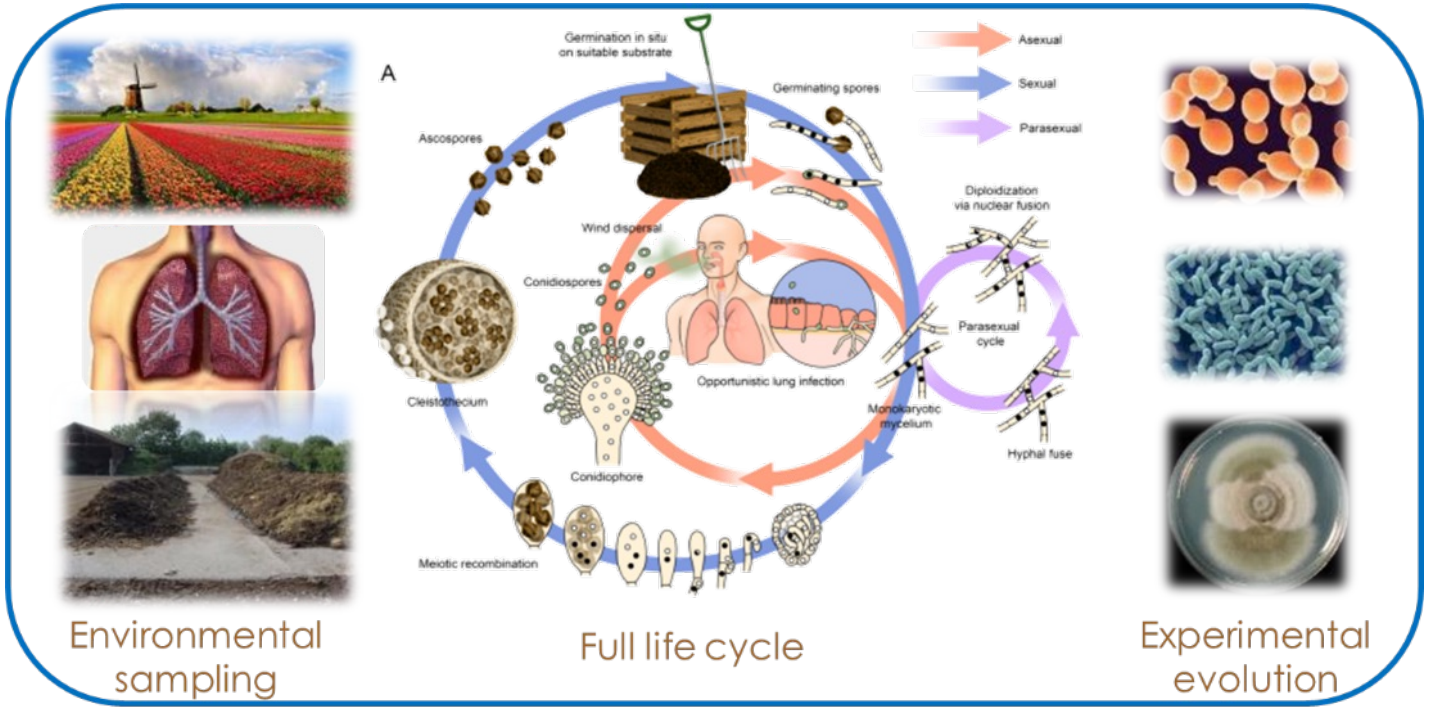
**a potential (One-)health problem emerges**

# Disentangling AMR evolution

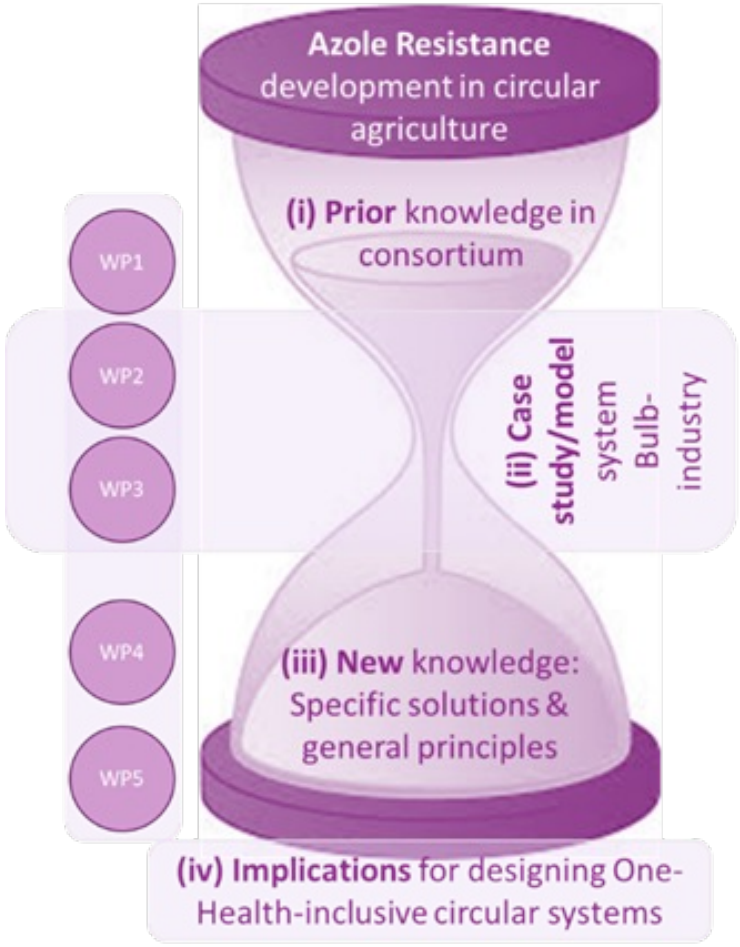
## Separating the communalities from the specifics

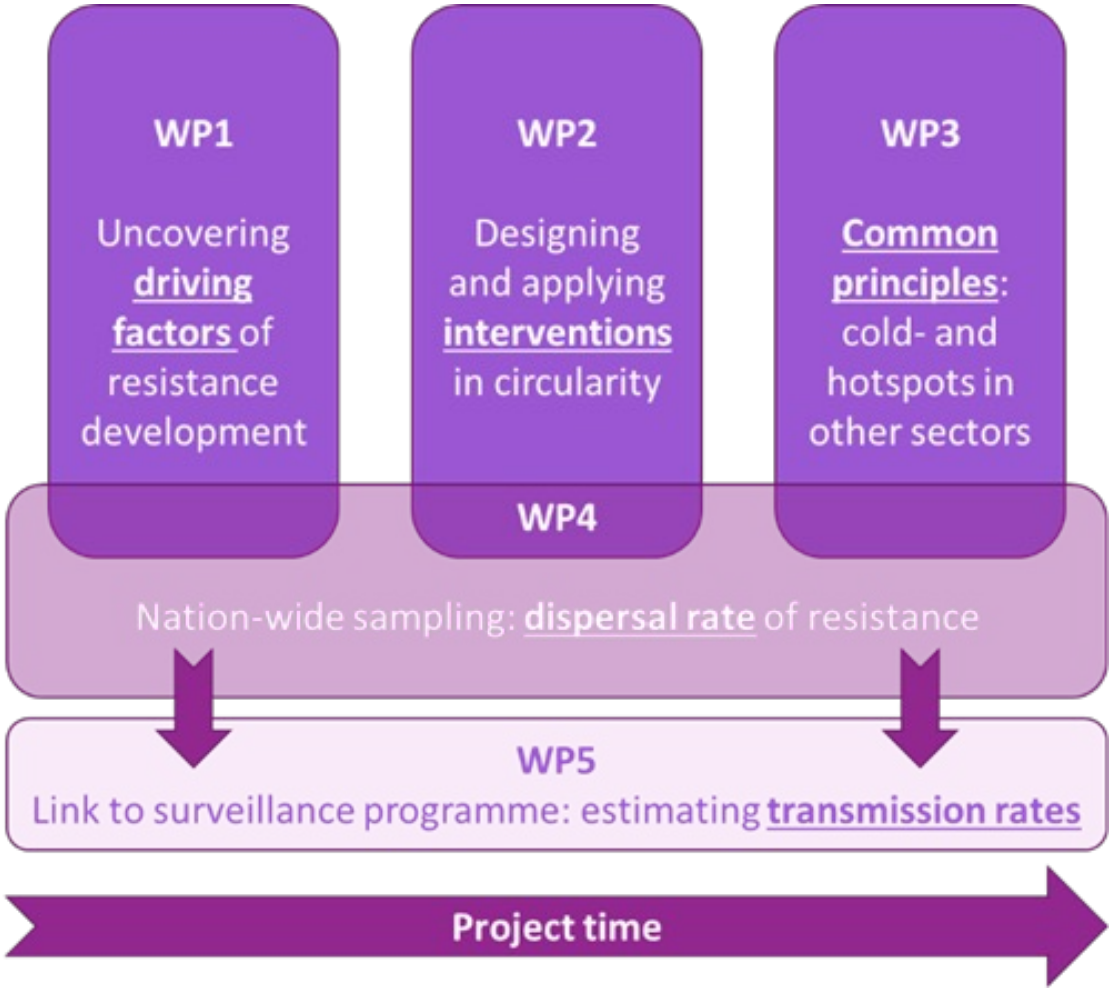
Relevant factors

- Resistance mechanisms
- Ecological settings
- Biology of the species
- Nature of the selection pressure



