# Environmental Azole Resistance in Target and Non-Target Fungal Pathogens in Crop Production

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# Introduction

- Ergosterol (and some other sterols) are important constituents of fungal cell membranes that regulate membrane stability and permeability.
- DMI fungicides inhibiting key enzymes involved in fungal sterol biosynthesis including CYP51 are very effective in managing many fungal diseases of crops.
- DMIs are critical for plant disease management and are the foundation of modern disease control programs.
- DMIs represent over 30% of the agricultural fungicide market worldwide.
- Multi-site mode of action fungicides are being limited or phased out by regulatory agencies.

# Known mechanisms involved in DMI resistance

(1) **Mutations in the target enzyme C14 demethylase** (CYP51) result in decreased binding affinity of fungicides

(2) **Overexpression of CYP51** genes

(3) Decreased intracellular fungicide concentration mediated by **increased energy-dependent efflux**

# Characteristics of DMI Resistance in Targeted Plant Pathogens

- **Resistance to DMIs evolves slowly, and resistance levels are often low compared to other fungicides (e.g., MBCs, QoIs, APs).**
- **Fitness penalties are often associated with DMI resistance.**
  - Higher use rates often overcome resistance.
  - Minimal usage diminishes resistant sub-populations that results in restoring of efficacy.
- **Resistance is not absolute. There are numerous case studies where resistance was reported, but the DMI is still in use today –**
  - Penicillium decays of citrus - Resistance in *Penicillium* spp. to the postharvest fungicide imazalil (imidazole) reported in the 1980s (Promotor insertion - overexpression of CYP51).
  - Brown rot of stone fruit - Resistance in *Monilinia fructicola* reported to propiconazole (triazole) reported in the 2000s (“Mona” insert for overexpression of CYP51 without fitness penalties).
  - Cherry leaf spot – Resistance in *Blumeriella jaapii* to fenbuconazole (triazole) caused by a long-interspersed nuclear element (“LINE”) promotor insertion - overexpression of CYP51.

# Managing DMI Resistance in Targeted Plant Pathogens and Non-Target Fungi of Agricultural Crops

- To maintain their field performance, limit DMI applications to a maximum of two - three times per year
  - Low-cost generics encourage increased usage over other modes of action (MOA)
  - Lack of alternatives (regulatory cancellation of older MOAs) results in over-usage of DMI fungicides
- Use DMI fungicides in a mixture or in alternation with other effective fungicides of different modes action to break the selection process any single MOA.
- DMI fungicide should only be applied as preventative rather than curative treatments (Use when target and non-target populations are low instead of high).
- Fungicide Resistance Action Committee (FRAC) provide sensible and practical use guidelines for different crop pathogen systems.
- Monitoring of sensitivity in target pathogens – Multiple organizations involved that provide annual updates on resistance levels by crop or region.

