Does the use of azole fungicides in crop protection encourage resistance in the human fungal pathogen *Aspergillus fumigatus*?

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Introduction

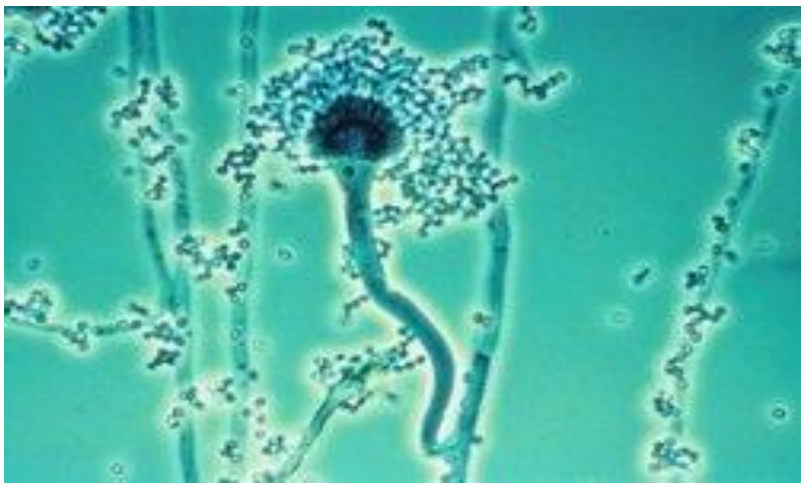
Background

A recent UK Government report highlighted the problem of antibiotic resistance, and that patients were at risk because no effective alternatives were available. The report concluded that the problem was as serious as climate change. No mention was made of fungicide resistance, even though affecting control of several human fungal pathogens, including *Candida, Cryptococcus* and *Aspergillus.* Several oral azole fungicides provide front-line treatment of these diseases. Although echinocandins are an alternative, they are less effective. Azoles are largely fungistatic rather than fungicidal, and so long-term therapy is often needed to contain infection to manageable levels. However, for immunocompromised patients following transplant surgery, and HIV patients, their lack of an immune system means these infections become fatal.

Aspergillus fumigatus

Resistance to the pathogen *Aspergillus fumigatus* (Figure 1) causes particular concern. Although found in soils and composts, its airborne spores are inhaled, and in patients with on-going lung conditions infection results in *Chronic Pulmonary Aspergillosis.* Infection can become invasive and spread to other organs, especially in immunocompromised and HIV patients, and, if untreated, this is invariably fatal. Even in patients with azole sensitive strains the mortality rate is around 40% and in patients with resistant strains this rises to 88%. *A. fumigatus* is the largest cause of deaths in patients due to fungi.

Figure 1. ***Aspergillus fumigatus***



Emergence of azole resistance in Aspergillus fumigatus

Azole chemistry exploits inhibition of the pivotal 14α-demethylase (CYP51) step in the sterol biosynthesis pathway, and although a large number of products are available, those used in

medicine are not the same as those used in crop protection. During the 1980s, two oral azoles, fluconazole and itraconazole (Figure 2), became available and were successfully used to control Aspergillosis. In 1989, the first itraconazole resistant *A. fumigatus* isolate was recovered from an American patient. Other reports of resistance soon followed and were linked to, either point mutations in the target CYP51 or increased drug efflux. Cross resistance to newer and more effective azoles , such as voriconazole and posaconazole, was not always the case. But in 1998, resistant isolates were obtained from a number of patients in the Netherlands, which combined a point mutation with a tandem promoter repeat (TR_{34}L98H), and which conferred cross resistance to all available azoles. *A. fumigatus* strains with this resistance mechanism quickly spread worldwide (Figure 3).