# Scientific approach



# It is NOT just the Dutch bulb sector

| Genetic analysis of the cyp51A promotor region and structural gene of 41 representative azole-resistant A. fumigatus isolates. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Resistance mechanism on azole targeted gene cyp51A | |
| Materials | Samples Code | Total A. fumigatus CFU/g | Freq TEB Resistance | Freq ITR Resistance | Number of colonies | Selection | Promoter insertion | Coding region |
| B-wood | NG_HAB_BW_200817_2 | 552000 | 0.03 | 0.03 | Colony 1 | ITR | 34 | L98H* |
| | | | | | Colony 2 | ITR | 0 | None |
| B wood | AL_HAB_BW_201009_2 | 2300 | 0.28 | 0.24 | Colony 1 | ITR | 0 | None |
| | | | | | Colony 2 | TEB | 46 | Y112F/T289A/G448S* |
| B-wood | AL_HAB_EC_201113_1A | 1600000 | 0.22 | 0.16 | Colony 1 | ITR | 46 | Y112F/T289A* |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| C wood | AL_HAC_BW_201009_1A | 4750 | 0.19 | 0.05 | Colony 1 | ITR | 46 | Y112F/T289A* |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A* |
| Strawberry | DB_AB1_20201228_1B | 5400000 | 0.31 | 0.20 | Colony 1 | ITR | 46 | Y112F/T289A* |
| | | | | | Colony 2 | ITR | 34 | L98H* |
| Strawberry | DB_AB3_20201228_1B | 43200000 | 0.13 | 0.13 | Colony 1 | ITR | 34 | L98H* |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Strawberry | DB_AB2_20201228_1A | 54000000 | 0.03 | 0.01 | Colony 1 | ITR | 0 | None |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Onion | DA2_UI_20201210_1A | 13800000 | 0.78 | 0.45 | Colony 1 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Onion | EM_UI_20210106_1A | 10800 | 0.19 | 0.19 | Colony 1 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| | | | | | Colony 2 | ITR | 34 | L98H* |
| Potato | OB_AA1_20210108_1B | 7560000 | 0.21 | 0.25 | Colony 1 | ITR | 34 | L98H* |
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| B-wood | NG_HAB_BW_200817_2 | 552000 | 0.03 | 0.03 | Colony 1 | ITR | 34 | L98H* |
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| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Strawberry | DB_AB2_20201228_1A | 54000000 | 0.03 | 0.01 | Colony 1 | ITR | 0 | None |
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| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Onion | EM_UI_20210106_1A | 10800 | 0.19 | 0.19 | Colony 1 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
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| Strawberry | DB_AB3_20201228_1B | 43200000 | 0.13 | 0.13 | Colony 1 | ITR | 34 | L98H* |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Strawberry | DB_AB2_20201228_1A | 54000000 | 0.03 | 0.01 | Colony 1 | ITR | 0 | None |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Onion | DA2_UI_20201210_1A | 13800000 | 0.78 | 0.45 | Colony 1 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Onion | EM_UI_20210106_1A | 10800 | 0.19 | 0.19 | Colony 1 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| | | | | | Colony 2 | ITR | 34 | L98H* |
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- Also, in samples including wood-chipping, strawberry, potato, onion, resistant *A. fumigatus spores* were found
- For a subset, for single spore colonies presence of known *cyp51A* mutations was assessed

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| | | | | | Colony 2 | ITR | 0 | None |
| B wood | AL_HAB_BW_201009_2 | 2300 | 0.28 | 0.24 | Colony 1 | ITR | 0 | None |
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| | | | | | Colony 2 | ITR | 34 | L98H* |
| Strawberry | DB_AB3_20201228_1B | 43200000 | 0.13 | 0.13 | Colony 1 | ITR | 34 | L98H* |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Strawberry | DB_AB2_20201228_1A | 54000000 | 0.03 | 0.01 | Colony 1 | ITR | 0 | None |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Onion | DA2_UI_20201210_1A | 13800000 | 0.78 | 0.45 | Colony 1 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| | | | | | Colony 2 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| Onion | EM_UI_20210106_1A | 10800 | 0.19 | 0.19 | Colony 1 | ITR | 46 | Y112F/T289A/S363P/I364V/G448S* |
| | | | | | Colony 2 | ITR | 34 | L98H* |
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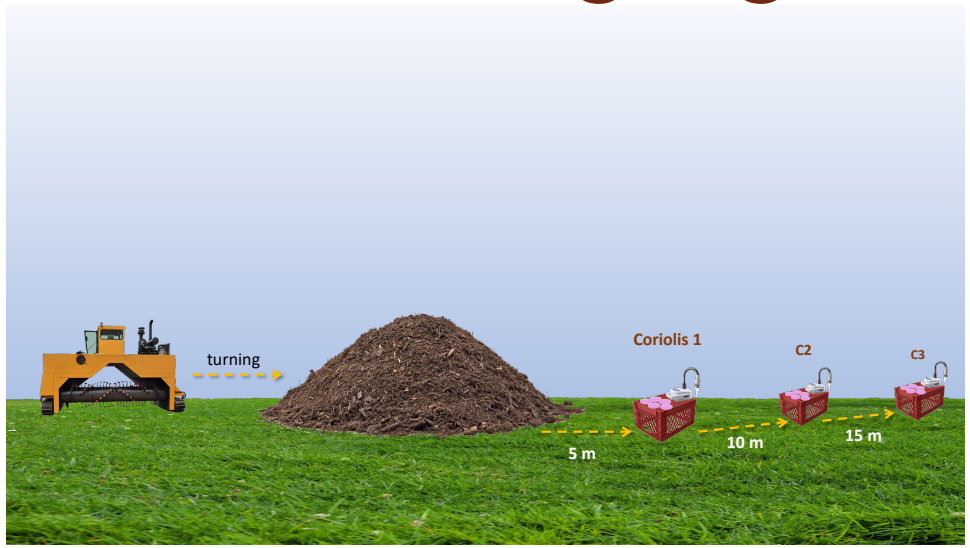
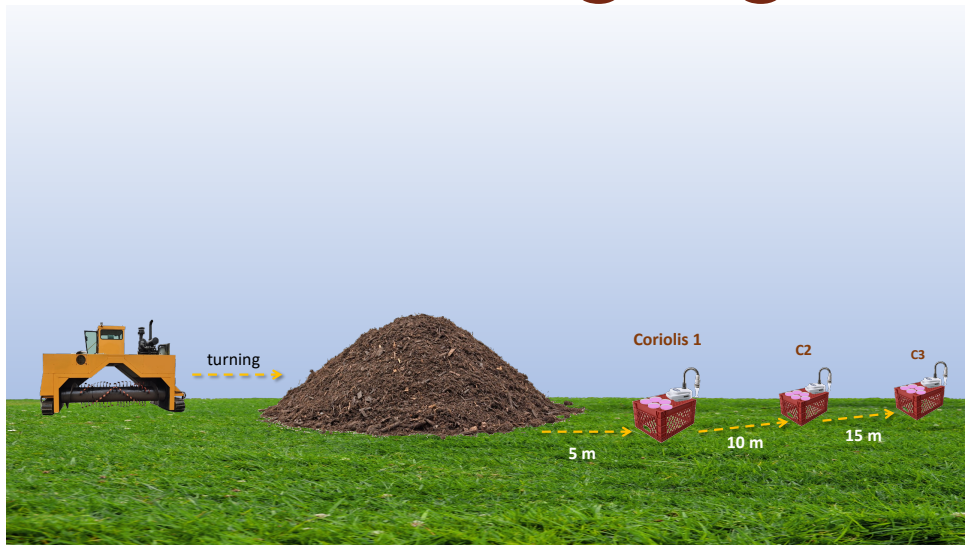
- Also, in samples including wood-chipping, strawberry, potato, onion, resistant *A. fumigatus spores* were found
- For a subset, for single spore colonies presence of known *cyp51A* mutations was assessed
- Similar tandem repeat-mediated resistance mechanisms were found as reported in clinical azole-resistant isolates

