The Role of Plant Agricultural Practices on Development of Antimicrobial Resistant Fungi Affecting Human Health: Proceedings of a Workshop Series (2023)

DETAILS

194 pages | 6 x 9 | PAPERBACK

ISBN 978-0-309-69767-5 | DOI 10.17226/26833

CONTRIBUTORS

Elizabeth Ashby, Anna Nicholson, and Tamara Haag, Rapporteurs; Forum on Microbial Threats; Board on Global Health; Health and Medicine Division; National Academies of Sciences, Engineering, and Medicine

SUGGESTED CITATION

National Academies of Sciences, Engineering, and Medicine. 2023. *The Role of Plant Agricultural Practices on Development of Antimicrobial Resistant Fungi Affecting Human Health: Proceedings of a Workshop Series*. Washington, DC: The National Academies Press. https://doi.org/10.17226/26833.

NATIONAL ACADEMIES *Sciences Engineering Medicine*

NATIONAL ACADEMIES PRESS
Washington, DC

The Role of Plant Agricultural Practices on Development of Antimicrobial Resistant Fungi Affecting Human Health

Elizabeth Ashby, Anna Nicholson, and Tamara Haag, *Rapporteurs*

Forum on Microbial Threats

Board on Global Health

Health and Medicine Division

Proceedings of a Workshop Series

NATIONAL ACADEMIES PRESS 500 Fifth Street, NW Washington, DC 20001

This activity was supported by contracts and awards between the National Academy of Sciences and American Society of Tropical Medicine and Hygiene, Burroughs Wellcome Fund, Centers of Disease Control and Prevention (Contract No. 75D30121F00095), EcoHealth Alliance, ExxonMobile Foundation, Infectious Diseases Society of America, Johnson & Johnson (Contract No. C2021020002), Merck & Co., Inc., National Institute of Allergy and Infectious Diseases (Contract No. HHSN263201800029I, Task No. HHSN26300011), New Venture Fund (Contract No. NVF-NGDF-NAT10-Subgrant-017387-2022-04-01), Sanofi Pasteur, U.S. Agency for International Development (Grant No. 7200AA18GR00003), Uniformed Services University of Health Sciences (Award No. 5R13FD006897-03), U.S. Food and Drug Administration (Award No. 5R13FD006897-03), U.S. Department of Veterans Affairs (Contract No. 36C24518D0171). Any opinions, findings, conclusions, or recommendations expressed in this publication do not necessarily reflect the views of any organization or agency that provided support for the project.

International Standard Book Number-13: 978-0-309-XXXXX-X
International Standard Book Number-10: 0-309-XXXXX-X
Digital Object Identifier: https://doi.org/10.17226/26833

This publication is available from the National Academies Press, 500 Fifth Street, NW, Keck 360, Washington, DC 20001; (800) 624-6242 or (202) 334-3313; http://www.nap.edu.

Printed in the United States of America.

Suggested citation: National Academies of Sciences, Engineering, and Medicine. 2023. *The role of plant agricultural practices on development of antimicrobial resistant fungi affecting human health: Proceedings of a workshop*. Washington, DC: The National Academies Press. https://doi.org/10.17226/26833.

The **National Academy of Sciences** was established in 1863 by an Act of Congress, signed by President Lincoln, as a private, nongovernmental institution to advise the nation on issues related to science and technology. Members are elected by their peers for outstanding contributions to research. Dr. Marcia McNutt is president.

The **National Academy of Engineering** was established in 1964 under the charter of the National Academy of Sciences to bring the practices of engineering to advising the nation. Members are elected by their peers for extraordinary contributions to engineering. Dr. John L. Anderson is president.

The **National Academy of Medicine** (formerly the Institute of Medicine) was established in 1970 under the charter of the National Academy of Sciences to advise the nation on medical and health issues. Members are elected by their peers for distinguished contributions to medicine and health. Dr. Victor J. Dzau is president.

The three Academies work together as the **National Academies of Sciences, Engineering, and Medicine** to provide independent, objective analysis and advice to the nation and conduct other activities to solve complex problems and inform public policy decisions. The National Academies also encourage education and research, recognize outstanding contributions to knowledge, and increase public understanding in matters of science, engineering, and medicine.

Learn more about the National Academies of Sciences, Engineering, and Medicine at **www.nationalacademies.org**.

Consensus Study Reports published by the National Academies of Sciences, Engineering, and Medicine document the evidence-based consensus on the study's statement of task by an authoring committee of experts. Reports typically include findings, conclusions, and recommendations based on information gathered by the committee and the committee's deliberations. Each report has been subjected to a rigorous and independent peer-review process and it represents the position of the National Academies on the statement of task.

Proceedings published by the National Academies of Sciences, Engineering, and Medicine chronicle the presentations and discussions at a workshop, symposium, or other event convened by the National Academies. The statements and opinions contained in proceedings are those of the participants and are not endorsed by other participants, the planning committee, or the National Academies.

Rapid Expert Consultations published by the National Academies of Sciences, Engineering, and Medicine are authored by subject-matter experts on narrowly focused topics that can be supported by a body of evidence. The discussions contained in rapid expert consultations are considered those of the authors and do not contain policy recommendations. Rapid expert consultations are reviewed by the institution before release.

For information about other products and activities of the National Academies, please visit www.nationalacademies.org/about/whatwedo.

PLANNING COMMITTEE ON THE ROLE OF PLANT AGRICULTURAL PRACTICES ON DEVELOPMENT OF ANTIMICROBIAL RESISTANT FUNGI AFFECTING HUMAN HEALTH[1]

SALLY MILLER (*Co-chair*), Distinguished Professor of Food, Agricultural and Environmental Sciences in Plant Pathology, The Ohio State University
PAIGE WATERMAN (*Co-chair*), Professor of Medicine; Vice Chair of Clinical Research, Uniformed Services University of the Health Sciences
MARIN BREWER, Associate Professor, University of Georgia
TOM CHILLER, Chief, Mycotic Diseases Branch, US Centers for Disease Control and Prevention
JULIUS FAJARDO, Senior Plant Pathologist, Office of Pest Management Policy, Office of Chief Economist, US Department of Agriculture
SUSAN JENNINGS, Senior Advisor for Public Health, Office of Pesticide Programs, US Environmental Protection Agency
RAMANAN LAXMINARAYAN, Director, One Health Trust
JEFFREY LEJEUNE, Food Safety Officer, Food Systems and Food Safety Division, Food and Agriculture Organization of the United Nations
MERVALIN MORANT, Program Manager, Food, Agriculture and Veterinary Defense, Department of Homeland Security
PHILIP TAYLOR, Training Manager and Global Plant Clinic Coordinator, CABI

Forum on Microbial Threats Staff

ELIZABETH ASHBY, Associate Program Officer
CLAIRE BIFFL, Research Assistant
CHARLES MINICUCCI, Research Assistant (*until January 2023*)
JUSTIN HAMMERBERG, Senior Program Assistant (*until November 2022*)
JULIE LIAO, Director, Forum on Microbial Threats
JULIE PAVLIN, Senior Board Director, Board on Global Health

[1] The National Academies of Sciences, Engineering, and Medicine's planning committees are solely responsible for organizing the workshop, identifying topics, and choosing speakers. The responsibility for the published Proceedings of a Workshop rests with the workshop rapporteurs and the institution.

FORUM ON MICROBIAL THREATS[1]

PETER DASZAK (*Chair*), President, EcoHealth Allicance
KENT E. KESTER *(Vice Chair)*, Vice President of Translational Medicine, International AIDS Vaccine Initiative
EMILY ABRAHAM, Director, External Affairs and Policy, Global Public Health at Johnson & Johnson
KEVIN ANDERSON, retired
CHRISTOPHER BRADEN, Acting Director, National Center for Emerging Zoonotic Infectious Diseases, U.S. Centers for Disease Control and Prevention
DANIEL BAUSCH, President, American Society of Tropical Medicine and Hygiene and Senior Director, Emerging Threats & Global Health Security, FIND
CRISTINA CASSETTI, Deputy Division Director, Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases
ANDREW CLEMENTS, Senior Scientific Advisor, Emerging Threats Division, U.S. Agency for International Development
MALICK DIARA, Public Health Manager, Corporate Medicine and Occupational Health Department, ExxonMobil
SCOTT F. DOWELL, Deputy Director for Surveillance and Epidemiology, Bill & Melinda Gates Foundation
MARCOS A. ESPINAL, Assistant Director, Pan American Health Organization
EVA HARRIS, Professor, Division of Infectious Diseases and Vaccinology; Director, Center for Global Public Health, University of California, Berkeley School of Public Health
JON H. HEINRICHS, Associate Vice President and R&D New Targets, Franchise Head in Portfolio Strategy and Execution, Sanofi Pasteur
ELIZABETH D. HERMSEN, Lead, Global Antimicrobial Resistance & Antimicrobial Stewardship Medical Affairs, Pfizer
MARK G. KORTEPETER, Vice President for Research, Professor of Preventive Medicine and Medicine, Uniformed Services University of the Health Sciences
MICHAEL MAIR, Acting Assistant Commissioner for Counterterrorism Policy; Acting Director, Office of the Chief Scientist, U.S. Food and Drug Administration

[1] The National Academies of Sciences, Engineering, and Medicine's forums and roundtables do not issue, review, or approve individual documents. The responsibility for the published Proceedings of a Workshop rests with the workshop rapporteurs and the institution.

