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GUEST COMMENTARY

Critical Annotations to the Use of Azole Antifungals for Plant Protection

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Fungal infections of plants cause a considerable loss of crop yields worldwide. In addition some fungi, such as *Fusarium* spp., while growing on plants are able to produce mycotoxins which can seriously harm the consumers. Hence, it is understandable that antimycotics are used in agriculture to control fungal growth on plants and fruits. Antimycotics are used also to prevent or to ease the problem of postharvest spoilage of these plants and fruits.

Various compounds have been described for their antimycotic activity against a broad range of fungi. Many of these compounds are potentially useful in plant protection. Among them, azoles are widely applied, besides dithiocarbamates, strobilurins, and benzimidazoles. Thousands of tons of azoles are sold annually for the purpose of plant protection. According to the instructions of manufacturers, about 100 g/ha should be used in the field. In other words, approximately 10 mg of azoles are finally applied on 1 m^2 of plant surface. Multiple applications per year are sometimes necessary, for example, during rainy seasons.

SOME REASONS FOR THE MASSIVE USE OF AZOLES IN AGRICULTURE

There are several obvious advantages of azoles over other antimycotic agents. Azoles are not only inexpensive but they also have a broad spectrum of antifungal activity. They are effective against mildews and rusts of grains, fruits, vegetables, and ornamentals; powdery mildew in cereals, berry fruits, vines, and tomatoes; leaf spots and flower blights in flowers, shrubs, and trees; and several other plant pathogenic fungi. Owing to a systemic action against invaded fungi, azoles, in contrast to other available antimycotics, are not just applied in preventing plant infection but also for treatment.

One particularly interesting feature of azoles is their long-lasting stability. Some azoles could remain active in certain ecological niches, e.g., in soil and water, over months with only slight changes in their chemical structures, e.g., loss of some side chains. The half-time of triadimenol, a primary metabolite of triadimefon, ranges from 110 to 375 days in soil (18). Consequently, azole residues have been detected in various food items, for example, commercial strawberry samples (26), grapes (1), or peppermint (5), reaching peak values of up to 0.5 to 0.8 mg/kg. High levels of azole residues were also detected in carrots during routine monitoring (6). Although the pesticide residues found in bulk samples have not reached health hazardous toxic levels, the amount of such remnant pesticides could, however, vary significantly in single items. It has been reported that the peak values in single apples can reach up to 2.16 mg/kg (6). Thus, there is evidence that considerable amounts of residues of antimycotic agents could persist in at least certain food items for quite a long time.

COMMON ANTIFUNGAL MECHANISM OF ALL AZOLES AGAINST ALL FUNGI, BOTH PLANT AND HUMAN PATHOGENS

Within the group of azoles various chemical derivatives are available, differing either in their characteristic imidazole or triazole ring or in the side chain. At the moment, some of these derivatives are either used medically or are under clinical evaluation (22). Other derivatives are used in agriculture but not in medicine. All azoles, irrespective of their distinctive chemical structure and variable biological properties, interact and target to the same active site in a fungal enzyme (7). Their mechanism is based on interference with the activity of fungal lanosterol 14α-demethylase, a member of the cytochrome P450 family. Fungal lanosterol 14α-demethylase is responsible for transforming lanosterol to ergosterol, which is an essential constituent of fungal cytoplasmic membrane. The inhibition of ergosterol formation would result in fungal cell wall disorganization and, finally, stop fungal growth. The mode of action of azoles, therefore, is fungistatic rather than fungicidal. (The term "fungicides," which is used in agriculture for this type of pesticide, is misleading). The strength and efficiency of antifungal activities vary strongly among the different azole derivatives. This is illustrated by the different MICs, ranging from about 0.075 to 8 mg/liter for azole-susceptible fungal strains. This implies that on plant surfaces and certain food items, the concentration of azole residues could exceed or reach the MICs for most normal plant as well as human pathogenic fungi and persist for several months.