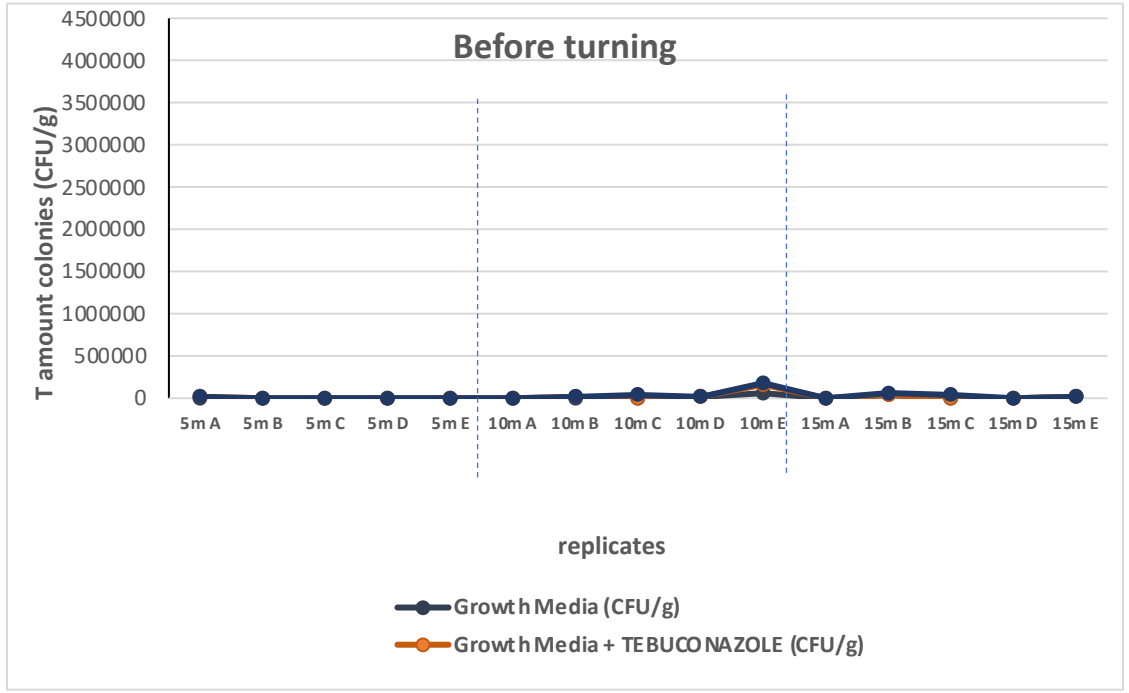
# Some highlights: air-sampling during turning




- Air-sampling around organic waste-heaps

# Some highlights: air-sampling during turning

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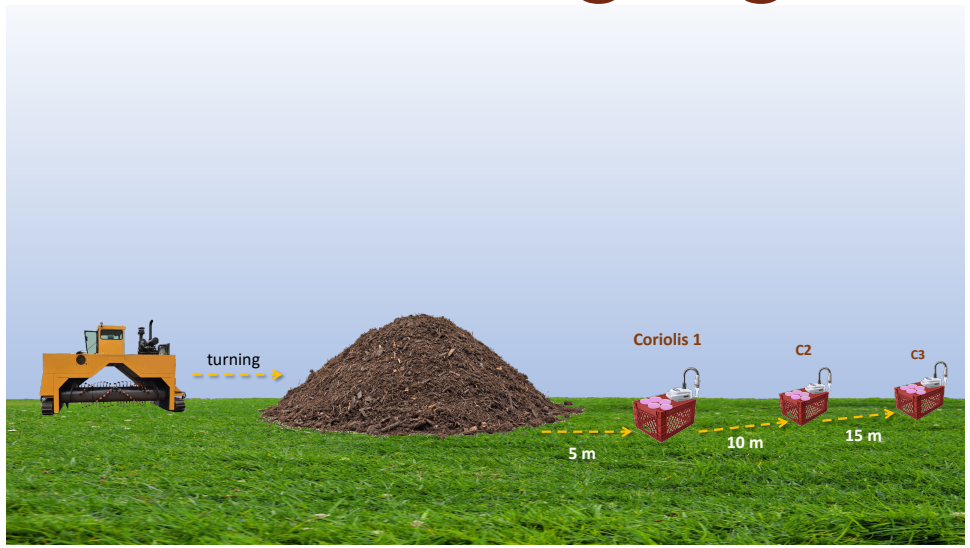


