INSTITUTE FOR AGRICULTURE AND TRADE POLICY

Fungicide Resistance

Risk and Consequence in Modern Agriculture

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We want to thank the Center for Environment and Agriculture in Holland for bringing this issue to our attention in 2012. We also want to thank Patrick Tsai for his helpful comments and contributions in writing this paper.

PREFACE: FUNGICIDE RESISTANCE: REVENGE OF THE THIRD KINGDOM

The old taxonomy that came down from the great Swedish biologist Carl Linnaeus eventually classified fungi as the third kingdom, following animals and plants. It is a system that has fallen out of favor in the wake of new knowledge we've gained from the study of genes and DNA. But Linnaeus' classification is still used and helps us recognize the importance of fungi. Given the number of news reports in 2014 on fungal diseases affecting crops and animals, there should be little doubt that trouble is brewing in the third kingdom.

Bats, salamanders, Norway Maples, Hawthorns, wheat, corn, soy beans, coffee, potatoes, bananas, cats, cattle, honey bees—and the list goes on—no longer experience the beneficial effects of fungicides, leaving populations vulnerable to decimation by fungal disease. Not to be left out, humans are also at greater risk from fungi that have grown resistant to treatment. With threats to ecosystems, food security and public health, we are facing a trifecta of losses to the biological world that requires much greater public attention to factors that are increasing and accelerating the spread of disease.

What are those factors? They include: industrial agriculture based on multi-year mono-cropping; the overuse and inappropriate use of fungicides to increase crop yields; the globalization of trade and the worldwide movement of crops, livestock and pests without any effective biosecurity protocols; and finally, climate change which has shown to be a determining factor in the geographical reach and spread of fungal disease.

The threats posed by fungicide resistance are not new and they will not be going away. Chemical and pharmaceutical companies are responding by developing new and more powerful pesticides. They have turned to genetic modification for what is likely to be very short-term fixes that could complicate matters and create new and greater risks. We believe far more fundamental changes are needed in how we grow and trade our food to effectively deal with these dangerous threats to food security and public health.

The Institute for Agriculture and Trade Policy (IATP) is publishing this primer on fungicide resistance as part of an effort to help raise the alarm and provide basic information about fungicide resistance. We look forward to working with others around the world who, in many cases, know a great deal more about this issue than we do, and share our concern over the need to act.

–Dale Wiehoff, IATP

EXECUTIVE SUMMARY

Fungi are ubiquitous and vital members of nearly all ecosystems on our planet, from untouched wilderness to carefully managed agriculture. In our interaction with these creatures we have experienced both extraordinary benefits—including harnessing the process of fermentation—and terrible losses—including the Irish Potato Famine. The majority of fungal species are detritivores, quietly decomposing and recycling organic matter in soil or water. But some species are parasites, and they can threaten the health of humans, our crops and livestock, and wild species. There are a variety of methods available for managing fungal pathogens, and each presents a mixture of costs and benefits. Chemical fungicides are the most consequential of these management options. One of the potential costs of these chemicals is the risk of the fungal target evolving resistance to the fungicide, until it is no longer an effective treatment.

In this document, we present the essential background information necessary to understand the risk of fungicide resistance, and provide two case studies of fungicide use and resistance. These examples provide a portrait of modern agriculture in which widespread and indiscriminate fungicide applications have led to an increasing risk of fungicide resistance; reducing our ability to protect ourselves and our crops from the substantial, and sometimes catastrophic, effects of pathogenic fungi. To go along with this increased risk of resistance, recent trends in global trade and climate change have increased the incidence of dangerous emerging fungal diseases.

Our first case study takes us to The Netherlands, where a ubiquitous soil fungus, *Aspergillus fumigatus*, which can infect immunocompromised humans, began to show resistance to the azole family of medical fungicides. These chemicals are widely used in agriculture and medicine, and numerous lines of evidence indicate that this fungus evolved resistance to agricultural products before infecting humans. The consequences of this resistant fungi are sobering: 12 weeks after receiving a diagnosis, a staggering 88 percent of patients with a resistant infection had died. Although this environmental origin of this resistance has not been proven, it is now the leading hypothesis, and strikingly illustrates the serious consequences of resistant fungal pathogens.

The second case study is set on the vast corn belt of the American Midwest, where fungicides were rarely used before 2007. However, recent volatility in corn price have driven farmers to abandon non-chemical control options in pursuit of higher yields, leaving fungicides as the only recourse in the face of disease. Strobilurins, a common family of fungicides began to be marketed in corn to promote general plant health, even in the absence of disease, and the use of fungicides in corn was hugely expanded. However, a number of careful studies showed that these chemical applications generally fail to provide any financial dividends, and strobilurins have proven to high risk for the development of resistance. The widespread use of fungicides under these conditions shows vividly the considerable risk for the development of resistance that is created within the modern agricultural system.

