# Azole-resistant *Aspergillus fumigatus* in agronomic settings: hotspots & coldspots

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# ARAf (Azole-resistant *Aspergillus fumigatus*) in context

- ARAf presents a significant, widespread hindrance to treatment of Invasive Aspergillosis with medical azoles
- Azoles are also used in the environment: agriculture/horticulture, material protection, veterinary
- Medical azole antifungals and agricultural azole fungicides can be cross-resistant
- *A.fumigatus* is not a target for treatments with azole fungicides in agriculture/horticulture, but “collateral” exposure of the fungus to azole residues can occur
- Key questions: where, how and to what extent can azole fungicides select & amplify ARAf in agronomic settings?

# What is an agronomic "hotspot" for ARAf?

- An agronomic setting in which the following criteria are fulfilled:

| Requirement | Comments |
|---|---|
| Favorable conditions for growth and multiplication of *A. fumigatus* | Availability of a suitable organic substrate capable of supporting a sizable population of *A. fumigatus*; prevailing conditions of temperature and humidity provide optimal growth conditions, leading to a competitive advantage for *A. fumigatus* within the fungal/microbial community |
| Exposure of *A. fumigatus* to residual concentrations of DMI fungicides that are selective for resistant genotypes | Residue levels of a specific DMI fungicide exceed its Minimum Inhibitory Concentration (MIC) for wild-type (sensitive) *A. fumigatus*, leading to selection of resistant *A. fumigatus* genotypes |
| Mass release of airborne spores of *A. fumigatus* into the environment | Selection of resistant genotypes leads to preferential reproduction and release into the air spora, resulting in an "amplification" of resistance over background levels |

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# Which (aspects of) agronomic settings can comprise an ARAf "hotspot"?

*microorganisms*

MDPI

*Review*

## Selection and Amplification of Fungicide Resistance in *Aspergillus fumigatus* in Relation to DMI Fungicide Use in Agronomic Settings: Hotspots versus Coldspots

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**Abstract:** *Aspergillus fumigatus* is a ubiquitous saprophytic fungus. Inhalation of *A. fumigatus* spores can lead to Invasive Aspergillosis (IA) in people with weakened immune systems. The use of triazole antifungals with the demethylation inhibitor (DMI) mode of action to treat IA is being hampered by the spread of DMI-resistant "ARAf" (azole-resistant *Aspergillus fumigatus*) genotypes. DMIs are also used in the environment, for example, as fungicides to protect yield and quality in agronomic settings, which may lead to exposure of *A. fumigatus* to DMI residues. An agronomic setting can be a "hotspot" for ARAf if it provides a suitable substrate and favourable conditions for the growth of *A. fumigatus* in the presence of DMI fungicides at concentrations capable of selecting ARAf genotypes at the expense of the susceptible wild-type, followed by the release of predominantly resistant spores. Agronomic settings that do not provide these conditions are considered "coldspots". Identifying and mitigating hotspots will be key to securing the agronomic use of DMIs without compromising their use in medicine. We provide a review of studies of the prevalence of ARAf in various agronomic settings and discuss the mitigation options for confirmed hotspots, particularly those relating to the management of crop waste.

**Keywords:** *Aspergillus fumigatus*; DMI fungicides; azoles; resistance; agriculture; mitigation

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# Patterns emerging from studies of ARAf distribution & frequency in agronomic settings

- Existence of a “background” proportion of ARAf within the environmental *A.fumigatus* (air, soil) population
- ARAf is no more frequent in/above agronomic settings than in samples from urban settings
- No clear distinction between organic & azole-treated crop settings in terms of recovery of ARAf
- Limited occurrence of ARAf (or indeed *A.fumigatus*) in the growing crop
- Waste derived from ARAf-”free” crops can become a “hotspot”, carrying large numbers of ARAf colony-forming units

# Flower bulb waste: an example of an ARAf "hotspot"

Image: courtesy of Leon Jansen

- Stockpiling plant waste can create a hotspot, depending on how the waste is managed
- Waste piles are considered likely to provide the conditions for sexual reproduction/ generation of ARAf mutants

| Setting | Matrix sampled | Cyp51A mutation(s) of ARAf isolates | Country[3] |
|---|---|---|---|
| Flower field | Soil | $TR_{34}$/L98H, $TR_{46}$/Y121F/T289A | CO |
| | Waste piles / compost | $TR_{34}$/L98H, $TR_{34}$/L98H/S297T, $TR_{46}$/Y121F/T289A, $TR_{46}$/Y121F/M172I/T289A/G448S, $TR_{46}$/Y121F/T289A/S363P/I364V/G448S, $TR^{3}_{46}$/Y121F/M172I/T289A/G448S, $TR^{4}_{46}$/Y121F/M172I/T289A/G448S | NL |
| | | $TR_{34}$/L98H, $TR_{46}$/Y121F/T289A, $TR_{34}$/L98H/F495I, $TR_{34}$/L98H/S297TF495I, $TR_{92}$/Y121F/M172I/T289A/G448S, $TR_{46}$/Y121F/T289A/I364V | NL |
| | | $TR_{46}$/Y121F/T289A, $TR_{46}$/Y121F/T289A/M172I/G448S $TR^{3}_{46}$/Y121F/T289A/M172I/G448S | NL |

# Cereals: an example of an ARAf "coldspot"

- Cereal crop soil, grain and straw do not carry large ARAf (or *A.fumigatus)* populations
- The most frequent "environmental" ARAf genotypes are present in azole-treated cereals soils at similar frequencies to urban air

| Setting | Matrix sampled | Cyp51A mutation(s) of ARAf isolates | Country[3] |
|---|---|---|---|
| Cereals | Soil | $TR_{34}$/L98H, WT | DE |
| | | $TR_{34}$/L98H, WT | DE |
| | | F46Y/M172V/E427K | DE |
| | | $TR_{34}$/L98H, F46Y/M172V/E427K | FR |
| | | $TR_{34}$/L98H | FR |
| | | $TR_{34}$/L98H | FR |
| | | - | GR |
| | | - | HU |
| | | - | IT |
| | | $TR_{34}$/L98H, G54E | IT |
| | | $TR_{34}$/L98H | UK |
| | | - | UK |
| | | $TR_{34}$/L98H | UK |
| | | $TR_{34}$/L98H, $TR_{46}$/Y121F/T289A, F46Y/M172V/E427K | UK |
| | | F46Y/M172V/E427K | UK |
| | Fallow soil | WT | DE |
| | Soil and leaves | $TR_{34}$/L98H, WT | IT |
| | Grain | - | NL |
| | Straw | - | FR |
| | Hay | - | FR |
| | Air sample | D100G, V101I, N102H | UK |

# Implications / conclusions

- When positioning fungicides in agronomic settings, the crop protection industry will consider the risk of selection of resistance in human pathogenic fungi in those settings, informed by the expanding knowledge base
- “Integrated disease management” of ARAf in crop waste is unlikely to be a simple extension of fungicide resistance management of target pathogens in the crop
- Understanding crop waste management processes & options will be key to determining avoidance / mitigation strategies