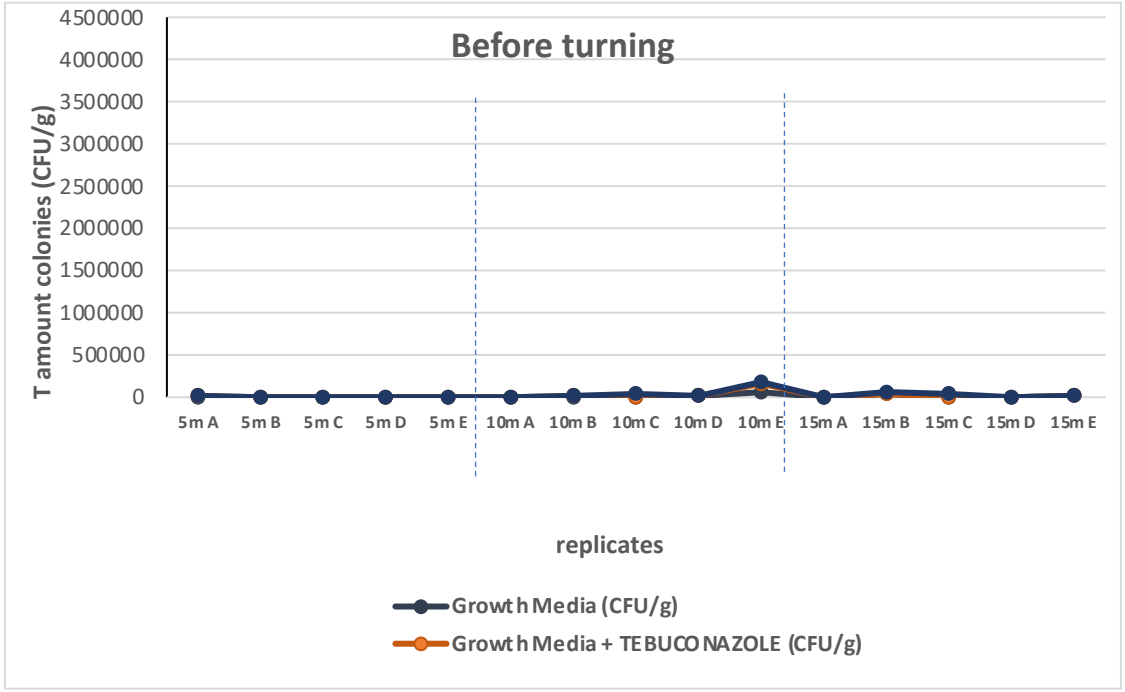


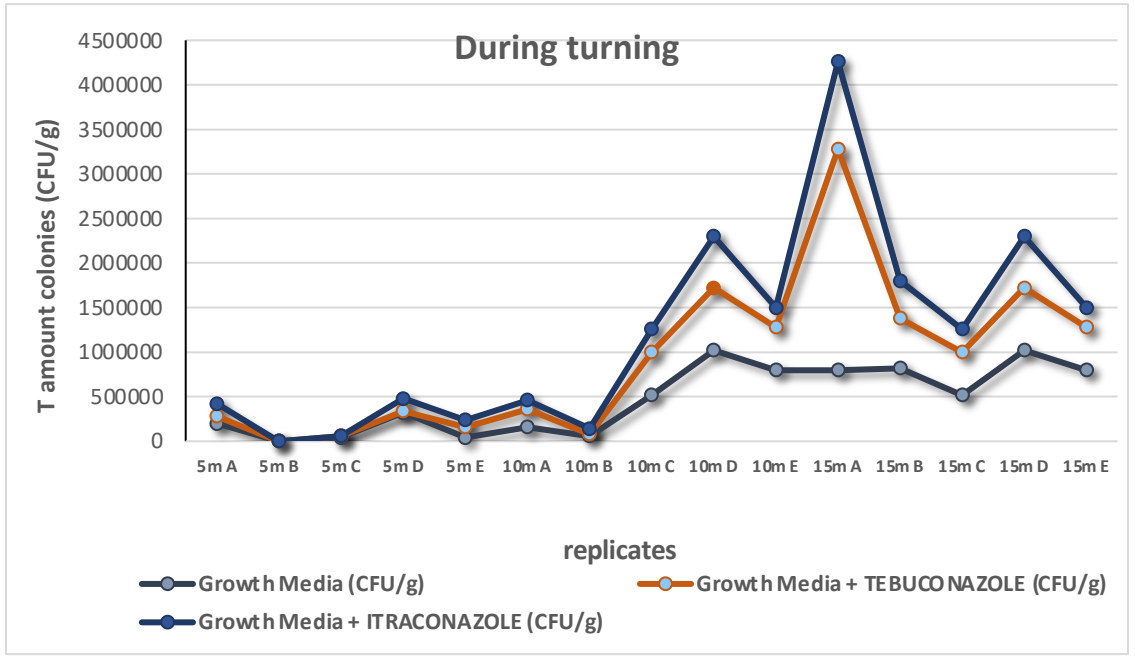


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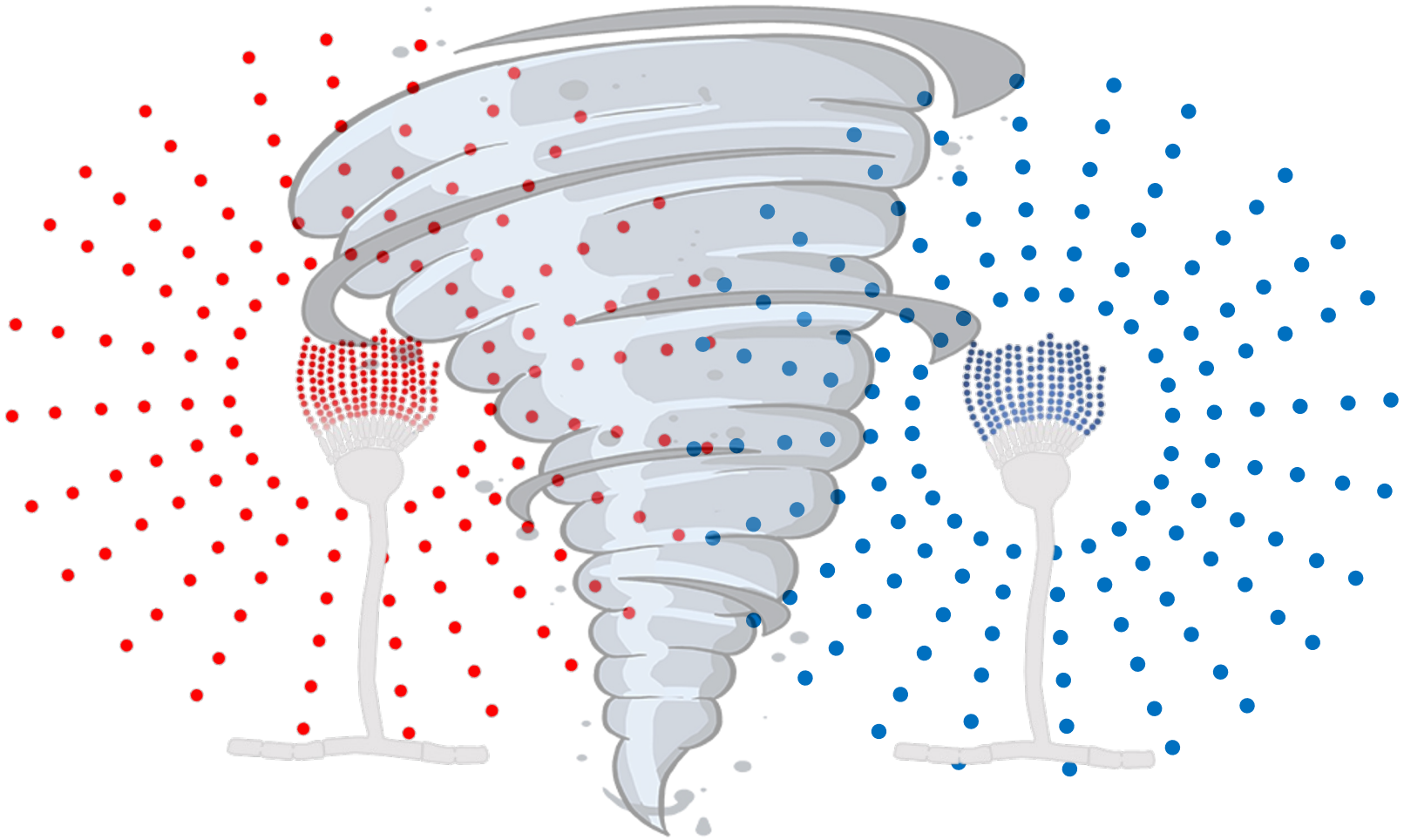
- Profusive spore release from heaps when turned

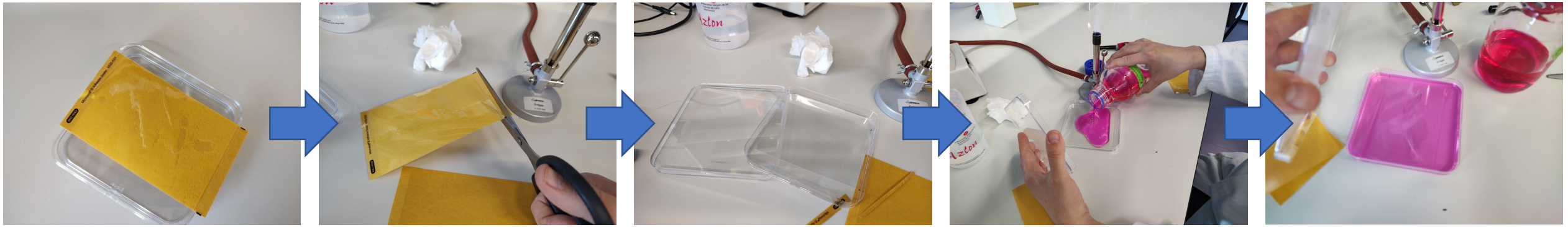
# How to air sample?



# How to air sample?

## A promising new method

- Incubate at 48C for three days



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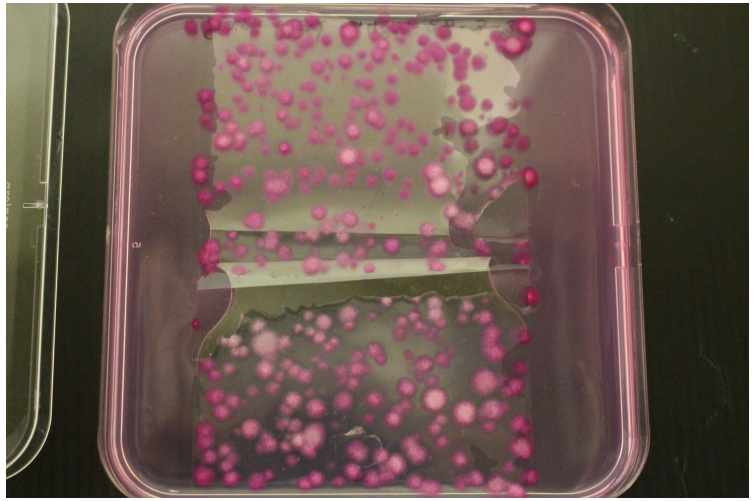

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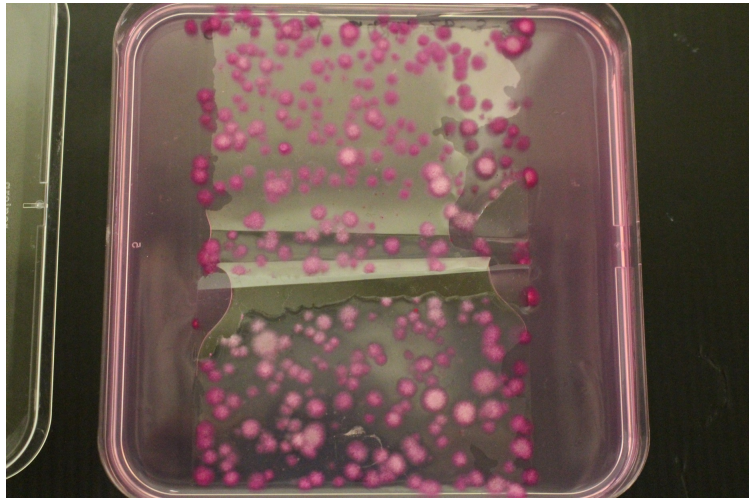