JONNA A. K. MAZET, Vice Provost—Grand Challenges; Chancellor's Leadership Professor of Epidemiology & Disease Ecology, University of California, Davis
VICTORIA McGOVERN, Senior Program Officer, Burroughs Wellcome Fund
SALLY A. MILLER, Distinguished Professor of Food, Agricultural and Environmental Sciences in Plant Pathology, The Ohio State University, College of Food, Agriculture, and Environmental Sciences
SUERIE MOON, Director of Research, Global Health Centre; Visiting Lecturer, graduate Institute of International and Development Studies; Adjunct Lecturer, Harvard T.H. Chan School of Public Health
RAFAEL OBREGÓN, Country Representative, UNICEF Paraguay
KUMANAN RASANATHAN, Unit Head for Equity and Health, Department of Social Determinants of Health, World Health Organization
GARY A. ROSELLE, Executive Director, National Infectious Disease Services Program, Veterans Health Administration, U.S. Department of Veterans Affairs
PETER A. SANDS, Executive Director, The Global Fund to Fight AIDS, Tuberculosis & Malaria
THOMAS W. SCOTT, Distinguished Professor, Department of Entomology and Nematology, University of California, Davis
MATTHEW ZAHN, Deputy Health Officer, Orange County Health Care Agency

National Academies of Sciences, Engineering, and Medicine Staff

ELIZABETH ASHBY, Associate Program Officer
CLAIRE BIFFL, Research Assistant
CHARLES MINICUCCI, Research Assistant *(until January 2023)*
JUSTIN HAMMERBERG, Senior Program Assistant *(until November 2022)*
JULIE LIAO, Director, Forum on Microbial Threats
JULIE PAVLIN, Senior Board Director, Board on Global Health

Reviewers

This Proceedings of a Workshop was reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the National Academies of Sciences, Engineering, and Medicine in making each published proceedings as sound as possible and to ensure that it meets the institutional standards for quality, objectivity, evidence, and responsiveness to the charge. The review comments and draft manuscript remain confidential to protect the integrity of the process.

We thank the following individuals for their review of this proceedings:

DAVID W. DENNING, The University of Manchester; Global Action For Fungal Infection
SALLY A. MILLER, The Ohio State University

Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the content of the proceedings nor did they see the final draft before its release. The review of this proceedings was overseen by **HUGH H. TILSON,** University of North Carolina. He was responsible for making certain that an independent examination of this proceedings was carried out in accordance with standards of the National Academies and that all review comments were carefully considered. Responsibility for the final content rests entirely with the rapporteurs and the National Academies.

Contents

Boxes and Figures

BOXES

FIGURES

Acronyms and Abbreviations

ACT	AMR Codex Texts
AFST	antifungal susceptibility testing
AI	artificial intelligence
AIRE	autoimmune regulator
AMR	antimicrobial resistance
AMU	antimicrobial use
ARLN	Antimicrobial Resistance Laboratory Network
ARAf	azole-resistant *Aspergillus fumigatus*
BARDA	Biomedical Advanced Research and Development Authority
BTK	Bruton's tyrosine kinase
C5	component 5
CABI	Centre for Agriculture and Bioscience International
CARD9	caspase recruitment domain family member 9
CBC	cap-binding complex
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
CLABSI	central line-associated bloodstream infections
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CPA	chronic pulmonary aspergillosis
CYP51	sterol 14-alpha-demethylase
DHODH	dihydro-orotate dehydrogenase

DMI	demethylation inhibitor
DON	deoxynivalenol
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
FDA	U.S. Food and Drug Administration
FHB	*Fusarium* head blight
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FRAC	Fungicide Resistance Action Committee
GAFFI	Global Action for Fungal Infections
GLP	Principles of Good Laboratory Practice
GM-CSF	granulocyte-macrophage colony-stimulating factor
GPI	glycosyl-phosphatidyl inositol
ICU	intensive care unit
INA	impact network analysis
InFARM	International FAO Antimicrobial Resistance Monitoring
IPM	integrated pest management
IUCLID	International Uniform Chemical Information Database
JAK	Janus kinase
LAC	Latin America and the Caribbean
LAMMN	Latin American Medical Mycology Network
LMIC	low- and middle-income country
MALDI-ToF	matrix-assisted laser desorption/ionization time-of-flight
MER	market entry reward
MIC	minimum inhibitory concentration
NASS	National Agricultural Statistics Service
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NTM	nontuberculous mycobacteria
OECD	Organisation for Economic Co-operation and Development
OSU	The Ohio State University
PCR	polymerase chain reaction
PPE	personal protective equipment

QJS	Quadripartite Joint Secretariat
QoI	quinone outside inhibitor
ROS	reactive oxygen species
SA	South Asia
SBI	sterol biosynthesis inhibitor
SDHI	succinate dehydrogenase inhibitor
SEA	Southeast Asia
SNP	single nucleotide polymorphism
SSA	Sub-Saharan Africa
SYK	spleen tyrosine kinase
TB	tuberculosis
TISSA	Tripartite Integrated Surveillance System for Antimicrobial Resistance and Antimicrobial Use
UN	United Nations
UNEP	United Nations Environment Programme
USDA	U.S. Department of Agriculture
USDA/ARS	U.S. Department of Agriculture Agricultural Research Service
WHO	World Health Organization
WOAH	World Organisation for Animal Health

1

Introduction

As the use of antimicrobials in agriculture has become a globally widespread and standard practice, the impacts on human, animal, and ecosystem health have become more pronounced. Antimicrobial resistance (AMR) is now one of the most pressing global health threats as microbes affecting humans, animals, and plants become less responsive to standard treatments (WHO, 2021). The use of fungicides that belong to the same chemical class as antifungal medicines in crop production is an area of concern that has garnered the attention of global health entities such as the World Health Organization (WHO) and U.S. Department of Health and Human Services (HHS, 2021; WHO, 2021). Use of these fungicides in agriculture may promote the development of resistant fungi in the environment that could have implications for human health. However, several questions remain as to the practices that promote resistance, the effects of resistant fungi in the environment, and how this phenomenon might affect human health.[1]

For decades, antimicrobials have been the cornerstone of control and treatment of many diseases in humans, animals, and plants alike. Fungal plant pathogens rarely infect animals and humans; however, some have been known to cause disease in immune-compromised people or animals, and several plant pathogenic fungi produce toxins that are harmful to humans

[1] The planning committee's role was limited to planning the workshop, and the Proceedings of a Workshop has been prepared by the workshop rapporteurs as a factual summary of what occurred at the workshop. Statements, recommendations, and opinions expressed are those of individual presenters and participants, and are not necessarily endorsed or verified by the National Academies of Sciences, Engineering, and Medicine, and they should not be construed as reflecting any group consensus.

and animals (WHO, 2018). Furthermore, these plant pathogens share the same environment with saprotrophic fungi that can also cause opportunistic infections in susceptible individuals. Antimicrobials are widely used in crop agriculture to protect plants from yield- or quality-limiting diseases. Recent estimates indicate that 20–40 percent of global crop production is lost to pre- and post-harvest diseases and pests, with climate change predicted to worsen this destruction (FAO, 2021a; Savary et al., 2019). Fungi and oomycetes cause the majority of plant diseases, and fungicide use is common in commercial crop production and landscaping, as well as in-home gardening. While limiting the use of antimicrobials is foundational to mitigating AMR, steps to address this issue must be balanced with the need to address diseases and infections that threaten agricultural production. Food production, human health, and environmental stability depend on sustainable solutions for mitigating diseases that affect crops while reducing risk of AMR (FAO, 2016).

Although fungi can cause a spectrum of human infections, systemic antifungal therapies available for use in human and veterinary medicine are limited. Of the three drug classes currently used to treat systemic infections, azoles are the most diverse class of antifungals. Several different azole compounds and formulations are also used to control fungal diseases in plants. Widespread and long-term use of azoles on crops has resulted in the selection of fungal phytopathogens and saprophytes (i.e., environmental isolates) that are resistant to these fungicides. Although the same agents are not employed for medical purposes, structural similarities of these compounds that belong to the same chemical class give rise to concerns that the use of these fungicides could result in cross-resistance with azoles reserved for use in human medicine. *Aspergillus fumigatus (A. fumigatus)*, an environmental fungus that can cause aspergillosis (a lung infection) in humans and is primarily treated with azole antifungal drugs. Azole-resistant *A. fumigatus* are much more difficult to treat and result in higher chances of patient death (CDC, 2021a). Although more research is required, this example demonstrates how human health can be affected by AMR that may have arisen through agricultural practices.