These case studies are simply two examples of a much larger trend: the over-reliance on simple chemical solutions to complex ecological and evolutionary challenges. We need to abandon this myopic approach to agriculture because unnecessary fungicide applications increase the risk of resistance, the consequences of which may be quite dire indeed.

FUNGAL BIOLOGY

Fungi represent one of the three great kingdoms of multicellular life of this planet. More closely related to animals than plants, but truly distinct from both, these creatures are vital to many human interests, but can also cause catastrophic, almost unthinkable damage. Fungi are responsible for bread and beer and wine, but also the Irish potato famine and the near extinction of the American Chestnut (*Castanea dentata*). To know how to best share our world with these organisms, to avoid their depredations while appreciating their benefits, we must first gain an understanding of their biology.

There are some 100,000 species of fungi that have been formally described, but the total number of species have been estimated to be over 5 million.[1] This figure does not include the Oomycetes, an unrelated group of organisms who share a similar biology and ecology, and which are included in most analyses of fungi in agriculture, including this one. The vast majority of these species are saprotrophs, meaning they survive by decomposing organic matter within soil ecosystems, obtaining the energy, carbon and nutrients that they need from dead and decaying plants and animals. To thrive in this environment, fungi have a unique physical structure, made up of microscopic filaments, called "hyphae," that are each a single cell in diameter. These filaments are connected in dense network called a "mycelium." This structure gives fungi an extraordinary amount of surface area for its volume, allowing it to interact very closely with its immediate soil environment, secreting enzymes, and absorbing nutrients and water across all of its surfaces.[2]

In addition to this dispersed, vegetative and microscopic life stage, fungi have distinct, and often macroscopic reproductive stages. These include the recognizable mushrooms that we generally associate with fungus. While there are a wide variety of reproductive strategies among the fungi, all groups

produce asexual spores, genetically identical to the parent fungus, and nearly all have sexual stages that can combine with other individuals to produce genetically unique spores. All spores are then dispersed into the environment to grow into new hyphae, and another mycelium, continuing the effort of decomposing organic matter and recycling carbon and nutrients within global ecosystems.[3]

Not every fungal species follows this path as a solitary soil saprotroph however. Some fungi have adapted to become exceptional symbiotes, meaning that they have formed a close relationship with another species (often a plant). Some of these relationships are mutualistic, where both partners benefit, while others are pathogenic, with the fungus growing at the expense of its host, sometimes killing it in the process. The classic case of fungal mutualism are the so called mycorrhizal fungi. This diverse assemblage of fungi form close attachments to the root systems of plants, often extending hyphae into the root cells themselves. The fungi then use the exceptional surface area of their mycelia to collect water and nutrients for the host plant, and in return they extract energy-rich sugars from the root. These associations occur in an estimated 80 percent of plant species, and are essential for many human crops, including corn.[4,5]

Our crops are similarly affected by pathogenic fungi. These diseases have been known since antiquity, and can cause significant damage to yields in nearly every crop. These fungi use their distributed body plan to integrate themselves into plant tissues and cells, secreting chemicals to evade plant immune responses and enzymes to destroy and digest plant tissues. These fungal pathogens are essentially treating a living plant as though it was dead organic matter to be decomposed. They can then produce large volumes of spores to infect and destroy new hosts. Fungal diseases do not only affect crops of course. Humans, our livestock, and wild plants and animals are all subject to pathogenic fungi. To combat this threat, humans have developed a number of techniques to protect ourselves and our domesticated species. Chief among these techniques is the use of chemical fungicides.[6]