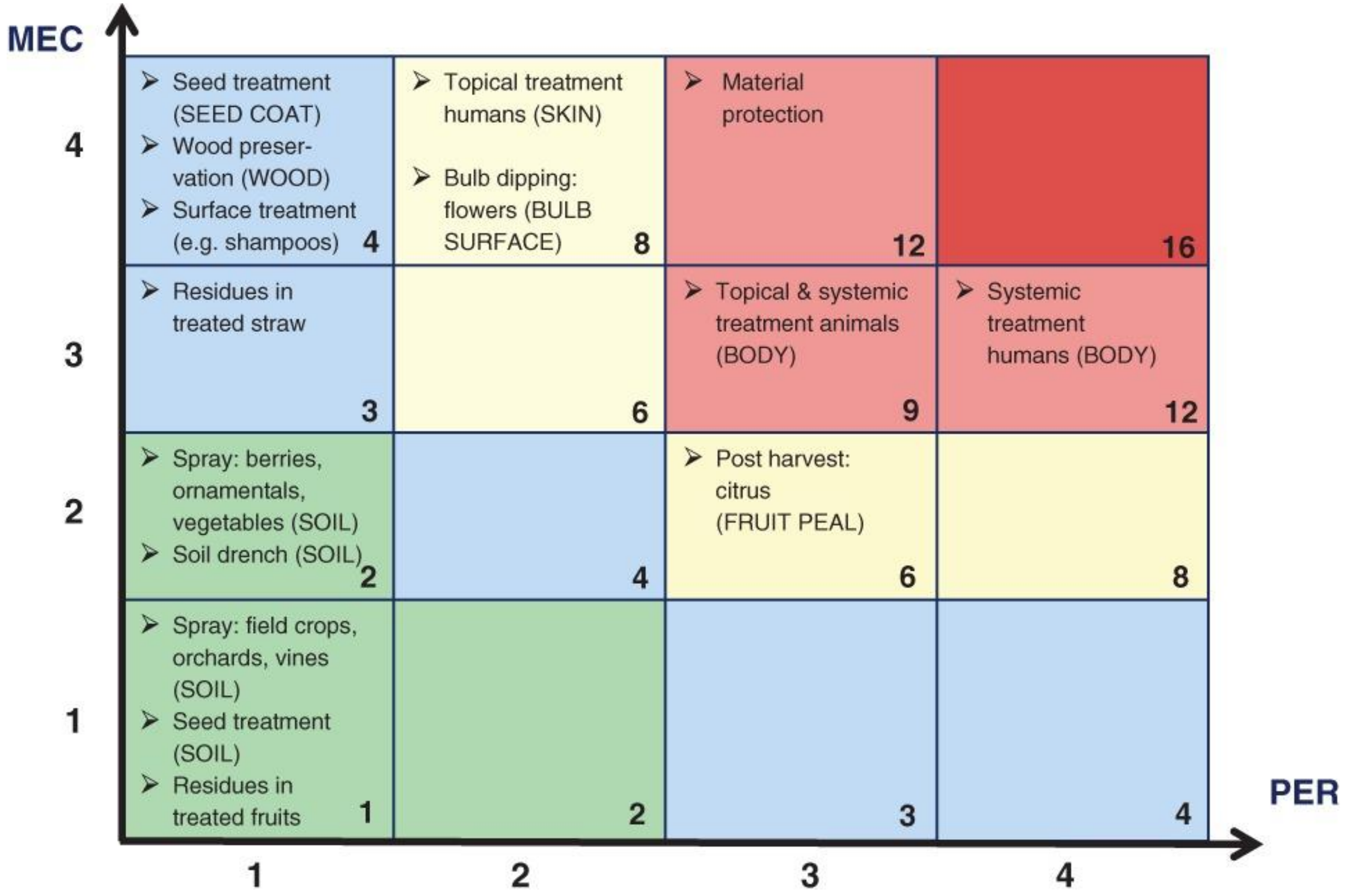
# Azole resistance - an emerging problem in the opportunistic mold *Aspergillus fumigatus* (a non-target organism)

"The recovery of *A. fumigatus* isolates resistant to the medical triazoles from azole-naive patients and from the environment strongly indicates an environmental route of resistance selection."

- *A. fumigatus* grows optimally at 37°C (human body temperature) and can grow at up to 50°C, with conidia surviving at 70°C.
- These temperatures are regularly encountered in self-heating compost heaps.
- Air-borne spores of the fungus are ubiquitous in the atmosphere.
- People inhale an estimated several hundred spores of *A. fumigatus* per day.
- Spores are quickly eliminated by the immune system in healthy individuals.

# Assessment of selection and resistance risk for DMI fungicides

- Maximum exposure concentrations (MEC) are highest during medical and certain fruit, seed, and wood preservation treatments as compared to applications for plant protection.
- Most mutations in the target gene *cyp51* of DMI-resistant isolates are different in *A. fumigatus* ($TR_{34}$/L98H, TR46/Y121F/T289A) than in plant pathogens (A379G, I381V).
- ASR=MEC × PER for resistance to DMIs in *A. fumigatus* is estimated to be highest for medical uses.
- Still, environmental origin of DMI-resistant spores from certain sites cannot be ruled out.




Assessment of selection and resistance risk during exposure of *A. fumigatus* to DMIs in the environment and medicine. MEC, maximum exposure concentration; PER, pathogen exposure risk ; ASR, assumed selection risk (ASR = MEC × PER). Scale: 1–2, very low risk; 3–4, low risk; 5–8, medium risk; 9–12, high risk; 13–16, very high risk.