In the United States and other high-income countries, the sale and use of fungicides is regulated based on efficacy evaluation and risk assessments for human health and the environment (Environmental Protection Agency [EPA], 2022 #211). U.S. statistics on pesticide use are generated from self-reporting by farmers responding to surveys or specific census requests.[2] In contrast, regulations for pesticide use in low- and middle-income countries (LMIC) may be less stringent or not fully enforced due to lack of capacity.

[2] U.S. Department of Agriculture survey data and reports are available at https://www.nass.usda.gov/ (accessed August 31, 2022).

Additional surveillance is required to gain a better understanding of how pesticides are used globally, how their use influences AMR, and how human and plant pathogens interact to affect various aspects of health.

Various strategies have been developed to prevent or mitigate AMR development in agriculture to limit its deleterious effects on crop production. "Integrated pest management" (IPM) is an approach to reduce the use of antimicrobials in conventional plant production systems and maintain their effectiveness. A systems approach, IPM is designed to minimize economic losses to crops, as well as risks to people, animals, and the environment. The main components of IPM for plant diseases are (1) accurate diagnosis and monitoring, which can also include disease modeling and predictive systems to guide the timing of pesticide applications, (2) use of disease-resistant crop varieties, (3) exclusionary practices that prevent the introduction of pathogens into a crop, (4) site selection and soil improvement to maximize plant health and minimize environmental factors that favor pathogens, (5) crop rotation and other cultural practices to prevent pathogen buildup, (6) use of biological and biorational products, and (7) judicious use of pesticides, including both antibiotics and fungicides.

While promising, the successful implementation of IPM and other approaches to counter the spread of AMR from agricultural practices hinges on closing several critical knowledge gaps. These include accurate measurements of antimicrobial use and their regulatory guidance in different contexts (e.g., high-income countries vs LMICs), understanding of AMR mechanisms in the environmental microbiota that may be consequential for human health, and data on the effectiveness of current surveillance tools and systems. This public workshop series will provide an opportunity for researchers and policymakers working at the intersection of plant, animal, and human health to share the latest knowledge, advance the ongoing discussions, and explore ideas that can improve the mitigation strategies to contain the spread of AMR.

In opening remarks, Paige Waterman, interim chair of medicine and vice chair for clinical research at the F. Edward Herbert School of Medicine at the Uniformed Services University of the Health Sciences, Bethesda, emphasized that the workshop represents a collaboration of experts across the spectrum of infectious diseases in humans, plants, animals, and microbiomes. Antimicrobials have both beneficial and harmful effects on humans, animals, and environment. As the global population continues to adjust to the ramifications of SARS-CoV-2, AMR poses a pressing global health threat, according to WHO and other expert entities. She remarked that antimicrobials, including antifungals, play a critical role in the control and treatment of human and animal diseases and in preventing diseases in plants. Although fungi cause a majority of plant diseases, antifungal stewardship in limiting or moderating the use of antifungals is needed to

limit further resistance in humans, animals, and plants. This workshop provided an opportunity to bridge gaps in combined knowledge about antifungal resistance and identify connections between the environment, plant agriculture, and human health in mitigation approaches.

WORKSHOP OBJECTIVES

On June 21, 22, and 27, 2022, the Forum on Microbial Threats at the National Academies of Sciences, Engineering, and Medicine held a 3-part public workshop series titled *The Role of Plant Agricultural Practices on Development of Antimicrobial Resistant Fungi Affecting Human Health*. The first two parts were virtual meetings, and the third was a hybrid meeting held in Washington, DC. The aims of the workshop were to consider (1) the magnitude of environmentally induced/selected antimicrobial resistance (AMR) in agricultural practices worldwide, with a focus on plant crop production, (2) the practices that contribute to AMR in human pathogens, (3) surveillance strategies, and (4) mitigation strategies. The workshop featured invited presentations and discussions to explore the following questions:[3]

- What is the magnitude of antifungal use in crop production in high-, middle- and low-income countries? How are such uses regulated?
- What are the mechanisms of AMR in plant pathogens and non-target environmental microbiota? How might this influence AMR in human pathogens?
- Which practices promote, prevent, or reduce the development of AMR in plant production environments, specifically in fungal pathogens? How does this affect risk of produce contamination with AMR pathogens?
- Are sampling and testing technologies for AMR surveillance in plant production systems adequate? What further evidence is needed to inform the use of antimicrobials worldwide? What further evidence is needed to understand the presence and effects of environmental AMR on human health?

ORGANIZATION OF THE PROCEEDINGS OF THE WORKSHOP

In accordance with the policies of the National Academies, the workshop did not attempt to establish any conclusions or recommendations

[3] The full Statement of Task is available in Appendix A. The workshop agenda is provided in Appendix B.

about needs and future directions, focusing instead on information presented, questions raised, and improvements suggested by individual workshop participants. Chapter 2 focuses on fungal disease, antifungal drugs, fungicide use in agriculture, and efforts to address AMR. Chapter 3 features an overview of fungal disease in humans, the emergence of antifungal-resistant infections, and implications and challenges in effectively treating these diseases. Chapter 4 explores the development of resistance in response to the agricultural use of fungicides. Chapter 5 discusses the role of fungicides in producing adequate and safe food supply. Chapter 6 highlights efforts to assess the extent and causes of fungal drug resistance and the role of regulatory bodies in addressing it. Chapter 7 explores nontraditional approaches to AMR research, including community science, simulation modeling, and network modeling. Chapter 8 focuses on research investigating the association between the use of agricultural fungicides and the emergence of antifungal-resistant infections, the factors contributing to the risk of cross-resistance, and mitigation efforts. Chapter 9 discusses the role of resistance in driving a continued need for new antifungal drugs, particularly those featuring novel modes of action, and the challenges inherent in antifungal discovery and development. Chapter 10 highlights agricultural technologies and strategies to mitigate the threat of resistance by reducing the use of fungicides and addressing conditions that foster development of resistant fungi.

2

Fungal Pathogens in Plant and Human Health

The workshop's opening panel focused on fungal disease and antifungal drugs, fungicide use in agriculture, and efforts to address antimicrobial resistance (AMR). It was moderated by Tom Chiller, Chief of the Mycotic Diseases Branch at the Centers for Disease Control and Prevention. Arturo Casadevall, Bloomberg Distinguished Professor and Alfred and Jill Summer Chair of the Molecular Microbiology and Immunology department at Johns Hopkins University, discussed the characteristics of fungi that have historically limited their pathogenic effect on humans, the ways in which these characteristics and the climate are changing—thereby increasing the threat of fungal disease—and the limited treatments currently available to address this threat. Tony Dorn, Environmental, Economics, and Demographics Branch chief at the U.S. Department of Agriculture (USDA), National Agricultural Statistics Service (NASS), described the history, methodology, quality measures, and data products of the NASS Agricultural Chemical Use Program. Tim Corrigan, Technical Officer for AMR and One Health at the World Health Organization (WHO), provided an overview of the Quadripartite Joint Secretariat (QJS) on AMR. He reviewed the partnership's aims, functions, strategic framework, and key activities.

ANTIFUNGAL DRUG USES IN HUMAN MEDICINE

Casadevall provided an overview of pathogenic fungi and their associated effects on humans. Featuring more than 6 million species, the kingdom fungi—the largest kingdom within biology taxonomy—includes major pathogens of plants, insects, invertebrates, and ectothermic (i.e.,

cold-blooded) vertebrates. Currently, fungi are affecting major ecosystems, causing significant declines in multiple animal populations. For instance, "white nose syndrome," caused by *Pseudogymnoascus destructans*, has devastated bat populations in North America. *Batrachochytrium salamandrivorans* has caused declines in European salamander populations, while *Batrachochytrium dendrobatidis* has led to catastrophic amphibian declines throughout the world. Additionally, fungi have had detrimental effects on snake and turtle populations.

Requirements for Fungal Human Pathogenicity

Overall, mammals are remarkably resistant to fungi, Casadevall noted. Relatively few fungal species are pathogenic for humans, with fewer than 10 species of major concern to human health. Some pathogenic fungi are host-associated, meaning that hosts spread them to other individuals; these include *Candida* spp., *Pneumocystis jirovecii*., and dermatophytes. Although host-associated fungi are often present without causing disease, disruption of the host-microbe relationship—particularly when it affects immunity—can result in pathogenic fungus-related disease. Other pathogenic fungi are environmental and tend to cause disease when the host is immunosuppressed or receives a large inoculum, such as *Histoplasma* spp., *Aspergillus* spp., *Cryptococcus* spp., *Coccidioides* spp., and *Blastomyces* spp.