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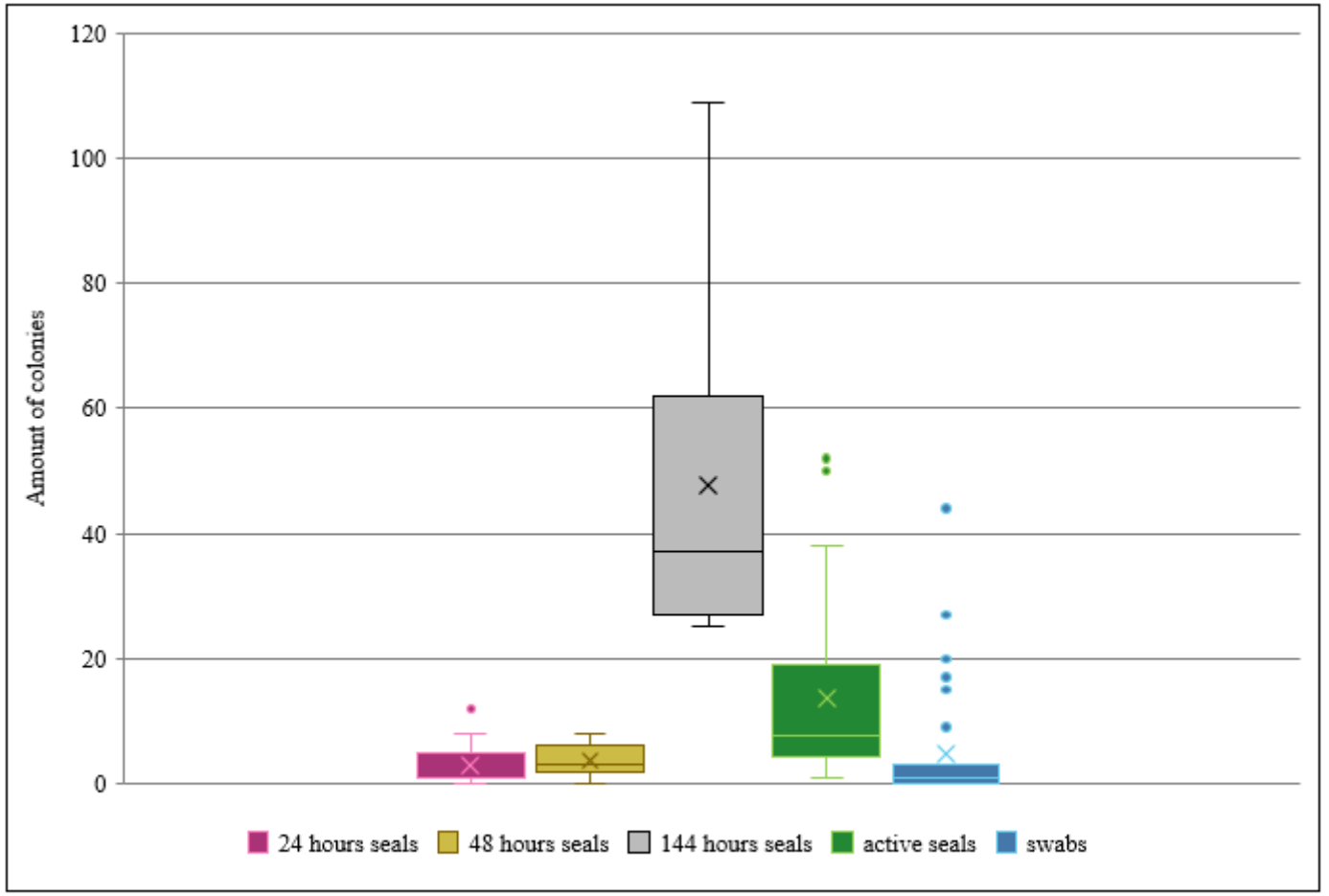


***Figure: Colony counts of different sampling methods.*** *The amount of colonies for respectively 24 hours, 48 hours, 144 hours seals, active seals and swabs.*

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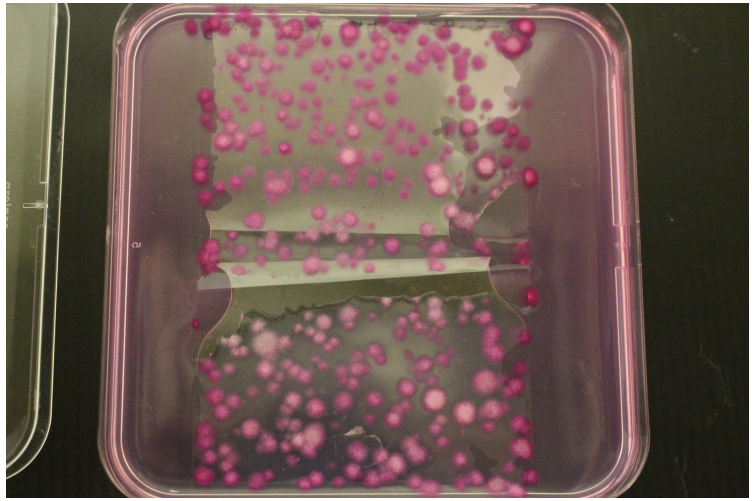

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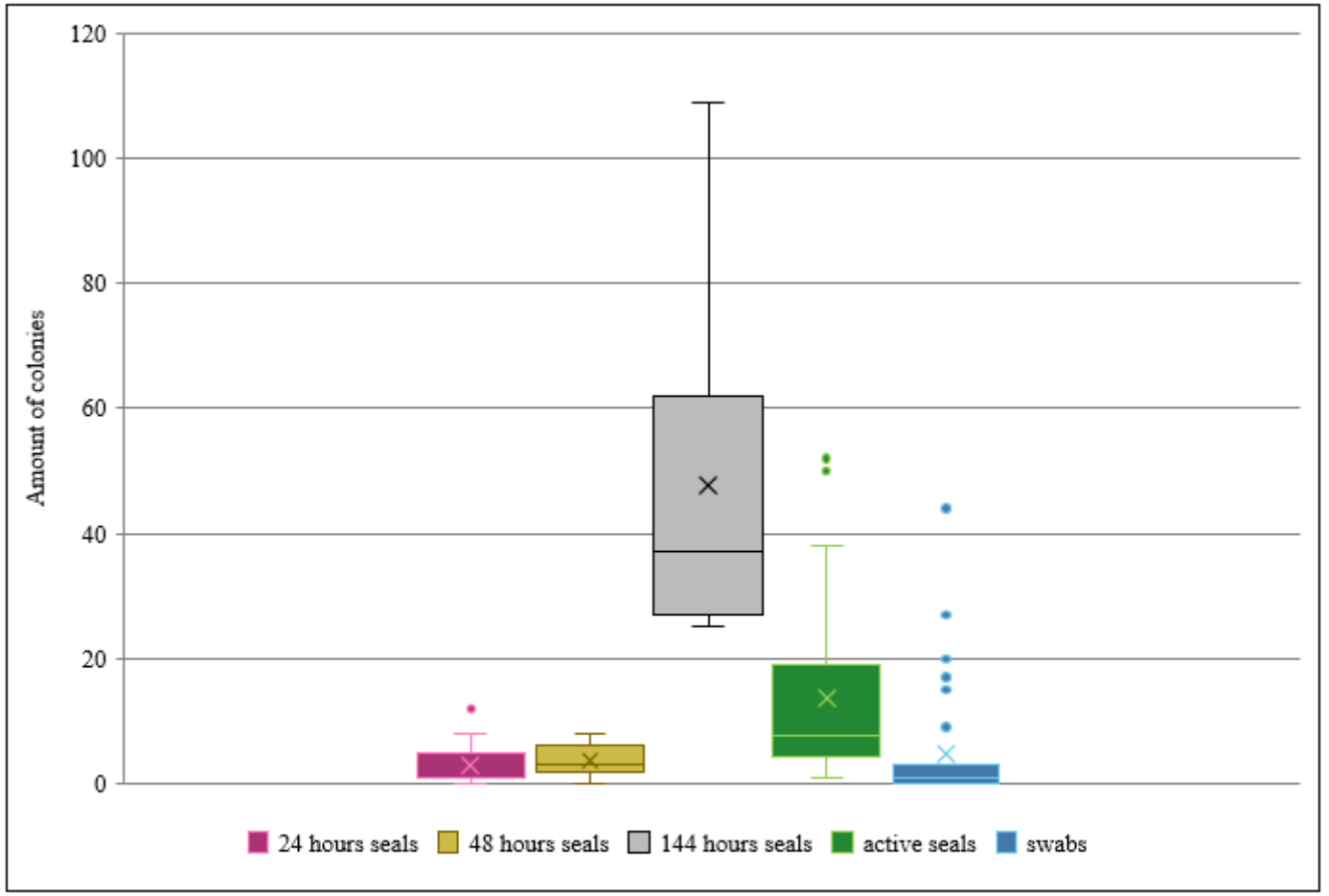


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# Population genetics of resistance mutations

Sex and recombination rate affect the dynamics of genetic variation

https://www.biorxiv.org/content/10.1101/2022.01.14.476329v1

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- Cross between fertile strains AfIR964 and AfIR974
- 14,113 segregating markers
- 195 offspring from several cleistothecia; 90X short read sequencing