Gisi, U. 2014. Pest Management Science 70:352–364

# Azole-resistant *Aspergillus fumigatus* isolates in the environment

Numerous potential sources and characteristics of azole-resistance have been investigated:

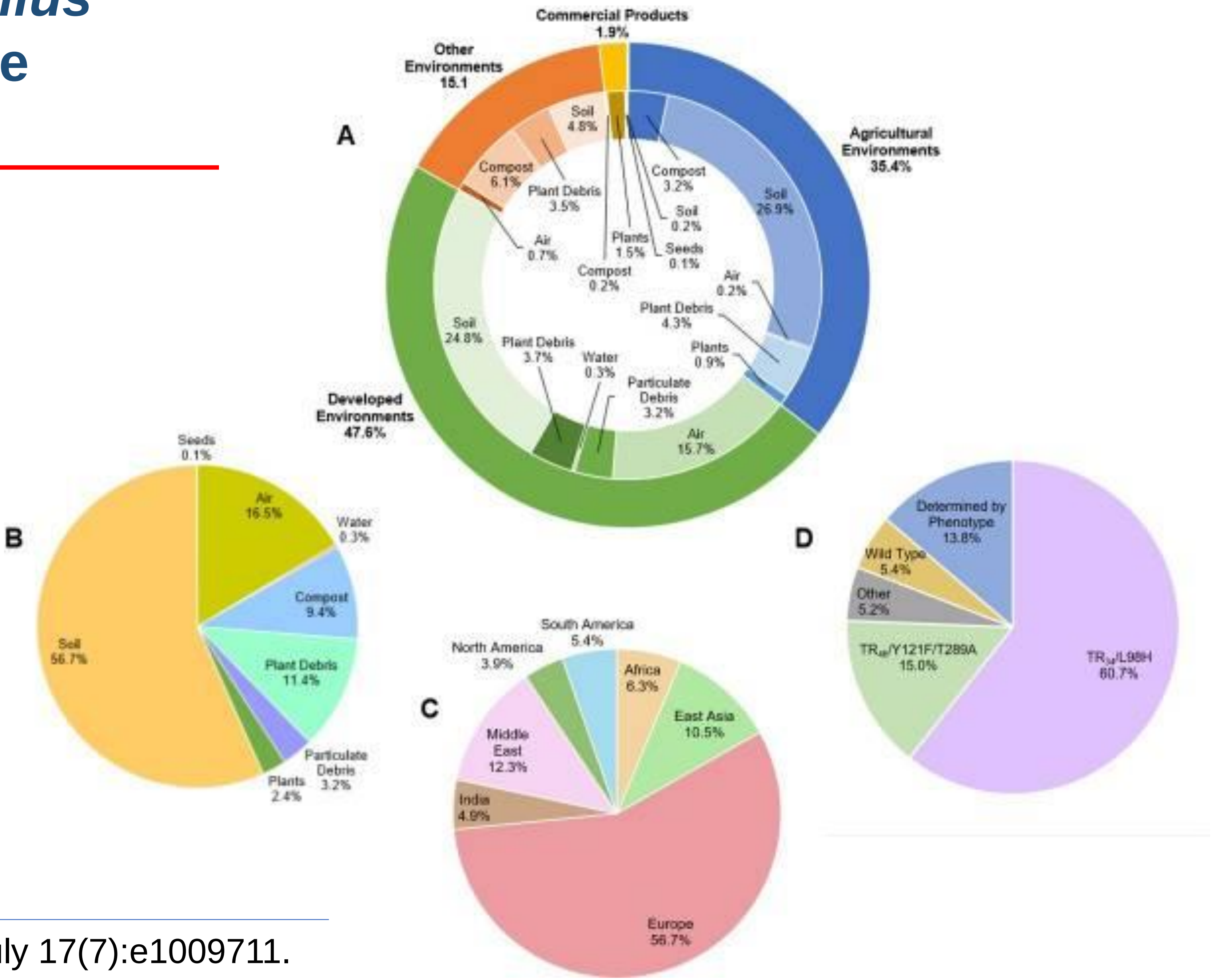
**A)** Environmental settings and substrates

**B)** Substrate sampled across all environmental settings

**C)** Geographic region

**D)** *cyp51A* alleles present

For environmental substrates, soils (57%), plant debris and compost (20.8%), and air (16.5%) are the main sources of resistance for 4 major settings.