To cause disease in humans, fungi must feature thermotolerance and the ability to survive and replicate within human hosts, stated Casadevall. Host-associated human pathogenic fungi have the capacity to survive mammalian temperatures, yet only 6 percent of fungi species are able to tolerate temperatures greater than 37 degrees Celsius (Robert and Casadevall, 2009). To cause systemic human disease, the organism must also have virulence factors that allow it to resist immune system clearance. A wide variety of such traits includes capsules, toxins, antioxidant systems, intracellular replication, and stress resistance. The combination of thermotolerance and the ability to survive, replicate, and evade sophisticated mammalian immune mechanisms is relatively rare in fungi, he noted.

The advanced nature of immunity in humans and mammals is such that fungal disease typically requires the interference of another disease or immunosuppressant medical treatment. In fact, fungal diseases were extremely rare until the advent of AIDS and immunosuppressant therapies for cancer and other diseases in the late twentieth century, Casadevall explained. These developments bolstered the ability of fungi to resist immune system clearance. Moreover, climate change could affect thermotolerance (Casadevall, 2020). As the climate trends toward warmer temperatures, microbial adaptations could enable fungi to survive above 37 degrees Celsius, thereby resulting in new fungal diseases.

Treatment and Prevention of Fungal Diseases of Humans

Fungal diseases in humans are often chronic, and systemic fungal diseases are typically lethal if untreated. Casadevall remarked that most fungal diseases are not reportable, thus prevalence figures are estimates with high uncertainty. Characterized by having a large burden of organisms, fungal diseases are often resistant to treatment, due to the high probability that some of those organisms have resistance mechanisms. Consequently, effective treatment generally requires prolonged therapy lasting months or years. Fungi and animalia are the most closely related of the classification kingdoms with few major differences to exploit, which hampers drug discovery and limits antifungal drug classes. One difference between the kingdoms is the presence of a cell wall in fungi; the drug class of echinocandins exploits this difference by targeting the cell wall (Sucher et al., 2009). Another difference is that ergosterol, found in fungi cell membranes, serves many of the same functions as cholesterol in animal cells. Aside from the presence of cell walls and ergosterol, the fungi and animalia have such similar biochemistry that it poses an intrinsic and fundamental problem to drug development.

Currently, no licensed vaccines are available to prevent fungal disease. Casadevall emphasized that the capacity to create vaccines for every major fungal pathogen already exists and has been demonstrated in animal models. However, fungal vaccines have not been developed. He added that vaccination—if developed—could serve as an important protection tool against antifungal resistance. Given the rising temperatures associated with climate change, new fungal diseases could emerge this century, some of which will likely arrive with inherent resistance to the drugs currently available. Casadevall added that most known organisms have not yet been tested for susceptibility to current antifungals. *Candida auris* is one example of an emerging fungus that has developed resistance to multiple drugs.

AGRICULTURAL CHEMICAL USE STATISTICAL PROGRAM

Dorn described the USDA National Agricultural Statistics Service (NASS) Agricultural Chemical Use Program's history, methodology, quality measures, and data products. In the early 1990s, NASS began to collect and publish chemical use data. This was partly in response to public outcry against the use of Alar, a growth regulator that posed a carcinogenic risk. The U.S. government responded by establishing initiatives on food safety and water quality. Although those initiatives have evolved over time, the need for reliable, timely, environmental data remains constant. Since 1990, NASS has routinely surveyed U.S. farmers to collect information on chemical ingredients applied to agricultural commodities via fertilizers, pesticides, and fungicides. The program collects data on fruits, vegetables, and major

field crops on a rotating basis that changes annually. Additionally, the program gathers information on pest management practices that farmers utilize to boost the effectiveness of pesticides or as alternatives to pesticides, thereby reducing dependence on agricultural chemicals.

Each year, the USDA Economic Research Service conducts the Agricultural Resource Management Survey from October through December, said Dorn. NASS is a partner in this initiative, contributing chemical use survey data for field crops. This integration allows for the analysis of fertilizer and pesticide data with farm financial information, farm household characteristics, and other agricultural production practices. Rather than survey the entire country, NASS selectively surveys the states responsible for 80 percent of a crop's production. For instance, states in the Midwest and Southeast grow the majority of U.S. corn and soybeans; Texas and states along the West and East coasts produce most of the country's fruits and vegetables. Due to limited resources, NASS is unable to survey every commodity annually. Instead, commodities are included in surveys on a rotating basis, with most commodities appearing in the annual survey every 2-3 years. In 2019, the Agricultural Chemical Use Survey collected data on fungicide use on 21 different fruit crops among the 12 states that produce the majority of those crops.[1] These data include total pounds of fungicide applied, specific active ingredients, and rates of use. Similarly, in 2020, NASS collected fungicide use data on 22 vegetable crops among the 18 highest producing states.

Survey data are made available in a variety of formats, said Dorn. The NASS website includes links to the Quick Stats 2.0 database, from which users can export data to Excel software.[2] This website includes pre-defined queries to expedite research for users. NASS also publishes two-page summaries of highlights from each survey on their website. Documents outlining quality measures and methodology are available for all crops included in Agricultural Chemical Use Program surveys. These documents include scope and purpose, survey timeline, sampling frames and methods, data collection and editing, analysis tools, non-sampling errors, nonresponse adjustment, outliers, estimators, estimation, and state-level sample sizes and response rates. Dorn stated that NASS strives to be as transparent as possible with these data.

Database modernization efforts are currently underway, with roll-out of a cloud-based data dissemination system expected in 2023. This system,

[1] More information about the 2019 Agricultural Chemical Use on Fruit Crops survey is available at https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/2019_Fruits/fruit-chem-highlights.pdf (accessed July 9, 2022).

[2] More information about NASS data products, the Quick Stats database, and pre-defined queries is available at https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/, https://www.nass.usda.gov/Data_and_Statistics/Pre-Defined_Queries/index.php, and https://quickstats.nass.usda.gov/ (accessed July 9, 2022).

which will replace the Quick Stats 2.0 database, will feature a new data taxonomy, structure, and a data dictionary for reference. Dorn explained that chemical use data taxonomy and structure do not always fit the structure NASS data products use for crop planting, harvesting, yield, and production data. The design of the new system is expected to be more user-friendly for locating chemical use data. To address user challenges related to searching for specific data within the existing database, an application programming interface will drive the new system, making it easier to sort, query, and locate data. Users will also be able to perform ad hoc queries on all years of historic data.

QUADRIPARTITE ALLIANCE JOINT ACTIVITIES ON ANTIMICROBIAL RESISTANCE

Corrigan, who serves as WHO Liaison Officer to the Quadripartite Joint Secretariat (QJS) on AMR, provided an overview of this partnership among the Food and Agriculture Organization (FAO) of the United Nations (UN), the United Nations Environment Programme (UNEP), WHO, and the World Organisation for Animal Health (WOAH). In 2010, FAO, WHO, and OIE (now WOAH) solidified a collaboration dedicated to applying a One Health approach to AMR, avian influenza, and rabies. This Tripartite Joint Secretariat on AMR worked closely with UNEP in the AMR area. On January 1, 2022, UNEP appointed a Liaison Officer to the QJS on AMR. The Tripartite officially expanded to a Quadripartite on March 17, 2022, with the four entities signing a memorandum of understanding to formalize their ongoing partnership and expand their collaboration beyond AMR to address the entire One Health sphere.

The QJS on AMR aims to accelerate a coordinated strategy on human, animal, and ecosystem health by drawing on the core mandates and comparative advantages of the four organizations to address the wide range of needs in the global response to AMR. Corrigan outlined the secretariat's key functions. The collaboration supports global promotion, advocacy, and political engagement with the Group of Seven and Group of Twenty members and the UN General Assembly. The QJS provides secretariat services and support to the global governance structures recommended by the UN ad hoc Interagency Coordination Group on AMR in their 2019 final report.[3] Additionally, the QJS coordinates the AMR Multi-Partner Trust Fund. Furthermore, the QJS coordinates and monitors workplan implementation and mapping

[3] Interagency Coordination Group on Antimicrobial Resistance. 2019. "No Time to Wait: Securing the Future from Drug-Resistant Infections." https://reliefweb.int/report/world/no-time-wait-securing-future-drug-resistant-infections-report-secretary-general-united (accessed September 30, 2022).

of gaps and opportunities. Corrigan noted that QJS convenes other partners in the sphere to collaborate.

In 2022, the QJS on AMR reached a major milestone with the publication of a strategic framework that presents the background and context for the collaboration between these four organizations, said Corrigan (WHO, 2022b) (see Box 2-1).