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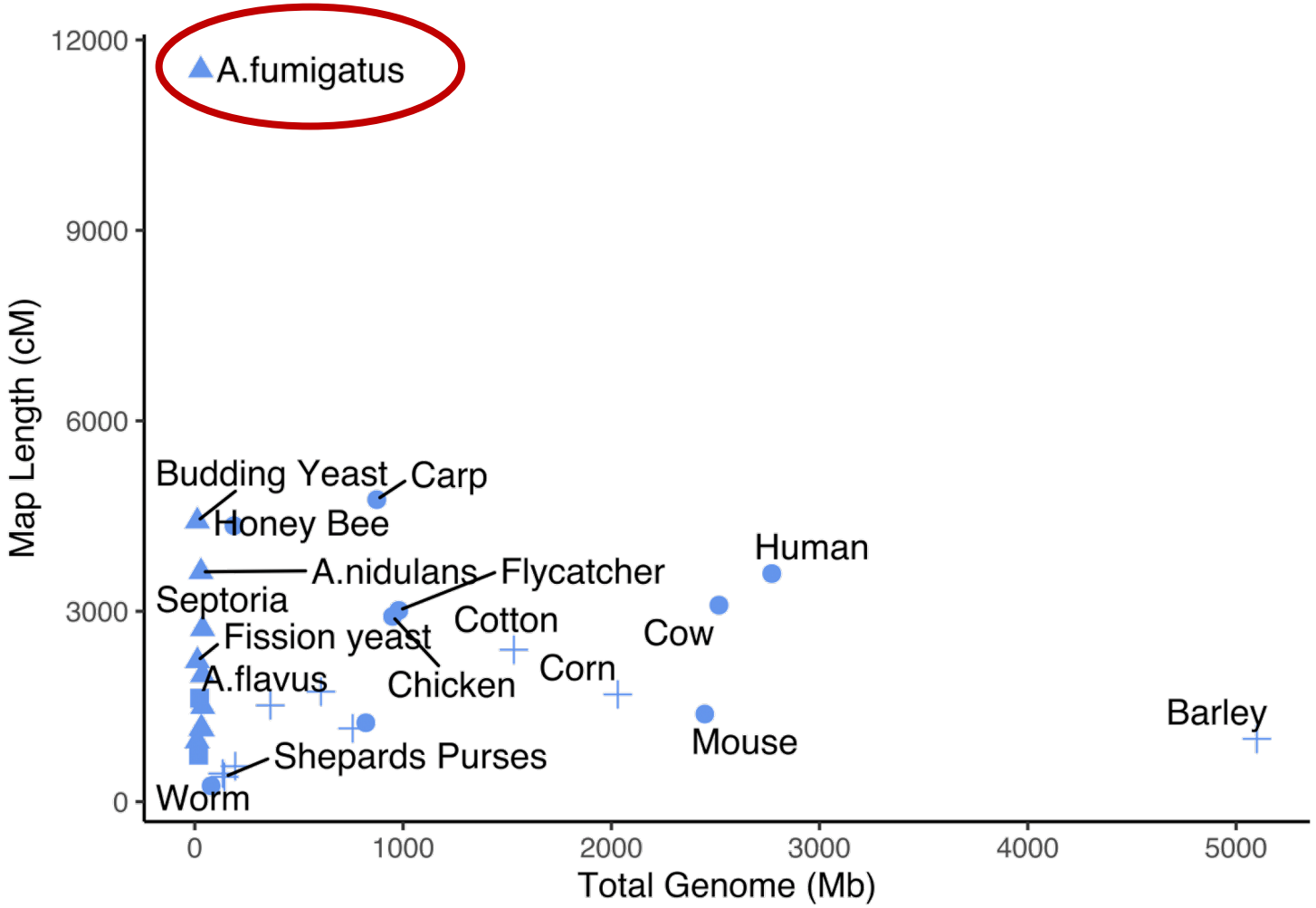


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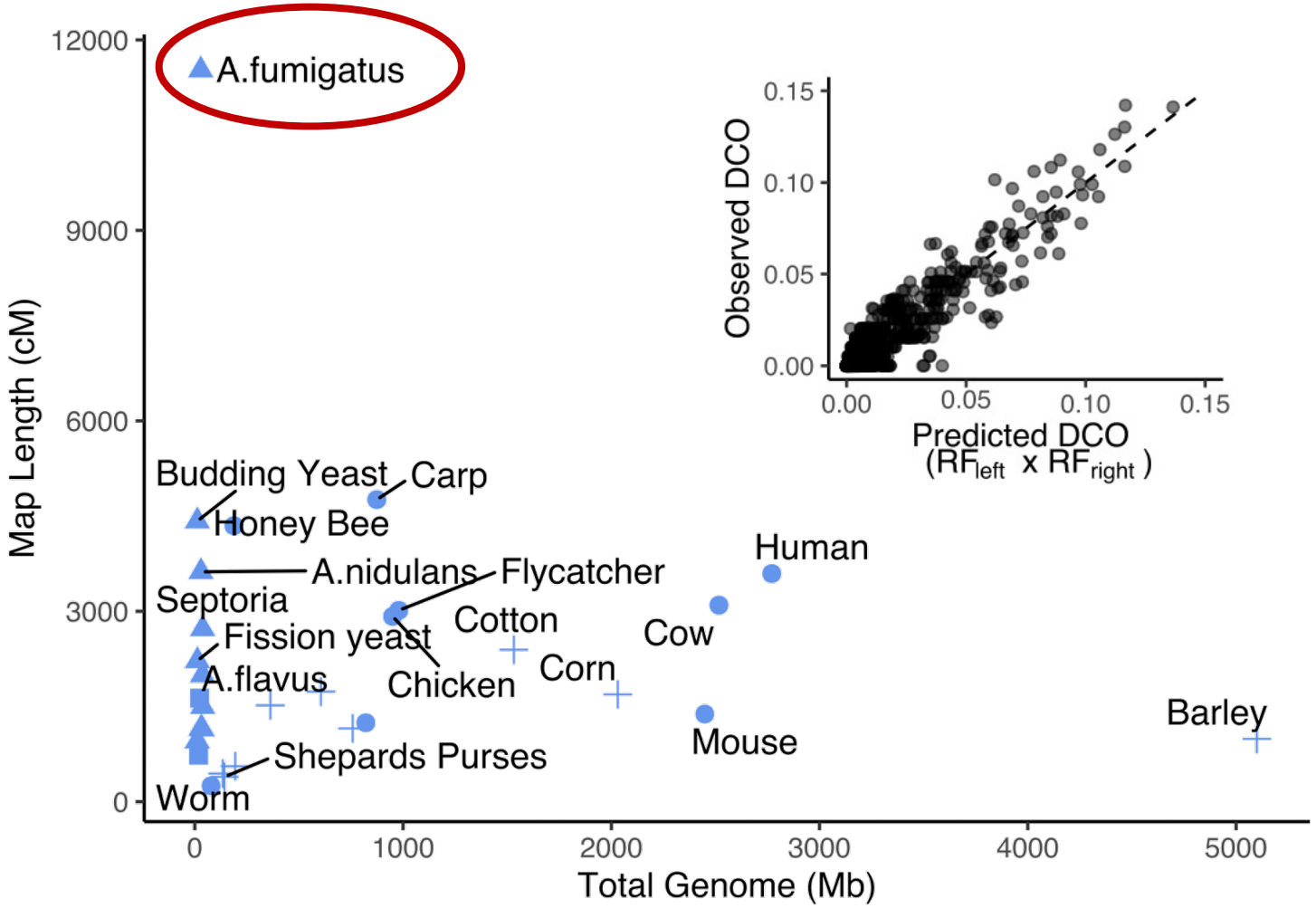


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- As for *A. nidulans*, no evidence for cross-over interference