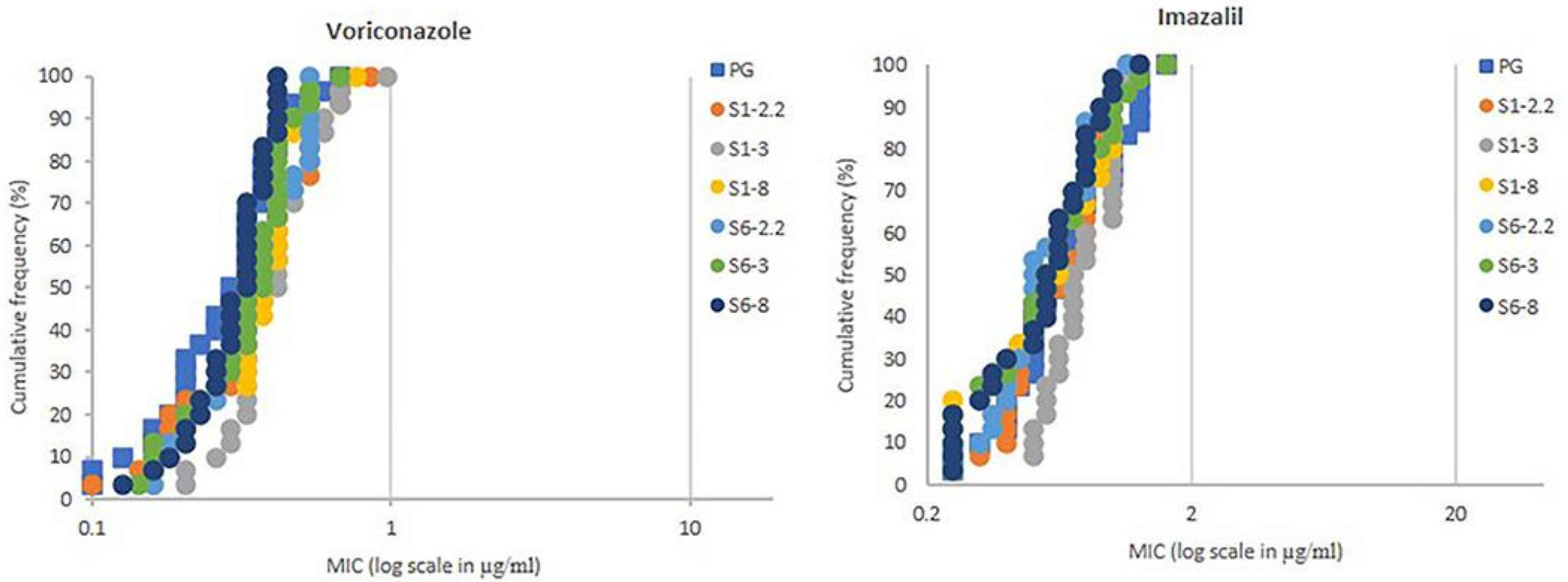



Burks, C., et al. 2021. PLoS Pathog. July 17(7):e1009711.

# Assessment of selection and resistance risk for DMI fungicides

**Study from the UK:**

- *A. fumigatus* was sampled from fungicide-treated (since the 1970s) and non-treated wheat fields and from grass lands with different fertilizer regimes.
- Isolates were tested for sensitivity to voriconazole and imazalil




## Results and conclusions:

- **No differences in azole sensitivity among isolate origins.**
- **Arable crop production is a “coldspot” for azole resistance development**:
  - Low numbers of pan-azole resistant isolates
  - Lack of new genotypes in soils of fungicide-treated commercial and experimental wheat crops.
- **Previously reported hotspots include flower bulb waste, green waste, and wood chippings.**

Fraaije, B. et al. 2020. Front. Microbiol., https://doi.org/10.3389/fmicb.2020.599233

# Conclusions

- DMI fungicides are used in both medicine and agricultural settings.
- Use in agriculture generally does not result in complete resistance in targeted pathogens.
- DMI usage can be regulated to minimize shifts in sensitivity after decades of usage.
- DMI resistance is found in medically important fungi (e.g., *A. fumigatus*) at an increasing rate.
- Potential sources include the environment:
  - Agricultural uses in plant protection have low risk.
  - Common sources include soil, plant debris and compost piles, as well as air in comparison to low numbers of pan-azole resistant isolates from soil
  - Lack of new genotypes in soils of fungicide-treated commercial and experimental wheat crops (two mutations predominate in resistant isolates - TR34/L98H, TR46/Y121F/T289A).
- Other selection pressures may be involved in the increased occurrence in DMI-resistance in *A. fumigatus*.