Fungicides and fungicide history

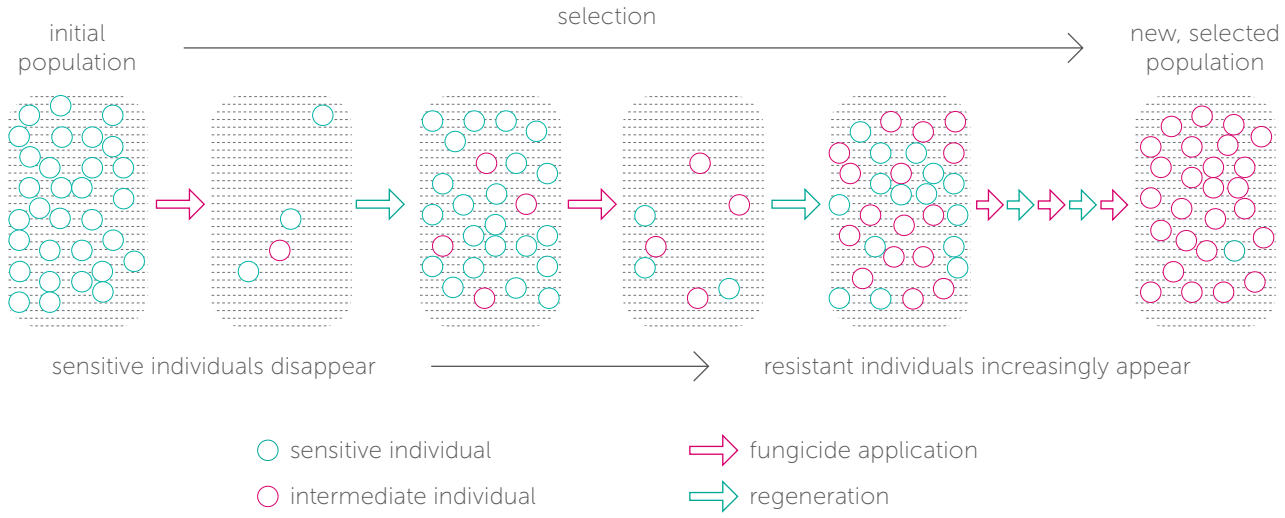
Fungicides, i.e., chemical compounds used to selectively destroy fungal pathogens, are a central component of modern agriculture. There are currently 150 or so fungicidal compounds on the market, although they are formulated and combined into a much larger number of individual proprietary products.[7] The benefits of any anti-fungal treatment, whether chemical or nonchemical, comes from its reduction in the fungal population and any subsequent increase in yield. This benefit must be weighed against the costs of the treatment, including the purchase price, application costs and any downstream costs to human or environmental health. In addition, chemical fungicides entail an additional cost: The risk of a fungi evolving resistance to the treatment, limiting or eliminating its effectiveness in the future. These costs have varied across time as new and more effective fungicides have been introduced, but generally have been low enough to warrant widespread use. Chemical fungicides are currently used annually on more than 80 percent of fruit and vegetables in the U.S. with the total benefits estimated at $13 billion a year.[8]

Since the "Bordeaux Mixture" (an aqueous mixture of copper sulfate and calcium oxide) was developed and popularized by the French botanist Millardet in 1885, chemical fungicides have been a powerful tool available to western agriculture. These early fungicides, including earlier copper formulations, were effective against a range of fungal pathogens, but could be damaging to the treated plants and irritating and expensive to apply.[9] Although these early treatments were generally prepared by the farmer and applied at very high concentrations (~10–20 kg/ha) resistance to these fungicides was not a major concern. Throughout the twentieth century, new families of fungicides have been introduced, each one generally more effective at lower doses than those that came before it. They have also typically boasted substantially reduced toxicity towards treated plants and nearby humans.[10,11]

In the 1970s, fungicide resistance began to be recognized as a serious risk when new families of fungicides lost effectiveness within just a couple of years of their introduction.[12] To deal with this risk, the companies within the fungicide industry came together to share research, monitor for resistance and provide consensus recommendations for users to reduce the risk of fungicide resistance. This group, now called the Fungicide Resistance Action Committee, or FRAC, has continued this important work with both new and existing fungicides.[13] New classes of fungicides, to which resistance has not yet evolved, remain a vital tool for providing effective pathogen control, often replacing older chemicals which many fungi have become resistant to.[14] This constant need for new fungicide chemistries is part of the cost of fungicide resistance, which needs to be considered against the benefits whenever chemical fungicides are applied.

Genetic variation exists in populations of pathogenic fungi, as it does in all organisms. This variation provides the seed from which fungicide resistance can develop. Random genetic mutations, an inescapable consequence of any biological reproduction, creates and sustains this variation in the genome. Purely by chance, some of these mutations will limit the effectiveness of a fungicide, usually by preventing the chemical from binding to its target inside the fungal cell, or expelling the chemical before it can do any harm. But this lucky mutant is still only a single spore or hyphal cell.[15,16] To

Figure 1: A schematic of the evolution of fungicide resistance. An initial population with negligible resistance changes until resistance is widespread due to repeated fungicide applications.



Adapted from Deising et al., 2008.

create a whole population of resistant fungal pathogens, natural selection must take place in which the environment favors the reproduction and proliferation of resistant forms. Individual fungicide applications can be considered the "selection events" that drive this process, selectively removing susceptible fungi.[17] However, any resistant mutants will survive these events and subsequently have the opportunity to grow and reproduce without competition from their fungicide susceptible peers. Each selection event furthers this process, leaving a fungal population with a greater proportion of resistance than the one that existed previously. After an application, this increasingly resistant population is allowed to proliferate and reproduce.[18]

Evaluating the risk of resistance

A detailed understanding of the theory behind resistance biology (outlined above) provides a framework for determining the risk of resistance evolving in a given system, based on the characteristics of the fungicide, the target pathogen, and the method by which it is used.[19] Some of these risk factors are listed below:

Pathogen risk factors:

- **LIFE CYCLE:** in fungi with short generation times, there are a greater number of opportunities for resistant mutants to arise. Furthermore, these diseases often require frequent fungicide treatments, further increasing the risk.[20]