MECHANISMS OF RESISTANCE TO ALL AZOLES

Most but not all fungi are primarily susceptible to azoles. Some fungi are intrinsically resistant because ergosterol is not required for their cell wall and membrane formation, e.g.,

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Pneumocystis spp. At least three different resistance mechanisms towards the azole group of antimycotics have been identified (24) and are summarized as follows: (i) exclusion or even active efflux from the fungi. Azole resistance is related to increased export from the fungal cell. Efflux pumps from the CDR family (members of the ATP binding cassette transporters) as well as MDR1 (a major facilitator) may be active. Several different CDR1 genes have been found in a fungal cell whereby some are involved in azole resistance. (ii) Resistance mechanisms may be based on structural alterations in the target fungal enzyme. (iii) Resistance may stem from overproduction of the target fungal enzyme.

Multidrug resistance, which means several resistance mechanisms occurring in one resistant strain, is also frequently observed. Genetic alterations may render an intrinsically susceptible strain resistant and, finally, result in the development of a permanent phenotype. Haploid fungal cells, such as *Candida glabrata*, might be more prone to such events (23). In contrast to bacteria, resistance carried on a plasmid, which would be able to spread easily from one cell to another, has not yet been described in fungi, so that in general the development of resistance in a population is more gradual. On the other hand, transient gene expression may temporarily render a strain phenotypically resistant. It has been observed that the production of CDR1 mRNA varies with different growth phases and physiological conditions, which in turn results in a phenomenon of growth cycle-dependent susceptibility of fungi (9).

INCREASING INCIDENCE OF RESISTANCE TO AZOLES AMONG FUNGAL HUMAN PATHOGENS

Azole resistance appears to be emerging as a serious problem in patients treated for yeast infections (24); in particular, the development of azole resistance in *C. glabrata* is becoming a major concern (4, 19). There are three ways by which patients may acquire azole-resistant fungi: (i) at the beginning, the infecting or colonizing strain is susceptible but mutates and develops azole resistance; (ii) the patient harbors a heterogeneous population and the inherently resistant variant is selected during treatment and exposure to antimycotics; or (iii) the patient acquires an inherently resistant strain from the external milieu. Azole-resistant *Candida* are found in patients not previously exposed to antifungal agents (24).

There is no doubt any more that resistance in fungal strains can develop in patients during prolonged treatment with azoles (13). On the other hand, resistant strains could also develop in the surrounding environment and gain access to humans afterwards. It was reported that a certain extent of airborne cryptococci taken up by AIDS patients and other immunocompromised patients were resistant prior to drug treatment (17). *Issatchenkia orientalis* (*Candida krusei*) and certain molds are even intrinsically resistant to some azoles because of a low uptake of these agents into the fungal cell (19).

ADDITIONAL PROBLEMS WITH FUNGI RESISTANT TO AZOLES

Yeasts which are highly resistant to azoles pose an increasing threat to patients (24). It has to be kept in mind that the acquisition of certain resistance mechanisms, such as efflux pumps, would at the same time lower the susceptibility of such strains to other nonrelated antimycotics. Some of these pumps, the major facilitators for example, can confer resistance to azoles as well as to a broad spectrum of other antifungals such as cycloheximide, benztriazoles, and other agents (24). In such a case the remaining options for an effective therapy are modest.

Other cellular functions also may be altered concomitantly with resistance properties. The increase in azole resistance might eventually lead to an increase in virulence. In a particular laboratory mutant of *Candida albicans*, the azole-resistant variant produced much higher amounts of extracellular aspartic proteinases, which represent important virulence factors; hence, this resistant mutant was much more virulent in mice than the azole-susceptible parent strain (3). Additionally, it has been reported that some yeasts are able to form hyphae even in the presence of azoles and are therefore more pathogenic than others (24). Moreover, phenotypic switching, which has been supposed to be involved in virulence, could also be hampered in azole-resistant fungi (20). Consequently, infection of humans by such resistant pathogens would be even more difficult to control by host defense mechanisms.

DOES THE USE OF AZOLES IN AGRICULTURE EXERT A SELECTIVE PRESSURE ON HUMAN PATHOGENIC FUNGI?

It is generally accepted that a strong and persistent antimicrobial pressure on a complex microbial population will lead to selection of resistant strains, particularly if the antimicrobial agents exert only a microbial static but not a microbicidal effect. This has been found to occur in fungi, too; for example, benzimidazoles, a group of antifungals which differ both chemically and biologically from azoles, heralded a revolution in the control of fungal plant diseases when they were initially introduced. However, benzimidazole-resistant strains, which were able to survive as fit as the naturally existing fungi in nature, emerged soon afterwards (15).