Global Collaborative Efforts Against Antimicrobial Resistance

Corrigan noted that a key joint venture is the AMR Multi-Partner Trust Fund. Thus far, the trust fund has been implemented in 10 countries—Morocco, Kenya, Zimbabwe, Senegal, Ghana, Cambodia, Indonesia, Ethiopia, Peru, and Tajikistan—with expansion to Bangladesh, Mongolia, Tunisia, Madagascar, and Cameroon taking place in 2022. The trust fund has raised $26 million to be used in joint activities conducted in collaboration with national governments. The aim of these activities is to catalyze

BOX 2-1
Strategic Framework for Collaboration on Antimicrobial Resistance

The Strategic Framework for Collaboration on Antimicrobial Resistance describes the catalytic role of the Quadripartite in the One Health response to AMR, including support provided to members, civil society, and other stakeholders. It provides a theory of change for this collaborative response and "outlines the roles, objectives, desired impacts at the country level, and the intermediate outcomes of the various quadripartite functions." Each of the four organizations has its own organizational mandates, action plans, or strategies guiding its sector-specific response. The framework complements these organization-specific mandates, bridging the overlapping goals to move work forward. The goal of the framework's theory of change is to preserve antimicrobial efficacy and ensure sustainable and equitable access to antimicrobials for responsible use in human, animal, and plant health, Corrigan noted. The objectives in achieving this goal are (1) to optimize the production and use of antimicrobials along the life cycle from research and development to disposal and (2) to decrease the incidence of infection in humans, animals, and plants to reduce the development and spread of AMR. The ultimate desired impact of these objectives is to build countries' capacity to "design and sustainably implement evidence-informed One Health responses to AMR." This framework provides the mechanisms and roadmap to address current One Health issues—as well as emerging or adaptable issues in the future—and will serve as the guiding document for QJS's work.

SOURCES: Presented by Tim Corrigan on June 21, 2022; (WHO, 2022b).

sustainable national multisectoral response and encourage domestic financing for One Health responses to AMR at the country level (WHO, 2022a). Corrigan described the trust fund as a mechanism for high-level goals at the global level to translate to impact at the regional and country levels. Currently, the governments of Germany, the Netherlands, Sweden, and the United Kingdom are the trust fund's main resource partners.

The Global Leaders Group on AMR, a high-level group driving political action, serves as a key global governance structure, said Corrigan. Sheikh Hasina, prime minister of Bangladesh, and Mia Amor Mottley, prime minister of Barbados, serve as co-chairs. The group has developed six priority areas and corresponding key performance indicators, which include political action, transforming systems, surveillance, financing, research and development, and environment dimensions.[4] Examples of the group's impact include helping to build the global consensus around reducing antimicrobial use in food systems, facilitating the Codex AMR negotiations to develop a framework of guidelines (FAO and WHO, 2022), and advocating for a UN General Assembly high-level meeting on AMR in 2024.

Integrated surveillance of antimicrobial resistance and use across sectors is a priority for the QJS on AMR, Corrigan stated. The QJS established a working group to review and revise currently available guidance documents, definitions, and approaches. This work will inform a proposal for the need, scope, and format of QJS guidance on integrated surveillance, which will include the optimal areas for investment. The working group will also define priority needs across sectors and in different settings, particularly in low- and middle-income countries, to develop approaches that are adaptable to different contexts rather than standardized. The QJS is currently in the process of establishing a Quadripartite Technical Group of external experts who will provide strategic and technical advice to the QJS and the Global Leaders Group. Furthermore, the Global Leaders Group has established an Integrated Surveillance Task Force led by Lothar Wieler, president of the Robert Koch Institute in Berlin, Germany. Corrigan remarked that the varied drivers of AMR (see Figure 2-1) constitute a One Health problem that requires One Health Solutions; the QJS has the appropriate mechanism and structures in place to help address these pressing issues.

[4] More information about the Global Leaders Group on Antimicrobial Resistance is available at https://www.amrleaders.org/resources (accessed July 24, 2022).

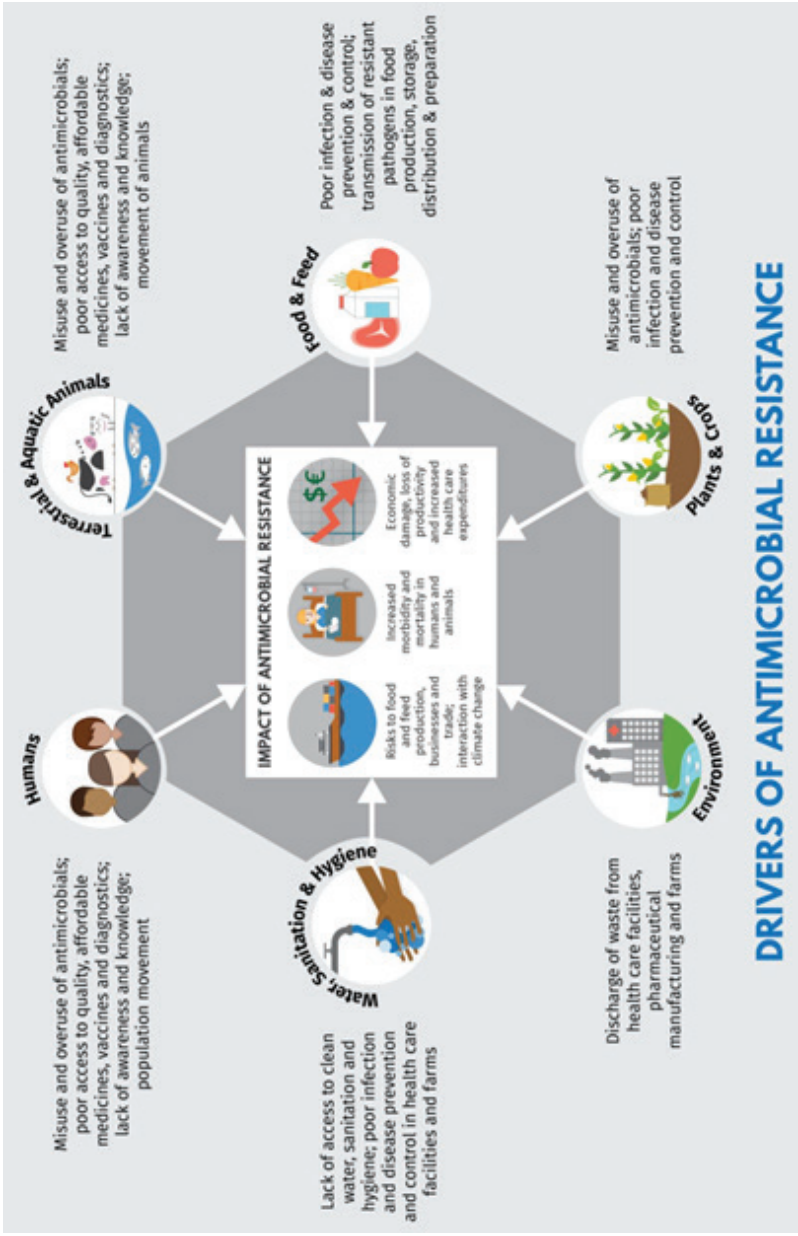

FIGURE 2-1 Drivers of antimicrobial resistance.
SOURCE: WHO, 2019.

DISCUSSION

Thermotolerance, Climate, and Human Body Temperature

Chiller noted that many fungi are unable to survive at 37 degrees Celsius—a feature that contributes to the relatively low number of mammalian fungal pathogens—and asked whether mammalian fungal pathogens are more prevalent in tropical regions. Casadevall replied that fungal diseases are indeed more prevalent closer to the equator and, accordingly, prevalence decreases further away from the equator. Replying to a query about the role of human body temperature in relation to fungal disease, Casadevall remarked that a recent study analyzed medical data spanning more than a century, finding that the human body temperature in the United States is decreasing over time (Protsiv et al., 2020). Thermometers from 150 years ago worked as well as their modern counterparts, thus the decrease cannot be attributed to a problem of measurement. The authors posited that the decrease of inflammatory diseases over the past century is a plausible explanation for decreasing body temperatures. Casadevall noted that medical challenges such as latent tuberculosis, worms, and exposure to air pollution levels that trigger lung inflammation were far more common 100 years ago than they are today. Moreover, body temperature varies across individuals, both above and below 37 degrees Celsius, so humans with lower-than-average body temperatures may not be protected against some organisms in some climates. Casadevall suggested that the current confluence of two negative trends—fungi adapting to warmer climates and human body temperatures declining—could portend trouble ahead.