- **REPRODUCTIVE CHARACTERISTICS:** Fungi that produce exceptionally large numbers of spores have a correspondingly high likelihood of producing mutants, some of whom could be resistant. Spores that can travel long distances increase the risk that resistant forms will spread once they have arisen.[21]

Fungicide risk factors

- **MODE OF ACTION:** Fungicides that interfere with a single product or process within the fungus (or "single-site" fungicides) can often be undone by a mutation to a single location in the fungal genome. Some older fungicides disrupt numerous processes within the fungal cell. These "multi-site" fungicides are much more difficult to evolve resistance to, and these fungicides have been able to be used for much longer before resistance has been observed.[22,23]

- **SYSTEMIC:** Some fungicides can be taken up by the treated plant and distributed throughout its tissues, providing protection to all of its parts, whether or not

they were directly sprayed by the chemical. The use of these "systemic" fungicides have been associated with far more resistance than would otherwise be expected. The cause of this is not clearly understood, and this pattern may be related to other factors.[24,25]

Usage risk factors

- **FREQUENCY:** The more regularly a fungicide is used, the more opportunities natural selection has to create and refine a resistant fungal population. Therefore, frequent applications are a major risk factor for resistance. Controlling the number of applications in a season is a major aim for fungicide management and labeling.

- **EXCLUSIVITY:** If a single fungicide is relied upon heavily for fungicide control, the chances of resistance emerging are dramatically higher than if a number of fungicides are used in tandem to control the same threat. Spontaneous mutants resistant to one of these chemicals is likely still susceptible to the others, and will therefore be unable to survive and spread. To maximize the benefit, the combined fungicides should come from distinct chemical families. To decrease the likelihood of a mutant resistant to both fungicides. Many marketed products are pre-mixed combinations to decrease the risk of resistance.

- **DOSE:** It has been generally accepted that high fungicide doses—by exterminating all fungal cells, even the slightly resistant ones—provide greater protections against resistance. For this reason, fungicide manufacturers have frowned upon the practice of applying lower than recommended doses to save costs. The theoretical and experimental evidence has not resolved this debate satisfactorily and the effect of dose on resistance may well depend on the exact method by which resistance evolves.[26,27]

These three aspects of risk should be considered together for any agricultural system in which a fungicide is used. The use of a high-risk fungicide will be more likely to cause problems when used against a high-risk pathogen than it would otherwise, while the same risky fungicide may be safely used in a low-risk agricultural system.[28]

In the early 1970's, concern over fungal resistance to the numerous chemicals then on the market to control fungal pathogens was negligible. The inorganic fungicides that had been on the market for decades had multi-site modes of action, and scattered reports of resistance to various products was not considered a major problem.[29] When resistance to the benzimidazole class of fungicides emerged less than two years after their introduction, the manufacturers of these products, that included DuPont and Janssen Pharmaceuticals, among others, provided no guidelines for managing resistance and failed to publish the results of early monitoring and research for another decade.[30,31] Shortly thereafter, widespread resistance was observed in another class of fungicides (the 2-aminopyrimidines), that also had only been in use for two years. This time, the company producing the affected products responded proactively, halting commercial use against resistant populations. Since this period, every new class of fungicide has been affected by resistance to some degree, often within a few years of their introduction. In a recent review, resistance was observed in 55 percent of the surveyed fungal diseases.[32]

The loss of effectiveness of chemical fungicides is particularly troubling at this time period. For the past two decades, fungal pathogens have been responsible for an increasing number of emerging infectious diseases, which have been responsible for significant and increasing crop losses, human health risks, and extinctions in wild species.[33] For instance, an outbreak of coffee rust (*Hemileia vastatrix*) in Central America has caused enormous losses: 50 percent of the 2013-14 crop was lost in the most impacted portions of Costa Rica, and 60 percent of trees in a Mexican research plot had more than 80 percent defoliation.[34] There have also been worrying outbreaks in human diseases. *Cryptococcus gatti*, a fungal disease native to Southeast Asia, recently infected patients in British Columbia, Washington and Oregon, occasionally proving fatal.[35,36] In wild species, the effects have been even more devastating. For instance, *Batrachochytrium dendrobatidis*, a fungal disease affecting amphibians has caused spectacular die-offs and extinctions in a wide number of frog species in Central America. In some areas, 40 percent of the original number of species were lost.[37]

These kinds of outbreaks have been increasing due to human activities. Recent patterns in global trade have increased the number of fungal pathogens moving across long distances, heightening the risk of novel and dangerous fungal pathogens. Global climate change and land use change have likely also driven some recent fungal outbreaks.[38,39] While concerted effort can and should change these anthropogenic, i.e., human-caused drivers, we must prepare for a future where these factors continue to increase the number of emerging infectious diseases in humans, our domesticated crops and livestock and wild species. To do that, we must be careful in how we use our fungicides, an important and powerful tool, so that they do not lose their effectiveness against new and existing threats.