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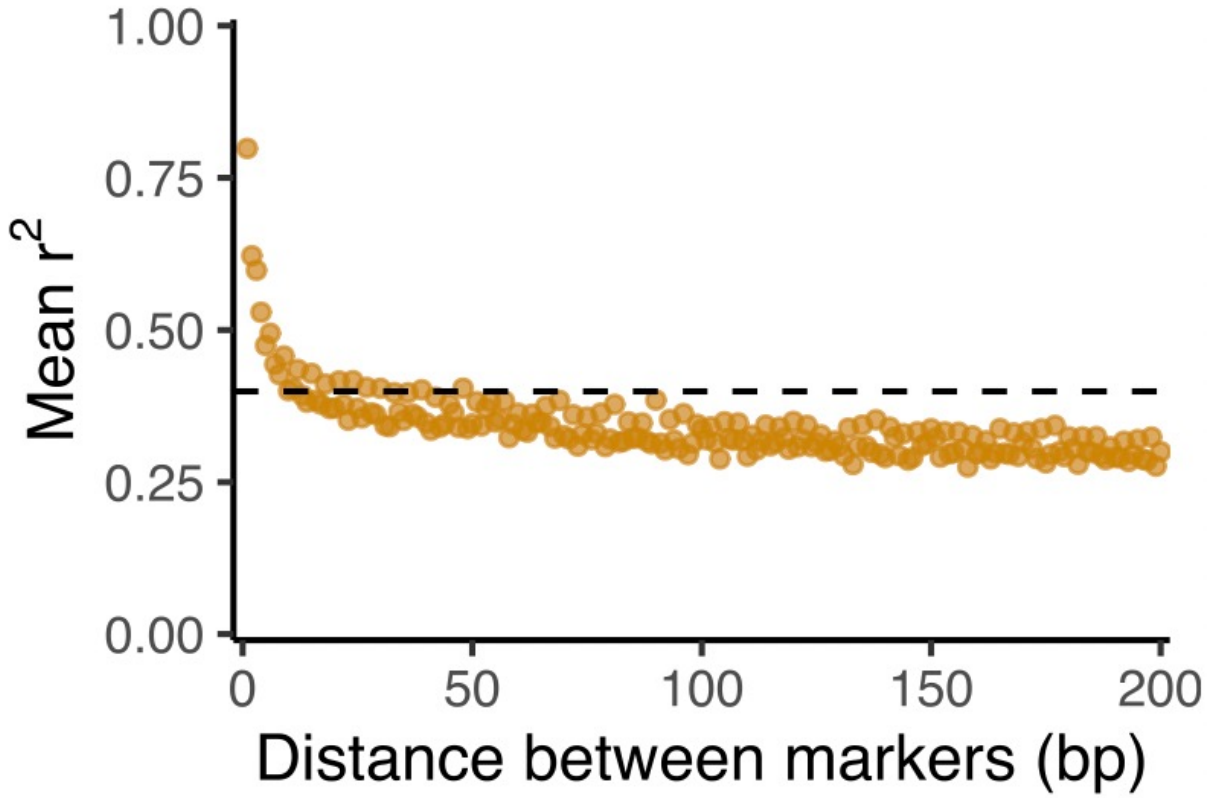
# Population genetics of resistance mutations

High recombination rate, rapid decay of linkage disequilibrium (LD)




- Using published* data from 175 sequenced individuals
- Very rapid decay of LD consistent with high recombination rates in *A. fumigatus*

*A. E. Barber *et al*., Nat Microbiol. 6, 1526–1536 (2021)

# Population genetics of resistance mutations

## High recombination rates and low LD facilitates genetic mapping

- Parental strains differ in Acriflavine resistance
- Offspring phenotyped on 50µg/mL acriflavine

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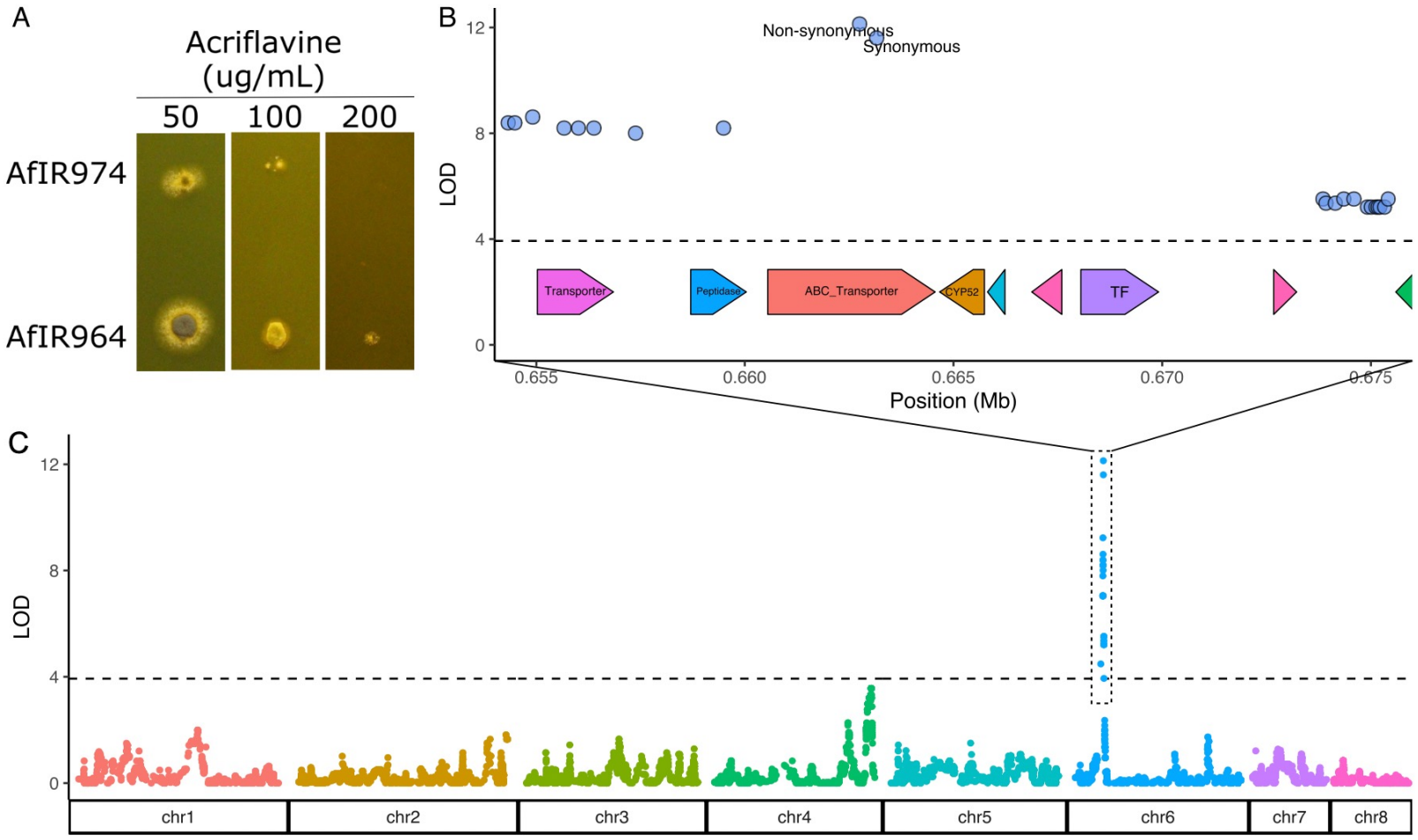


- Parental strains differ in Acriflavine resistance
- Offspring phenotyped on 50µg/mL acriflavine
- Single locus on chromosome 6 between positions 657kb and 675kb
- Presumed causal variant (Phe to Cys non-synonymous substitution)

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# Population genetics of resistance mutations

Can the tandem repeat and the non-synonymous polymorphism in the *cyp51* gene be combined through recombination?

- Crossed AFIR975 with previously* generated single mutants for $TR_{34}$ or L98H
- These single mutants were crossed with the expectation of 0.075% $TR_{34}$/L98H genotypes in the offspring
- Recombinants were selected on 10µg/mL itraconazole

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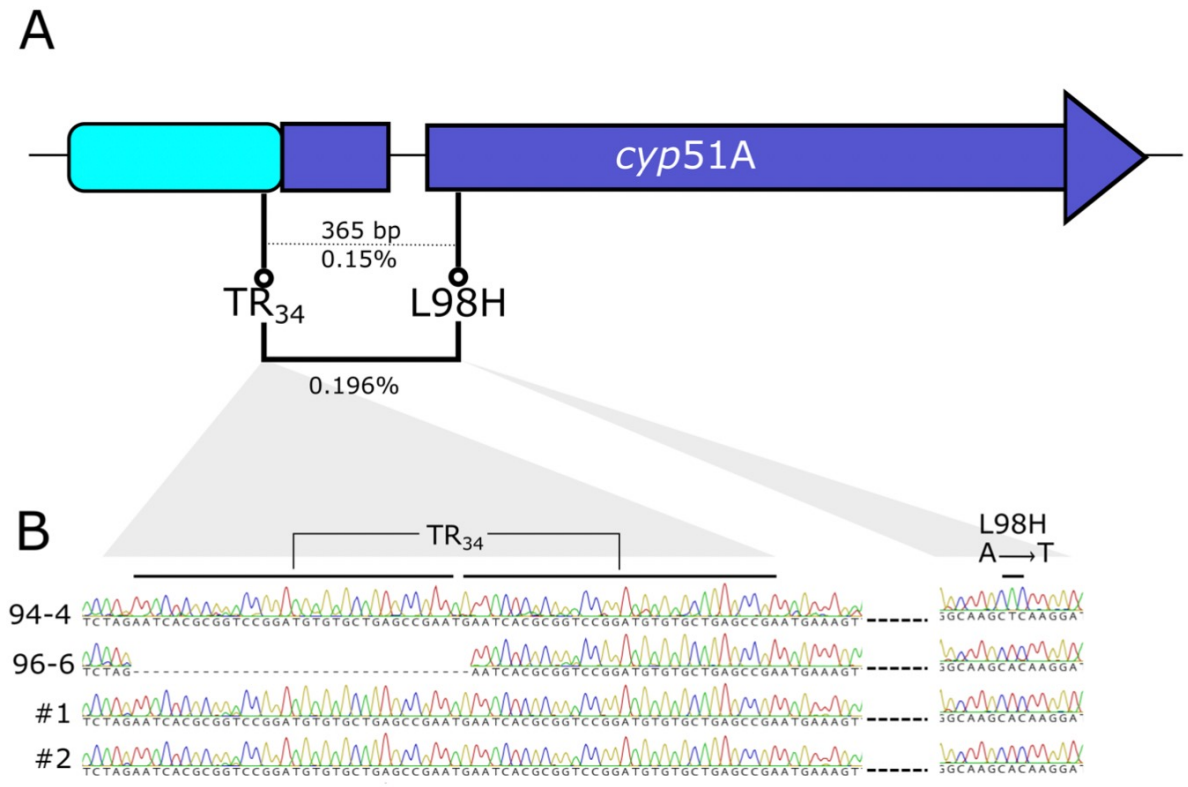