Given the extreme weather events in recent years in the American West, Australia, the American Midwest, and Western Europe, Chiller asked whether thermal tolerance in fungi evolves in response to such events or in response to a rise in mean or median temperatures. Casadevall replied that the fungal world is hypothermic in that mushrooms tend to be cooler than the environment and fungal colonies are always cooler than the environment, even at the microscopic level. He posited that this may be related to the increase in oxidative damage that occurs across kingdoms with higher temperatures. Spermatogenesis takes place in cooler areas due to the decreased likelihood of DNA damage, raising questions as to why some organisms appear to be thermally resistant. For example, *Aspergillus* appear to do well at 45 degrees Celsius. Casadevall surmised that they could be the result of an adaptation for a niche, such as compost piles. The earth has gone through periods of cooling and warming, thus selective events in the past may have created residual capacity for thermal tolerance despite the majority of the fungi kingdom appearing to prefer cooler regions.

NASS Survey Selection Criteria and Confidentiality Considerations

Chiller asked Dorn whether the states selected for NASS survey sampling were chosen due to high volume production per crop or by some other criteria. Dorn responded that production is a factor in quality measures, so commodities are selected based on the number of planted acres, rather than on production. Samples cover at least 80 percent of acres planted for each target commodity. Dorn added that surveys are voluntary and individual information is not disclosed. On occasion, not all data is published to protect the confidentiality of reporting farmers, which can create holes in the data. Chiller queried whether the USDA collects any pesticide data they do not make public. Dorn replied that NASS publishes or produces some type of results for all data items collected. The surveys require a time commitment from farmers in answering questions regarding applications of all pesticides, fertilizer, and fungicides for all fields and various crops over the span of a year. Therefore, NASS strives to produce results on these complex surveys for the benefit of the farmers and the public.

Addressing Fungicide Use

Chiller asked whether the QJS on AMR has any projects or activities addressing the role of fungicide in antifungal resistance. Corrigan stated that the QJS is not currently involved in any activities directly related to fungicide. However, a number of initiatives are underway in sector-specific responses linked to existing QJS activities, such as integrated surveillance. Additionally, joint activity in developing a prioritized research agenda for AMR may touch on fungicide. Although the QJS on AMR is not currently addressing fungicide directly, the strategic framework and biennial workplan allow for the addition of new issues and the identification of specific problems requiring action, said Corrigan.

Noting that fungi constitute as much as 80 percent of pathogens problematic in the agriculture sector, Chiller remarked on the critical role of fungicides in plant agriculture. He asked Corrigan whether the QJS on AMR has considered identifying certain crop groups as essential in evaluating their value—whether in terms of economics or calories—at the local, national, or international level. Corrigan replied that declaring a crop as essential remains outside the scope of the strategic framework and current direction of the QJS on AMR, which focuses on activities that can be addressed by all four organizations. Although relevant, this designation is outside of certain mandates of some of the QJS organizations. Dorn added that NASS does not set categories for products due to the range of specialty markets and variations in a product's importance across specific local settings.

Chiller asked about current trends in the agricultural sector that might be influencing fungicide use. Dorn responded that field application is outside the scope of NASS's data collection and presentation activities. However, given how expensive fertilizers have become for the agriculture industry, the current input costs of fertilizers and fuels likely factor into fungicide use. To generate returns from investing in rented land, farmers must consider their budgets and contain expenses to yield profit. The unexpectedly large increases in fuel and fertilizer input costs will likely continue to affect fungicide use. Dorn added that NASS will be conducting a 2022 census of agriculture that will collect data including overall expense information at the national, state, and county levels.

Candida auris and the Threat of New Fungal Diseases

Chiller asked Casadevall to describe his hypothesis on the emergence of *Candida auris*. Casadevall replied that it is simple but has not been proven and will likely be difficult to prove. *Candida auris* was unknown to medicine until 2007, when it was isolated in an individual's ear. It soon began causing disease in immunocompromised patients in three continents—South America, India, and South Africa—regions that have all experienced recent increases in median temperatures. He added that this organism has a significant amount of drug resistance when it emerges from the ground. *Candida auris* has been found in two locations in the Andaman Islands, in a beach and in a wild marsh. The organism found in the beach was temperature-adapted and possibly dropped there by humans. The marsh isolate was not as temperature tolerant as clinical isolates consistent with the notion that wild strains are adapting to temperature, which continues as these become human host adapted. Although climate change can be framed in terms of an average rise of degrees in temperature, the number of hotter-than-average days is also pertinent, because each hot day is a selection event that may spur change in organisms. His hypothesis is that many potentially threatening organisms are currently controlled by temperatures at which they cannot survive; however, as these organisms adapt, new fungal diseases previously unknown to medicine will emerge, as was the case with *Candida auris* (Casadevall, 2020).

Given that *Candida auris* is highly resistant to azoles, Chiller asked for theories on how this resistance developed. Casadevall responded that fungi have enormous biochemical variation in assembling a fungal cell. The biological diversity of fungal organisms, both in terms of how they assemble the fungal cell wall and where melanin and lipids are placed within the cell, contributes to a range of susceptibility. He added that resistance in *Candida auris* is not surprising, although the finding that amphotericins,

echinocandins, and azoles are indeed active against many of the fungal species is more unexpected.

Potential Fungal Disease Threats and Fungicide Stewardship

Chiller asked about the biggest concerns regarding fungal diseases in the coming century and about how antifungal or fungicide stewardship can be applied across human, animal, and plant settings. Casadevall's greatest concern is the limited attention devoted to fungal diseases, which represent relatively unfamiliar threats. For instance, humans constantly prepare for war, but place little attention on what is happening to animal species—particularly to frogs, bats, salamanders, turtles, and snakes— and to other kingdoms that have the potential to wreak major havoc on human life, he noted. Casadevall added that although major epidemics of fungal diseases have not yet occurred, they remain a potential threat. He likened this to the mistaken supposition, prior to the advent of HIV, that retroviruses did not cause human disease. Similarly, coronaviruses were previously believed to cause only mild colds, yet three coronavirus epidemics have already taken place this century. Casadevall expressed concern regarding the potential emergence of a fungal disease that is transmissible through human-to-human contact and is resistant to available antifungal drugs. Regarding fungicide stewardship, he highlighted a dilemma in azole fungicide use in agriculture. Reducing antibacterial use in animal husbandry may result in smaller cows, yet these smaller cows will continue to produce food supply. In contrast, fungicides are used to prevent the loss of crops that are required for the food supply. Therefore, it can be challenging to strike the appropriate balance between generating an adequate food supply and curing disease.

Dorn remarked that the effects of climate change on agriculture are part of a public discussion, but media attention rarely focuses on issues of crop loss and livestock effects of fungicides, despite future deleterious impacts on crop production that could be caused by new fungal diseases. Corrigan cautioned that the lack of awareness about AMR, coupled with a large burden of infections, could coalesce into a perfect storm in which people become sick with untreatable diseases, food supplies run low, and a socioeconomic collapse occurs. Thus, he urged the international community to more robustly examine the threat of antifungal resistance and included it among considerations regarding antimicrobial stewardship, guidelines, and data needs.

3

Fungal Diseases, Antifungal Resistance, and Human Health

The second panel of the workshop focused on fungal disease in humans, the rise of antifungal resistance, and implications and challenges related to effectively treating these infections. The panel was moderated by Tom Chiller, chief of the Mycotic Diseases Branch at the Centers for Disease Control and Prevention. Andrej Spec, associate professor, associate director of the Infectious Disease Clinical Research Unit, and medical director of the Invasive Mycoses Clinic at Washington University in St. Louis, described how fungal infections are contracted, their impact on human health, and current treatment options and challenges. David Denning, chief executive of Global Action for Fungal Infections and professor of Infectious Diseases in Global Health at the University of Manchester, United Kingdom, discussed invasive, chronic, allergic, and superficial aspergillosis—including barriers to accurate diagnosis and effective treatment—and provided an overview of increasing azole resistance. Brendan Jackson, medical epidemiologist and lead of the epidemiology team of the Mycotic Diseases Branch at the Centers for Disease Control and Prevention (CDC), discussed the prevalence and ramifications of increasing *Candida* resistance to antifungals. He described the emergence and characteristics of *Candida auris (C. auris)* and presented hypotheses of why resistance is increasing. Michail Lionakis, chief of the Fungal Pathogenesis Section and of the Laboratory of Clinical Immunology and Microbiology at the National Institute of Allergy and Infectious Diseases (NIAID), presented examples of how research on the host-fungal interaction is leading to the development of prophylactic and immune-based treatments.

IMPACT OF INVASIVE FUNGAL DISEASES AND ANTIFUNGAL DRUG RESISTANCE ON HUMAN HEALTH

Spec provided an overview of the medical classification of fungi and how fungal infections are contracted. He also discussed the global burden and mortality rates of fungal infections, current treatment options, and challenges related to resistance and drug development. He noted that the general public tends to associate fungi, in a medical context, with relatively benign issues such as toenail infections, despite the potential for fungal infections to cause life-threatening conditions.