The development of resistance in plant pathogenic fungi to azoles is more complex and is generated slowly in small steps. Although it has been considered that the risk of selecting azoles-resistant strains is low, there have been reports claiming that some plant pathogenic fungi have indeed acquired azole resistance (15).

It is anticipated that the excessive use of azoles in agriculture would not only influence the plant pathogenic species but also would inevitably attack susceptible species of the saprophytic flora. These innocent bystanders, however, actively regulate the growth of pathogenic fungi and consequently play a beneficial role (8). Furthermore, such a disequilibration in the ecology of the fungal flora might also affect the population of medically important fungi. One possible consequence is that certain naturally existing human pathogenic fungi might survive and multiply. In particular those strains which have acquired azole resistance will profit from the selective pressure. This would greatly increase the risks and chances for humans to encounter such resistant microbes.

Many potentially human pathogenic fungi such as *Coccidioides*, *Histoplasma*, *Aspergillus*, and *Cryptococcus* have their natural habitat in the environment, including plants and food

TABLE 1. Habitats of yeasts[a]

Source(s) of isolates	% of total[b]
Plants, leaves, flowers	21
Fruits, juices	9
Beer, wine	4
Other food items	8
Earth, sludge	11
Water	6
Air	1
Insects	12
Animals	8
Humans	16
Machines	3

[a] According to Schauer and Hanschke (16).
[b] Frequency of isolation from source(s) relative to other sources.

items (16). These facts are also true for most yeasts (Table 1). As a matter of fact, only a few species of yeasts exist as normal flora of humans. This means that in many instances the infecting fungal organisms are taken up from the surrounding environment.

Although there is at the moment no concrete proof of whether the azole-resistance phenotype of human pathogenic fungi is induced by the extensive use of these agents for plant protection, it is obvious that the population of resistant strains in the environment could be enriched by the exertion of such a prolonged selective pressure. Hence, the chance arises that an individual will be exposed to resistant fungi.

CAN THE DIETARY INTAKE OF AZOLES EXERT A SELECTIVE PRESSURE ON FUNGI COLONIZING HUMANS?

A second medical concern, possibly of minor importance, would be the influence of azoles towards the fungal flora of human consumers. It has been reported that the dietary intake of azole residues generally does not reach a toxic level (6) or cause any harmful effects to consumers. Otherwise, the use of such pesticides would be strictly prohibited by the Food Quality Protection Act (21). However, it can be argued that these antimicrobial agents might exert a selective pressure on the colonizing *Candida* spp., in at least certain scenarios.

CONCLUSIONS

As a matter of fact, there is still no clear evidence for a correlation between the agricultural use of azoles and the increase in antimycotic resistance in human pathogenic fungi. A lot more scientific studies should be carried out to further understand this issue. Further work should elaborate on, for example, epidemiological studies of resistant fungi in humans and nature, the effect of selection of resistant fungi by long-term application of inhibitory or subinhibitory concentrations of azoles, and the increase in fungal virulence induced by such mechanisms. Nevertheless, as long as there is no convincing proof indicating that the massive agricultural use of azoles is absolutely independent from the increasing incidences of resistant human pathogenic fungi, extra care should be taken in terms of the application of azoles in agriculture. A more judicious antimycotic usage in agriculture should be observed. Indeed, many alternative antimycotics are available and, in principle, these alternative agents could replace the presently used fungistatic azoles in agriculture. Some reports have pointed out that the need for azoles is not always compelling. For instance, treating *Fusarium*-contaminated cereal grains indeed did not reduce the mycotoxin load (10). Treating turf grass with azoles (14) is also not indispensable.

In the final risk assessment for the use of azoles, not only the toxicological aspects but also the possibility of induction and/or selection of resistant human pathogenic fungi should be taken into account. In the past few years, there have been strong discussions on the use of antibiotics for plant protection (11) and as growth enhancers in animal feed (25). At least in the latter case, there is a consensus that antibiotics which are of medical importance should no longer be used in animal feed (2).

The use of azoles clinically is of high priority, since there are only a few available alternatives in medicine for prophylactic and therapeutic treatment of yeast and other fungal infections. On the other hand, it is obvious that the need for antimycotics in medicine is increasing due to the rising incidences of fungal infections. Yeasts, for example, are the fourth most common cause of nosocomial infections (12).

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