Table 1: The origins of resistance in major fungicide classes. Earliest dates for practical resistance, and important resistant fungi are noted. Adapted from Brent, 2005.

Date first observed (approx.)	Fungicide or fungicide class*	Years of commercial use before resistance observed (approx.)	Main crop diseases and pathogens affected
1960	Aromatic hydrocarbons	20	Citrus storage rots, *Penicillium* spp.
1964	Organo-mercurials	40	Cereal leaf spot and stripe, *Pyrenophora* spp.
1969	Dodine	10	Apple scab, *Venturia inaequalis*
1970	Benzimidazoles	2	Many target pathogens
1971	2-Amino-pyrimidines	2	Cucumber and barley, powdery mildews *Sphaerotheca fuliginea & Blumeria graminis*
1976	Phosporothio-lates	9	Rice blast, *Magna-porthe grisea*
1977	Triphenyltins	13	Sugar beet leaf spot, *Cercospora betae*
1980	Phenylamides	2	Potato blight and grape downy mildew, *Phytophthora infes-tans & Plasmopara viticola*
1982	Dicarboximides	5	Grape grey mould, *Botrytis cinerea*
1982	Sterol Demethylation inhibitors (DMIs)	7	Cucurbit and barley powdery mildews, *S. fuliginea & Blumeria graminis*
1985	Carboxanilides	15	Barley loose smut, *Ustilago nuda*
1998	Quinone outside Inhibi-tors (QoIs; Strobilurins)	2	Many target diseases and pathogens
2002	Melanin Biosynthesis Inhibitors (Dehydratase) (MBI-D)	2	Rice blast, *Magna-porthe grisea*

Unfortunately, in many cases this has not been taking place. In the following two case studies, we see the risks that fungicide resistance can pose, decreasing our ability to manage fungal pathogens, even in the context of human health. We also observe the needless overuse of some fungicides, despite the very real risks of resistance.

AZOLE RESISTANCE IN HUMAN PATHOGENS

Aspergillus is a common genus of Ascomycete fungi (i.e., related to morels and truffles) containing nearly 200 distinct species. Like most fungi, they are saprophytes, decomposing dead organic matter and recycling carbon and nitrogen in soil and aquatic environments. *Aspergillus* is ubiquitous in nearly every environment, indeed, most people likely come into contact with hundreds of *Aspergillus* spores every day. Due to their ubiquity, different species have been employed by humans to produce soy sauce, sake, citric acid and pharmaceuticals.[40] However, this genus is not always beneficial. Some species, including *Aspergillus flavus* and *Aspergillus parasiticus* can contaminate common crops with dangerous toxins, while another species, *Aspergillus fumigatus,* can be a dangerous pathogen in susceptible human populations.[41]

Aspergillus infections, termed "aspergilloses," can come in a variety of forms, differing in severity from minor to life-threatening. Infection nearly always occurs through inhalation of spores, which are typically cleared from the respiratory system by various immune responses. Therefore, healthy patients are at little risk for aspergillosi.[42]

The more minor forms of aspergillosis (termed allergic bronchopulmonary aspergillosis (ABPA)) affect those suffering from severe asthma or cystic fibrosis. In these patients, a heightened immune response to *Aspergillus* spores triggers inflammation of the airways leading to damage of these sensitive tissues. A more severe aspergillosis (termed chronic pulmonary aspergillosis (CPA)) can affect those suffering from severe lung diseases, such as tuberculosis or emphysema. in these circumstances, the *Aspergillosis* gains a foothold within the lung and begins to grow and reproduce asexually. these growths can further reduce lung function, but do not spread to other organs. The last, and most dangerous type of infection is invasive aspergillosis (IA). In patients with reduced immune function, including those suffering from AIDS or myeloid leukemia, *aspergillosis* can grow unchecked, spreading throughout the lungs, into the bloodstream, and damaging organs throughout the body. If left untreated, invasive aspergillosis is often deadly. The primary treatment for these diseases are a family of fungicides called azoles.

Azole fungicides are currently the most widely used antifungal agents in medical, veterinary and agricultural contexts. Medical azoles include posaconazole and itraconazole, while agricultural azoles include tebuconazole and epoxiconazole. Numerous manufacturers market this family of chemicals, which interfere with the production of ergosterol, an essential structural component of fungal cell membranes.[43] The effectiveness of azoles against many different groups

of fungi has contributed to their popularity since they were first introduced in the 1970's. In agriculture, Azoles are used on many crops to prevent a diverse array of diseases, as well as in stored crops to prevent contamination.[44] These fungicides are now the primary agent used to control *Mycosphaerella graminicola*, which causes septoria leaf blotch in winter wheat. This fungus has already evolved resistance to two other families of fungicides used to control it in the past, and has begun to lose sensitivity to azoles as well.[45]