Fungal Morphology and Medical Classification

Human infections can be classified according to their causative agents into the categories of bacteria, viruses, fungi, protozoa, and helminths; the latter two are often grouped under the term "parasites." Fungi are medically classified into three groups: (1) yeast, (2) thermally monomorphic molds, and (3) thermally dimorphic molds. Yeast cells are round and similar in shape to bacteria, although they are larger (Spec et al., 2019). Hyphae form mycelia in the soil. Pseudohyphae are present in some species of fungi and share some characteristics of both yeast and hyphae. Some species create both yeast and hyphae, depending on the temperature. Yeasts are broad and varied. For example, *Candida* and *Cryptococcus* are both classified as yeasts, yet they are so widely diverged that *Cryptococcus* is more closely related to mushrooms than to *Candida*. Thermally monomorphic molds can be classified as zygomycetes, dematiaceous, dermatophytes, and hyaline hyphomycetes. Spec added that the classification of thermally monomorphic molds is largely driven by appearance under the microscope and in the patient (Spec et al., 2019).

How Fungal Infections Spread

Spec explained that fungi can be commensal or environmental. Commensals—including *Candida* and dermatophytes—live on humans all of the time, while all other groups of fungi come from the environment. Infections behave differently depending on whether they are commensal or environmental, thus management strategies vary dramatically. Commensals are part of the human microbiome and are common contaminants in culture. Often seen in overgrowth syndromes, commensals cause disease by overgrowing or growing in locations that become irritated. *Candida* commonly causes relatively innocuous and non-life-threatening diseases such as heat rash, tinea (commonly known as ringworm), vulvovaginal candidiasis, and thrush. However, *Candida* can be lethal if it enters the blood stream.

Environmental fungi spend the majority of their lifecycle in the environment, such as in soil or trees, said Spec. Fungi form spores, or conidia, that are released into the air and can be inhaled by humans.[1] These types of infections often start with pneumonia, which may be at a subclinical level that presents as a long-lasting cold. While the typical cold lasts 3–5 days, a fungal infection may last 10–15 days or longer. The infection may or may not disseminate from the lungs to the rest of the body, with dissemination location and frequency varying widely. Some fungal infections—particularly with *Cryptococcus* and *Coccidioides*—are prone to causing meningitis. Some fungi are more prone to causing bone or skin disease and others can cause visceral disease. *Histoplasma* is prone to causing disease in the liver, spleen, intestines, and other organs. Often, the method of a fungus's dissemination is highly dependent on the individual's underlying immune system, yet fungi can travel to virtually anywhere in the body. Although the vast majority of human fungal infections are contracted through the air, rare occurrences of direct inoculation can occur when an object is driven below the skin. Spec remarked that some of the most horrific diseases he has managed were in patients with otherwise typical immune systems who had fungi forced underneath their skin through trauma. Additionally, more people are now being treated with immunosuppressant therapies for chronic or potentially fatal conditions—such as cancers—thus there is a larger pool of people at risk of contracting serious fungal infections.

Global Burden and Mortality Rates of Fungal Infections

Spec remarked that fungi receive relatively little public attention relative to bacteria and viruses, despite the likelihood that fungal infections will become more prominent during this century. Currently, fungal infections are responsible for 1.5 million annual deaths worldwide (Hagan, 2018). Many types of fungal infections are on the rise, such as *Aspergillus*, which is experienced an annual increase in incidence of approximately 4.4 percent in France from 2001-2010 (Bitar et al., 2014). In addition, fungal infections generate significant economic cost. For instance, direct health care costs related to fungal infections in the United States totaled $7.2 billion in 2017. This figure increases to $11.5 billion when accounting for productivity and life, and to more than $48 billion if assessed using the value of statistical life rate (Benedict et al., 2022b).

Spec noted that the high cost of fungal infections, when assessed in terms of the value of statistical life, is largely due to the high mortality rates associated with those infections. For example, although many people do

[1] One example of how fungal infections disseminate in the body is available at https://www.cdc.gov/fungal/diseases/blastomycosis/causes.html (accessed July 26, 2022).

not associate *Candida* with invasive disease, the mortality rate of *Candida* bloodstream infections is approximately 43 percent (Mazi et al., 2022). The attributable mortality rate of these infections is between 10 and 25 percent. The frequency of *Candida* in hospitals is approximately half that of *Staphylococcus aureus*, but *Candida's* mortality rate is almost double that of *S. aureus* (approximately 20-25 percent). Spec added that the attributable mortality for these two diseases is the same for many subgroups of the patient population. However, far fewer resources are dedicated to researching and addressing *Candida* compared to *S. aureus*, despite the former's potential to cause fatal infection.

Infections caused by other species of fungi are also associated with high mortality rates, Spec emphasized. For instance, *Cryptococcus* has been estimated to have a mortality rate of approximately 15 percent in patients living with HIV or transplants (Hevey et al., 2019). This rate increases to almost 40 percent in people without HIV or transplants. He explained that infections in non-HIV, non-transplant patients are often diagnosed late and treated imperfectly. In sub-Saharan Africa—where access to drugs and supportive care is lower—the mortality of *Cryptococcus* increases to 40-60 percent in individuals with HIV (Tenforde et al., 2020). Spec surmised that the mortality rate of *Cryptococcus* is even higher among individuals who are not living with HIV.

Spec noted a large spike in the incidence of mucormycosis—a disease caused by the *Mucorales* fungi—during the 2021 wave of the delta variant of COVID-19 in India (Rao et al., 2021). This disease is present independent of COVID-19 and carries a mortality rate of close to 100 percent without surgical intervention. Surgeries for mucormycosis are often highly invasive, and mortality remains at around 70 percent at one year even with surgical care and treatment with the best available antifungals. Many survivors of the disease are left permanently disfigured, Spec added.

Fungal Infection Treatment and Resistance

Spec described how fungal infections are treated and the growing threat of treatment-resistant infections. Antifungal treatments can be classified into (1) polyenes, (2) azoles, and (3) echinocandins (Stevens, 2011). The polyenes currently include only one systemic medication, amphotericin B. This single medication has multiple formulations and carries a broad spectrum of activity and high potency. Only available as an intravenous therapy for invasive infection, amphotericin B is an extremely antifungal agent, stated Spec. Unfortunately, the drug is also highly toxic, and virtually every person who takes amphotericin B develops renal toxicity. Electrolyte wasting is also common; the loss of potassium and magnesium through urine can lead to arrythmias and death. Infusion reactions can include

severe muscle cramps, pain, and rigors, because the drug can begin to damage human muscle cells in the same way it damages fungi. Azoles, the most diverse class of antifungals, include drugs that have varying levels of broadness of activity. They are the only antifungals that can be taken orally, but treatment with azoles can be complicated. Because many of these drugs are not easily absorbed, it can be difficult to achieve and maintain effective therapeutic level in patients and may require continuous therapeutic drug monitoring. Additionally, azoles can cause challenging drug interactions, and many azoles have idiosyncratic side effects such as sunburns, high blood pressure, and even heart failure. They can also cause liver toxicity and they have a narrow therapeutic index. The newest class of antifungals, echinocandins, were developed approximately 20 years ago and are often referred to as the "good antifungal," because they are generally well tolerated and have few side effects. However, echinocandins must be administered intravenously and have a relatively narrow spectrum of activity that does not include many fungi.

Resistance generates further challenges in fungal treatment, Spec stated. The oral treatment options for several fungi—including *Candida krusei, Candida glabrata*, and azole-resistant *Aspergillus*—are poor or nonexistent. Moreover, some fungi have intrinsic resistance to all antifungals. These include *Scapulariopsis brumptii*, *Lomentospora prolificans*, *Scedosporium apiospermum/ Pseudoallescheria boydii*, *Candida auris*, and *Fusarium* isolates, especially *Fusarium solani*. Spec commented on the difficulty of seeing immunocompetent people experiencing horrible fungal infections for which no treatment options exist. Worryingly, antifungal resistance is increasing worldwide. Noting the central role echinocandins have played in addressing *Candida* infections, Spec emphasized that in one study, *C. glabrata* resistance to echinocandins increased from 4.9 percent in 2001 to 12.3 percent in 2010 (Alexander et al., 2013). Furthermore, new emergence of invasive infections due to fluconazole resistance in *Candida parapsislosis* has taken place in locations throughout the world (Souza et al., 2015). Spec highlighted the explosive growth of *C. auris* since its emergence in 2004. Over a short period of time, it has emerged in various communities—including within the United States—and now constitutes a significant portion of new cases of fungal infections in some locations (Rhodes and Fisher, 2019). A major concern is that, of the known isolates from this fungal species, 90 percent are resistant to fluconazole, 30 percent are resistant to amphotericin B, 10 percent are resistant to echinocandins, and 4 percent are resistant to all antifungals (CDC, 2020). Citing a worldwide report of significant presence of azole-resistant *Aspergillus,* Spec remarked that azole-resistant fungus is likely present in most places in the world (Lestrade et al., 2019b), even in settings where it has not yet been identified due to insufficient epidemiology work and analysis.