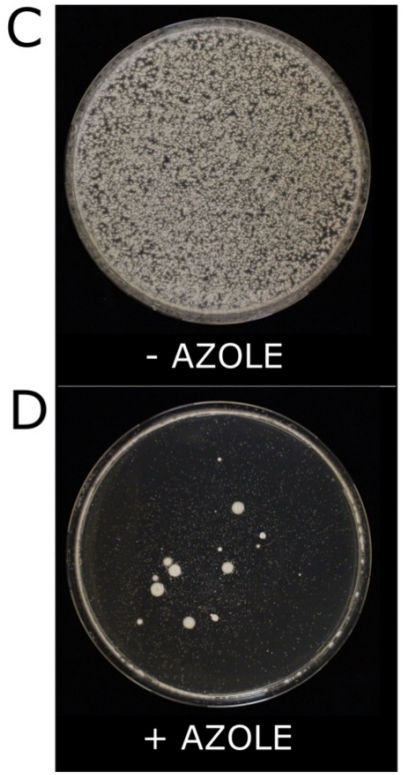


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# Linking the environment to the clinic: the smoking gun

Whole genome sequencing of a unbiased balanced set of samples

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## Whole genome sequencing of a unbiased balanced set of samples

| Haplotype | | |
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| | **Environmental** | **Clinical** |
| WT | 26 | 26 |
| WT R | 4 | 6 |
| TR34 | 24 | 33 |
| TR46 | 31 | 15 |
| TR92 | 0 | 2 |
| unclear | | 3 |
| **Total** | **85** | **83** |
| **Location** | | |
| | **Environmental** | **Clinical** |
| Location A | 31 | |
| Location B | 11 | |
| Location C | 40 | |
| Other farm | 3 | |
| AmsterdamUMC | | 38 |
| RadboudUMC | | 34 |
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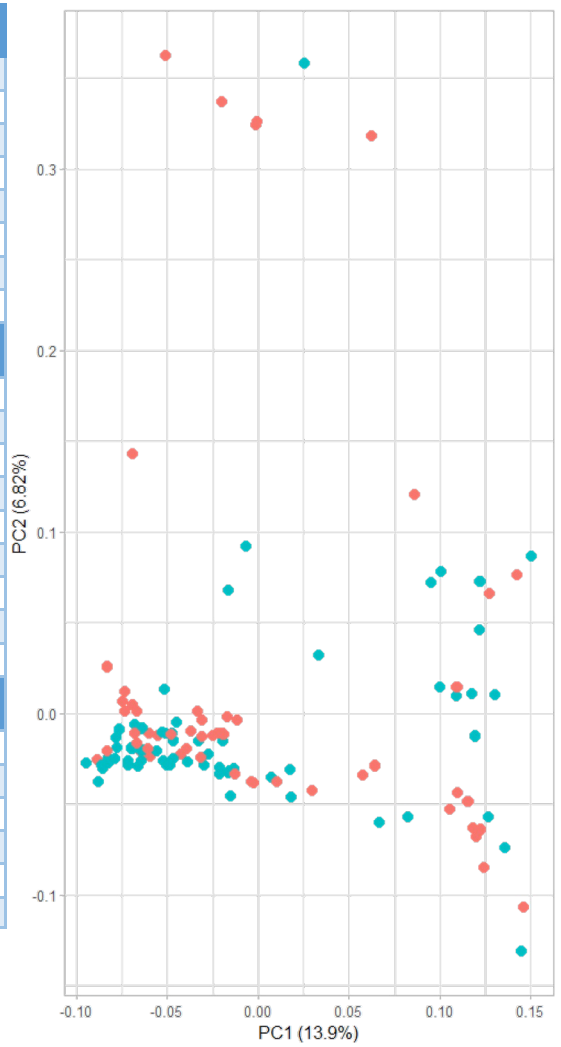


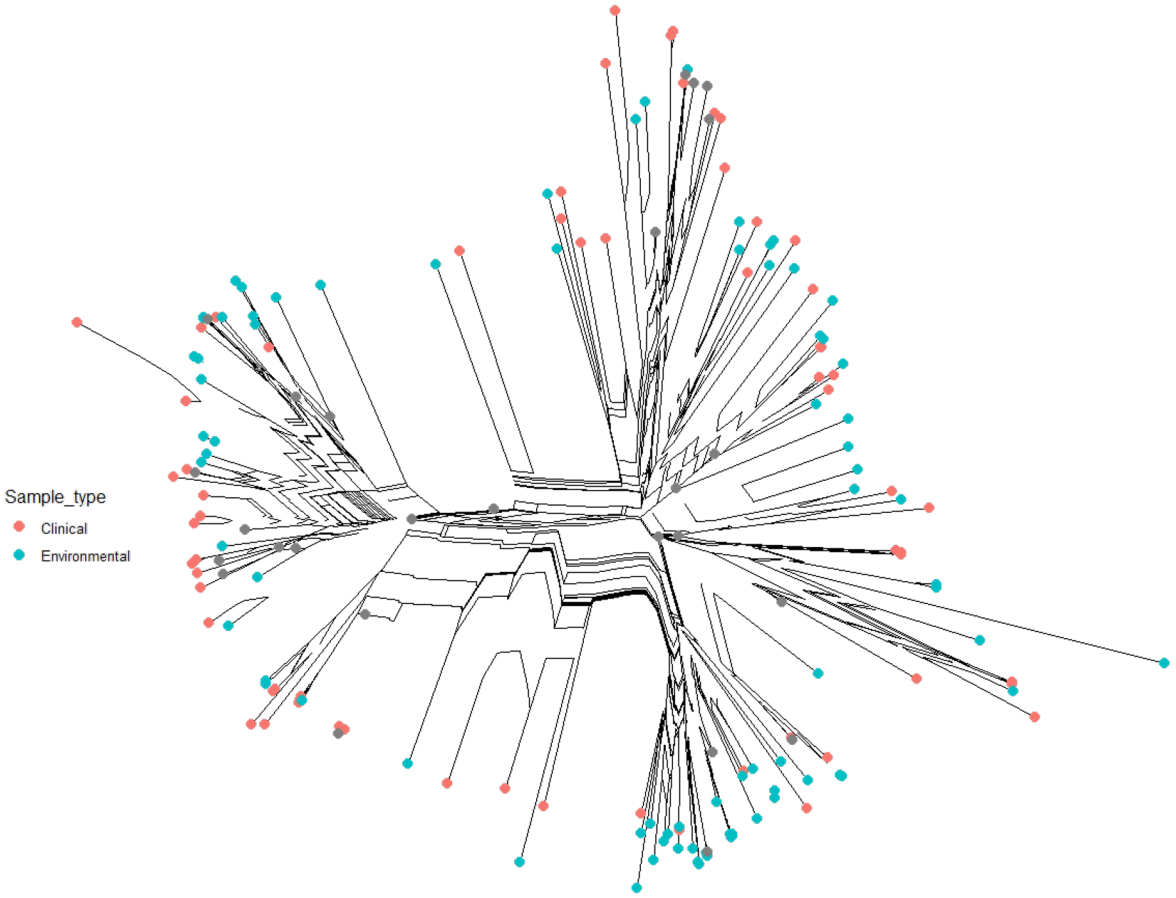


- No evidence of clinical/environmental separation

# Take home messages

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- Resistant *A. fumigatus* spores are ubiquitously present
  - Prevention of resistance development seems a lost cause
  - Prevention of transmission should be the new target

# Acknowledgements

## People


Ymke Nederlof


Sijmen Schoustra


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Paul Verweij


Eveline Snelders


Hylke Kortenbosch


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Bas Zwaan

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KAVB

NWO

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nefyto

BVOR

# Thank you

I am happy to take questions and comments