In a medical context, azoles have been used extensively to combat opportunistic human pathogens including *Candida albicans*, the most frequent cause of thrush, as well as *Aspergillus*. Resistance against azole medications has been observed in both of these fungi.[46] In *Aspergillus* in particular, azole resistance appears to have dramatic negative consequences. Mortality rates for azole-resistant invasive aspergillosis are sobering: 12 weeks after receiving as positive diagnosis, 88 percent of patients are dead.[47] Moreover, evidence is mounting that agricultural fungicides are responsible for some newly resistant strains of *Aspergillus.*

Azole resistant *Aspergillus* has been observed in medical contexts for some time. It tends to appear in patients with a recurring *Aspergillus* infection (CPA) that demands long term treatment with azole antifungals. These resistant fungi display a number of defining characteristics: They appear only after azoles have been used on a patient for some time, and they produce asexual spores in lung cavities, allowing a resistant mutant to quickly spread throughout the infected lung. The genetic mechanisms for this resistance are diverse, with each patient often presenting a unique mutation, indicating that the resistant fungi evolved independently within each individual.[48] However, since 1999, azole resistance has started to appear without these hallmarks of clinical origin. It has been hypothesized that this new strain developed resistance to agricultural azoles, and only subsequently infected humans.[49]

This new strain, named "TR34/L98H" as a description of the complex genetic mutation that gives it azole resistance, has appeared in patients who have had no prior contact with these medical fungicides (these patients are termed "azole-naive"). Additionally, many of these patients have the most severe aspergillosis (IA) which does not typically undergo asexual reproduction.[50] Since it was first observed, numerous lines of evidence, provided below, have converged to suggest that TR34/L98H may have originated in the environment. While the data is not yet conclusive, the environmental origin of this strain, driven by use of agricultural azoles, is now the leading hypothesis.[51]

Evidence for environmental origin

RESISTANCE BEFORE MEDICAL EXPOSURE: The presence of TR34/L98H in azole naive patients presents strong evidence that this strain has an environmental, and non-medical origin. 64 percent of patients in a Dutch study had no exposure to azole medications before they were found to be infected with resistant *Aspergillus*.[52] Moreover, no azole-susceptible, ancestral version of TR34/L98H has been found in a patient. this seems to imply that evolution of resistance occurred before the fungus entered the human population.[53]

CROSS RESISTANCE TO AGRICULTURAL PRODUCTS: The TR34/L98H strain is resistant to all three of the medical azoles used to treat aspergillosis. Additionally, susceptibility testing by Dutch researchers has determined that this strain is also resistant to five of the most common agricultural azoles which have similar molecular structures.[54] These chemicals have been widely used in Europe since the early '90s on wheat fields, grapevines, and numerous other agricultural products. This information suggests that TR34/L98H may have evolved resistance to these agricultural products, with resistance to medical azoles occurring as a side effect.

GENETIC DATA: The TR34/L98H strain has a set of two genetic changes that provides it with its broad resistance to azole fungicides. fungi that have developed resistance in medical contexts tend to have much simpler mechanisms.[55] It is therefore likely that this strain arose in an environmental context, where asexually and sexually reproducing fungi can spread and combine resistance mechanisms to greater effect. Researchers have attempted to genetically determine the time period when TR34/L98H first arose, This appears to have occurred around 1997, shortly after azole fungicides started to be used in European agriculture.[56]

ENVIRONMENTAL COLLECTIONS: *Aspergillus* with the TR34/L98H mutation has been collected from the environment across Europe. Compost and other gardening materials contained this resistant strain, as well as other environmental samples, including Norwegian fjord water, and soil samples from Denmark and the UK. Samples from natural areas, which are less likely to be exposed to agricultural fungicides, do not harbor the TR34/L98H strain.[57]

Despite the accumulated evidence, it cannot be said that the hypothesized environmental origin of TR34/L98H has been proven. One limitation of this hypothesis is that researches have been unable to induce the TR34/L98H into susceptible *Aspergillus* under laboratory conditions. Dutch researches have attempted to use agricultural fungicides, either singly or in combination to induce this mutation or something similar with no success.[58] This may be due to the the need for both sexual and asexual reproduction in the development of

this mutation. While both these processes occur in the environment, generally only asexual reproduction is allowed in laboratory conditions.[59,60]

While researchers attempt to determine the exact origins of TR34/L98H, it is not standing still. This resistant strain has been observed in clinical patients in Germany, India and China.[61] Environmental samples have been found in India as well, where azole fungicides are commonly used.[62] Moreover, a similar, but genetically distinct resistant form has now been observed in six Dutch hospitals, increasing the proportion of *Aspergillus* which is not affected by azoles.[63]