Figure 2. Azoles used in medicine and crop protection

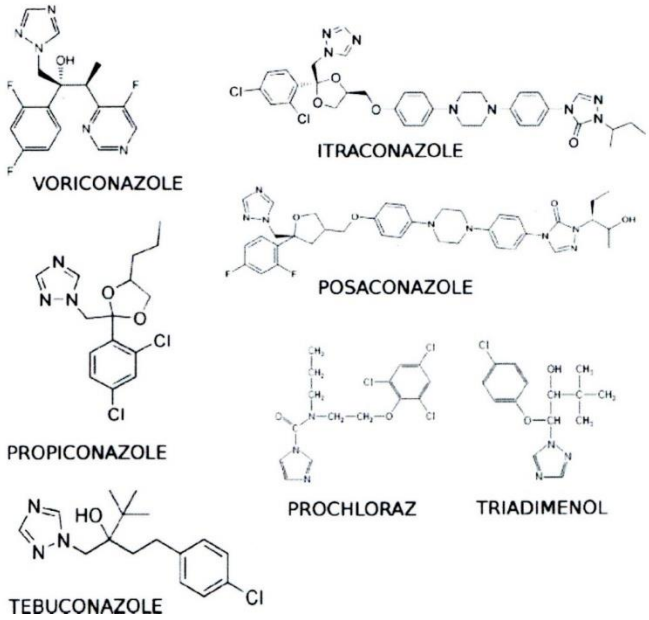


Figure 3. Spread of TR_{36} L98H resistance worldwide

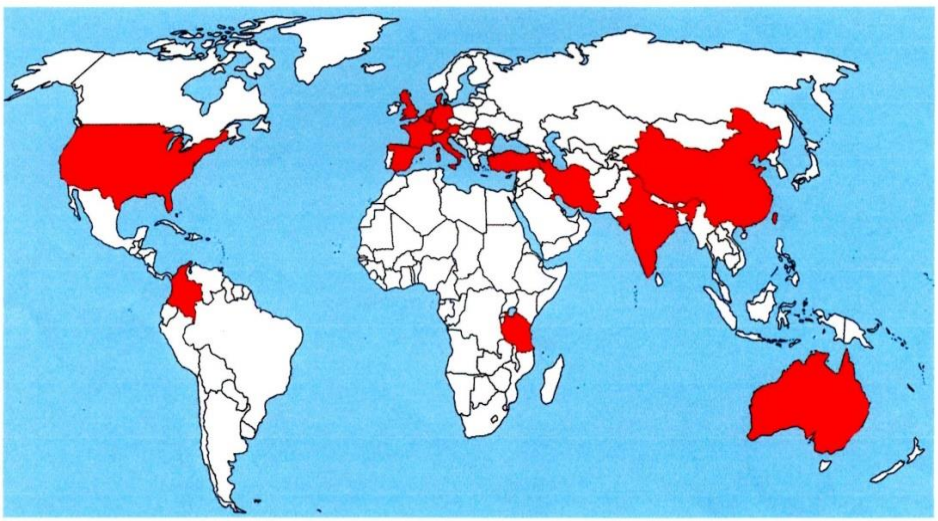


Figure 1. Shaded areas show countries that have reported the TR_{34}/L98H and TR_{46}/Y121F/T289A resistance mechanism in clinical or environmental *Aspergillus fumigatus* isolates.

Azole use in environment including crop protection

Apart from use in medicine, azoles are used to protect wood and textiles, and in animal and consumer health products. By far their greatest use is disease control in agriculture and horticulture, including prevention of post-harvest losses. Imazalil was the first azole introduced in the 1970s and was followed soon after by prochloraz, another imidazole. Both are still used today. A large number of triazoles (26+) were introduced from 1980s onwards, primarily for the control of cereal and fruit diseases. Today, azoles account for a quarter of all fungicide use wordwide, and are key components of fungicide anti-resistance strategies. Triazoles degrade slowly with half-lives between 20 and 200 days depending on soil type. Following a single spray to a young crop, *A. fumigatus* can be exposed to concentrations of 0.3 to 0.4 mg Kg^{-1} in the top 10cm of soil. For comparison, and equating a Kg with a litre, the concentration in blood serum is much higher at 11mg L^{-1} in patients receiving daily oral treatment for aspergillosis.

Evidence linking resistance with agricultural use of azoles

For:

1. Because azole resistant *A fumigatus* isolates were obtained from patients entering the clinic with no prior history of azole use, and resistant isolates were also recovered from the environment, at least from plant growing composts, but not field soils, it was suggested that azole use in crop protection had driven selection and spread of resistance in *A. fumigatus.*

2. Dutch workers correlated emergence of the TR_{36}L98H resistance with the introduction during the 1990s of five widely used triazoles.

3. Computer modelling of CYP51 confirmed that agricultural and medical triazoles had similar binding properties within the active site of the enzyme. Modelling did not include imidazoles and did not include interaction with leucine 98, which is not in the active site. Not surprisingly, as they have the same mode of action and resistance mechanisms, there was cross-resistance.

This position echoed medical concern for antibiotic resistance and use of antibiotics in livestock production, and prompted calls for restrictions on azole use in crop protection.

Against:

1. *A. fumigatus* is thermophilic, and although resistant isolates have been recovered from composts in protected cropping situations, no resistant isolates have been recovered from field soils. Indeed, *A. fumigatus* is seldom recovered from decaying organic residues in field cropping systems.

2. Exposure concentrations of azoles, at least in field cropping situations, are below levels likely to select for resistance, whereas, it is well established that resistance can evolve in hospitalised patients receiving azole therapy.

3. Agricultural triazoles are less active against *A. fumigatus* than medical ones, whereas imidazoles are equally active (Table 1). Imidazoles were being used widely in crop protection from the 1970s onwards, and although they could have selected for resistance, *A. fumigatus* resistance was not detected until the late 1980s.

Table 1. Azole sensitivity in itraconazole sensitive and resistant *A. fumigatus* isolates

Azole	Itraconazole-Sensitive EC50 (μg ml^{-1})	Itraconazole-Resistant EC 50 (μg ml^{-1})
Itraconazole	**0.13-0.23**	**2.0-16.0**
Voriconazole	**0.13-0.5**	**0.5-16.0**
Imazalil	**0.03-0.5**	**0.06-8.0**
Prochloraz	**0.05-0.5**	**0.13-16.0**
Propiconazole	**2.0-8.0**	**16.0-≥16.0**

4. To minimise the risk of resistance, azoles are generally used in crop disease control as mixtures (often pre-packed) with other modes of action such as chlorothalonil, folpet, QoIs, and SDHIs. There is no information on the activity of these fungicides against *A.fumigatus*, although they may equally well reduce the risk of resistance in *A. fumigatus*, as well as in target plant pathogens.

Conclusions

1. No critical evidence has emerged from appropriate field experiments, that not only detect *A. fumigatus* isolates within field cropping systems, but also show that their frequency increases following foliar sprays. Evidence that agricultural use of azoles are the origin, or increase the frequency, of azole resistance in *A.fumigatus* is lacking .

2. Restricting use of agricultural azoles would have serious impacts on disease control in many crops, and remove key tools in anti-resistance strategies.

This report is based on recent perspective article in Pest Management Science:

Derek Hollomon. Does agricultural use of azole fungicides contribute of resistance in the human pathogen *Aspergillus fumigatus*? DOI: 10 1002/ps 4607 (2017)