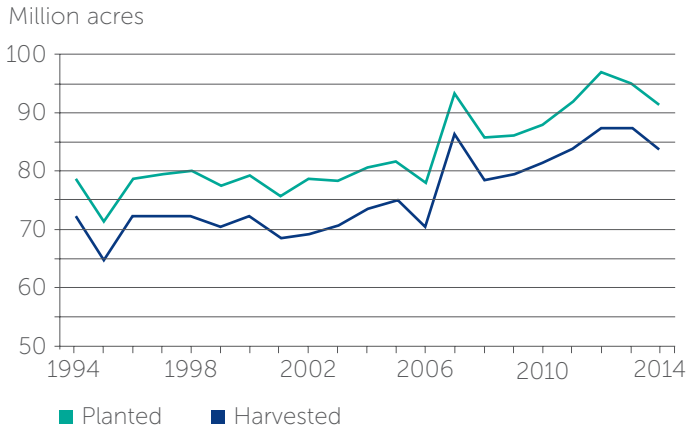
There are substantial costs associated with these resistant forms. In patients who have tested positive for azole resistant IA, mortality rates over 12 weeks are exceedingly high at 88 percent.[64] Other than azoles, there are no oral antifungals that can be used against aspergillosis, necessitating expensive and dangerous intravenous treatments.[65] TR34/L98H, and other resistant forms are unlikely to disappear, even if the use of agricultural fungicides is seriously rolled back.[66,67] This means that the best—and sometimes only—time to address fungicide resistance is before it appears. We must remain vigilant against improper overuse of these fungicides, otherwise they will no longer be available when we need them.

STROBILURIN FUNGICIDES IN CORN: NEEDLESS OVERUSE

Corn (*Zea mays*) is an immensely important cash crop produced throughout the North American great plains. This year, corn was planted over nearly 100 million acres in the United States, yielding 14.4 billion bushels of grain worth tens of billions of dollars. Production has increased over the past two decades. The ubiquity of this crop on the landscape creates tempting opportunities for the various diseases and pests that attack it and real demands for products to manage these pests. With the possibility for these organisms to reach large population sizes, and to disperse genetic material over a vast geographic extent, the development of resistance is a threatening possibility. The minimization of that risk ought to be a consideration in all management decisions.

Strobilurin fungicides are a class of fungicides first introduced to the market in 1996. By the end of the decade they comprised over 10 percent of the global fungicide market. These fungicides interfere with cellular respiration, preventing fungal cells from obtaining energy by burning fuel. Although other families of fungicides also attack this cellular respiration pathway, the specific binding site for strobilurins had not been targeted before. Because novel modes of action are infrequently discovered, even single-site modes of action like this one, these fungicides quickly grew in popularity. This trend was magnified because strobilurins proved effective against a wide range of plant pathogens, and in 1999, a single strobilurin fungicide, azoxystrobin, had sales totaling $415 million, higher than any other fungicide on the market.[68]

Figure 2: U.S. Corn Production. The area of the United States planted in corn has increased over the past two decades.



Adapted from USDA-NASS 2014.

Strobilurins are generally effective against a wide range of fungi that attack crop plants. Additionally, these fungicides are particularly effective against fungi early in their life cycle, while spores are germinating. Many other fungicide families are not effective at this stage, increasing the value of strobilurins. But the effects of these fungicides does not seem to end with the removal of disease. Numerous studies, particularly in wheat and barley, have shown that strobilurin applications provide a multitude of beneficial "plant health" effects: increased grain size, more efficient water use, stronger stems, greener leaves late in the season and, ultimately, higher yields. It is not yet clear whether these results are due to the direct effects of strobilurins on the physiology of the crops, or some effect on a pathogenic or nonpathogenic fungi that has escaped the notice of researchers in the field. Neither hypothesis has been fully explored, and some combination is also possible. However it cannot be doubted that these plant health effects have contributed to the popularity of strobilurins in a variety of crops.[69]

One of those crops is now hybrid corn, which saw very low amounts of fungicide applications before 2007. This changed rapidly, and foliar fungicides are now applied to more than 10 million acres of corn in the U.S.[70,71] There are a number of causes for this rapid increase, driven by interacting changes to corn growing economics and production practices. Prior to 2007, the numerous fungal pathogens of corn were managed using the non-chemical methods popularized by integrated

pest management (IPM), including the use of corn varieties that are less susceptible to fungi, regular crop rotation, and tilling to ensure that residue from previous crops (which often harbors fungal hyphae and spores) will not come into contact with the new crop.

Production practices that decrease tilling have recently been popularized due to their numerous environmental benefits, including reduced erosion and greenhouse gas emissions. The adoption of these practices has increased the amount of crop residue on the surface of the soil, increasing the likelihood of fungal diseases, and the need for fungicides. Additionally, some corn diseases have begun to occur in areas that were previously unexposed. This is expected to continue because of increasing global trade and a changing climate, further contributing to the need for fungicides.[72,73]

Increased volatility in corn prices since 2005, driven by global markets and biofuel requirements, has produced high prices for short periods of time. This has increased fungicide use for a number of reasons: Most simply, small yield benefits from a fungicide might pay for themselves when corn is expensive, but not be worthwhile when corn prices are low. High prices for corn also convinced many farmers to stop rotating corn with other crops such as soybeans. This continuous cropping increases the risk of pathogens, by providing a host population in the same location year after year. Lastly, as prices increased, more corn varieties were selected due to their yield alone, and varieties that were bred to be less susceptible to fungal pathogens were abandoned. All of these trends combined to create an environment where farmers had to rely exclusively upon fungicides to protect against fungal pathogens.[74]

In 2008 the EPA approved a supplemental label for the strobilurin fungicide marketed as "Headline" by the BASF corporation. This supplemental label claimed numerous plant health benefits for their product in corn as well as other crops.[75] Shortly after this label was approved, numerous academic researchers of plant pathology and integrated pest management raised concerns that strobilurin application in the absence of disease would increase the risk of resistance and other negative environmental effects, and that the yield benefits were not well established in corn. The researchers called this use of fungicides "a serious blow to IPM principles."[76]

Managing the risk of fungicide resistance is central in IPM and critical for responsible fungicide application. Particular care ought to be taken with fungicides that have provoked resistance in other contexts.[77] Although no strobilurin resistance has yet been observed in the fungal pathogens of corn, It has been observed in over 40 other crops where these fungicides have been used, including wheat, barley, soybeans and squash. In some instances resistance has evolved within two years of the fungicide being introduced.[78,79,80] The Fungicide Resistance Action Committee (FRAC) considers the strobilurin fungicides to have a high risk for resistance development.[81] The unnecessary application of these products will increase the likelihood that they will lose their effectiveness against fungal pathogens of corn or nearby crops.

The principles of IPM demand that disease/pest control be conducted in an economically cogent manner, so that the benefits of any action taken clearly outweigh the costs. Recent research has demonstrated that any yield benefits from strobilurin application to corn are marginal, and uncertain to outweigh the application costs.[82,83] Dozens of individual reports on the plant-health effects of strobilurins were conducted in corn grown across the midwest after these products came into frequent use. A number of meta-analyses compiled this body of research to understand the economic potential of this practice. In general, these compilations show that strobilurins can be beneficial: Roughly 80 percent of treated plots had a higher yield than untreated controls in one analysis. However,this yield increase was insufficient to offset the likely application costs in 45 percent of cases.[84] Another meta-analysis calculated the probability of losing money by applying various strobilurin fungicides under various economic conditions. For the vast majority (85 percent) of the conditions they modeled, there was a greater than even chance that the application would not pay off.[85] Even under the most generous conditions, there was a greater than 25 percent chance of losing money by applying strobilurins without evidence of fungal disease.[86]

It should be noted that this research does not imply that all strobilurin applications are unwarranted. In conditions where fungal diseases are observed (greater than 5 percent severity) 59 percent of applications are likely to pay off.[87] Monitoring of disease populations in the field is a principle of IPM and can provide indications when the use of strobilurin fungicides are worth their application costs. While large-scale monitoring of these diseases is possible and desirable, even simple observations of weather, timing and location can provide indications as to whether fungicide applications are warranted.[88]

Reducing unneeded strobilurin applications can certainly save money for farmers. But it will also substantially reduce the risk of resistance to these fungicides. Across the globe, novel fungal threats to humans, our crops and livestock, and natural ecosystems have been emerging and an alarming clip. In this context, the effectiveness of our fungicides ought to be carefully shepherded. In particular, the strobilurin fungicides—which provide broad effectiveness and present few environmental or health risks but constitute a high risk for resistance—should be used only when needed. Therefore the

widespread use of these products in the pursuit of often illusory yield benefits cannot continue across the vast expanse of the U.S. corn belt on which it now occurs.

In the strobilurin case study we've seen that current usage practices for some fungicides exceed any economic rationalization. This leads to an unacceptable increase in the risk of resistance arising. As demonstrated in the azole case study, widespread fungicide use can result in the appearance of resistance in unexpected systems, including medical contexts. When resistance appears, the consequences can be dire.

These are simply two examples of the many cases that demonstrate a widely applicable conclusion: Widespread and indiscriminate fungicide applications in modern agriculture have led to an increasing risk of fungicide resistance; reducing our ability to protect ourselves and our crops from the substantial, and sometimes catastrophic, effects of pathogenic fungi. These case studies are portraits of an agricultural system that wants simple chemical solutions to complex ecological and evolutionary problems. In an age where climate change and globalization are likely to increase the number and severity of fungal threats, this mindset cannot continue. A sensible, rational and precautionary approach to the use of our chemical fungicides would likely greatly reduce the risk of resistance occurring in existing systems, and ensure that when new fungal threats inevitably emerge, there are still effective practices available to protect crops and livestock, human health, and the ecosystems upon which we all rely.

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