Challenges in Antifungal Development

Spec emphasized that animal cells and fungal cells are closely related; thus, many antifungals are also "antihuman." Many of the antifungal drugs currently used in medical treatment are effective at killing fungi, but their therapeutic index is low. Spec noted the difficulty in identifying compounds that will kill fungus without the same deleterious effects on human cells. While right now there are more potential antifungal drugs are in the development pipeline than at any point in the past 20 years, some of these drugs belong to the same classes and others have already had their clinical trials terminated due to lack of efficacy (Rauseo et al., 2020). Although there is reason for optimism that better antifungal compounds will ultimately be available, these drugs are harder to develop than antibacterials, noted Spec. Therefore, it is important to protect current drugs against resistance, because outcomes tend to be much worse without therapy.

GLOBAL INCIDENCE AND PREVALENCE OF ASPERGILLOSIS AND INTRODUCTION TO AZOLE RESISTANCE

Denning described four groups of aspergillosis cases: invasive, chronic, allergic, and superficial infections. He reviewed the subpopulations most affected by aspergillosis, the scope of the disease, challenges in correctly diagnosing and effectively treating *Aspergillus* infections, and the rise of azole resistance.

Invasive Aspergillosis

Invasive aspergillosis is a life-threatening infection that can kill a person within 7 to 20 days. Difficult to diagnose, this disease poses a particular threat to people who are immunocompromised. People living with leukemia, transplantation, late-stage HIV, immunologic disorders, chronic obstructive pulmonary disease (COPD), and inherited immunodeficiencies are at a higher risk of developing invasive aspergillosis. Furthermore, complex hospital patients, including those in intensive care units (ICU)—particularly those in ICUs with renal dysfunction, respiratory failure, or chronic or temporarily compromised immune systems—or individuals hospitalized due to severe influenza or COVID-19 are more prone to aspergillosis. Denning noted that of these risk pools, patients with kidney transplants and stem cell transplants are at a relatively low risk (Herbrecht et al., 2012). People with heart transplants, late-stage HIV, and various leukemias are at intermediate risk. Patients with liver, lung, heart-lung, small bowel, or allogeneic stem cell transplants are among those at highest risk for aspergillosis. Denning added that aspergillosis is sometimes mistaken for a lung cancer relapse.

COPD is a disease most often related to smoking, but it can also be caused by smoke generated by indoor cooking (particularly affecting women), by occupational lung disease, and as a post-tuberculosis effect, said Denning. A southern Chinese study of approximately 300 hospitalized COPD patients found that *Aspergillus* spp. was present in nearly 20 percent of patients and probable invasive aspergillosis was found in 3.9 percent of patients (Xu et al., 2012). Although steroids are a risk factor for aspergillosis, only 13 percent of those who developed the disease were taking corticosteroids, he noted. In that study, 43 percent of the COPD patients with aspergillosis died (Xu et al., 2012). At the global level, the number of people with COPD worldwide is estimated at 550 million (Hammond et al., 2020). Approximately 10 percent of these individuals require hospitalization each year, with the mortality rate of hospitalized COPD patients ranging from 5 to 12 percent. Denning stated that the lower estimate of 1.3 percent of COPD patients developing invasive aspergillosis translates to 760,000 cases per year. The higher rate of 3.9 percent found in the Chinese study puts the number of invasive aspergillosis in COPD cases at approximately 2.25 million worldwide (Hammond et al., 2020).

Chronic Pulmonary Aspergillosis

The second group of aspergillosis cases is patients with chronic disease, primarily disease affecting the lungs and occasionally the sinuses, said Denning. People with lung disorders including COPD, sarcoidosis with asthma, and prior lung disease can develop aspergillosis as a complication. Aspergillosis can also set in after a person is cured of tuberculosis (TB). Chronic pulmonary aspergillosis (CPA) can be confused with TB or TB-like nontuberculous mycobacterial (NTM) infections because of similar clinical and X-ray presentations. Denning outlined three CPA challenges related to TB: (1) CPA can be present and the initial diagnosis of TB is incorrect, (2) CPA can occur as a co-infection with TB and NTM infections, and the CPA may go untreated if not fully investigated, and (3) CPA can follow TB as a sequela. In such cases, patients may be retreated with anti-TB therapy, which is unnecessary and ineffective in treating CPA. In research Denning carried out in Vietnam, he and his colleagues found that 54 percent of post-TB patients returning for care had CPA (Nguyen et al., 2021). Research in New Delhi found similar results, with 57 percent of people diagnosed with TB having CPA (Singla et al., 2021). Recent research conducted by Denning in Ghana also indicated CPA in approximately half of patients diagnosed with TB. He emphasized the similarity in how TB and CPA present on chest radiographs, with both diseases involving the upper lobes of the lungs. Severe cavitation can occur with TB and multiple cavities can present in patients with CPA. *Coccidioides* and *Histoplasma* can

also cause similar lung symptoms, although they are less common than *Aspergillus* infections.

Modeling of CPA and TB in India suggests that approximately 2.5 million patients present with new TB symptoms in India annually, resulting in about 500,000 deaths. However, in 2020, only about 54 percent of those cases were confirmed bacteriologically. The remainder were diagnosed based on chest radiographs and symptoms. Modelling these numbers based on recently published data, Denning predicted that approximately 200,000 of the 2.5 million new TB cases—about 10 percent—will actually be CPA misdiagnosed as TB. An additional 150,000 patients will develop aspergillosis during or immediately after TB therapy. An additional 250,000 individuals are expected to develop CPA within 2-5 years after TB therapy. He posited that nearly one-third of the 500,000 annual deaths attributed to TB in India may actually be caused by CPA (Global Action for Fungal Infections (GAFFI), 2022).

Allergic Aspergillosis

A third category of people prone to aspergillosis are those with fungal allergies, said Denning. Allergic bronchopulmonary aspergillosis is a severe allergy to *Aspergillus* that usually occurs in people with poorly controlled asthma and can cause mucus plugs in the airways. Other people are allergic to *Aspergillus* and molds, such as *Cladosporium* and *Alternaria,* and can develop severe asthma as a result of the fungal allergy. He noted that these groups of patients generally derive benefit from antifungal treatment, including azoles such as itraconazole and voriconazole. Allergic fungal rhinosinusitis—which causes nasal polyps and congestion—is usually not treated with antifungals. Allergic aspergillosis is more common than other types but is far less severe.

The global burden of invasive aspergillosis is estimated at approximately 850,000, compared to 1.5 to 3 million for chronic aspergillosis and 6 to 20 million for allergic aspergillosis, said Denning. The mortality rate of invasive aspergillosis is 100 percent without treatment and decreases to 30 to 85 percent with treatment. Chronic aspergillosis carries a mortality rate of approximately 75 percent without treatment and 45 percent with treatment. The mortality rate for both treated and untreated allergic aspergillosis is less than 1 percent, although some asthmatic deaths may be linked to this condition, Denning noted (Bongomin et al., 2017).

Superficial Aspergillosis

Keratitis, onychomycosis, and otitis externa are all forms of superficial aspergillosis, said Denning. Fungal keratitis is an infection of the

front of the eye. Recent modeling indicates that approximately 40 percent of cases are caused by *Aspergillus* and about 50 percent are due to *Fusarium* (Brown et al., 2021). The annual incidence of fungal keratitis is between 1 and 1.4 million people globally. More often than not, the infection causes blindness in the infected eye, and in approximately 10 percent of cases the eye must be removed or it perforates. Natamycin is the most effective treatment for this infection, which can also be treated using azole eyedrops. Onychomycosis is a fungal infection of the fingernails or toenails. The majority of the 300 million global cases of onychomycosis are due to dermatophytes, but up to 3 percent of cases are attributable to *Aspergillus* (Bongomin et al., 2018). This translates to approximately 10 million cases of *Aspergillus*-related onychomycosis, many of which are comorbid with diabetes. Acute otitis externa—commonly referred to as "swimmer's ear"—affects 1 in 250 people annually. Chronic otitis affects 3-5 percent of the global population, totaling 200-350 million individuals. Approximately 10 percent of otitis cases are fungal in origin, most often caused by *Aspergillus* spp. (Wiegand et al., 2019).

Resistance in *Aspergillus*

Denning explained that resistance involves two groups of patients: those who develop resistance while on therapy and those who breathe in a resistant fungus. A challenge in measuring resistance is the low culture yield for *Aspergillus*, with standard methodology resulting in a culture rate of only 20 percent (Vergidis et al., 2020). However, utilizing high volume culture and polymerase chain reaction testing can elevate the culture rate to 76 percent. Denning noted a gap in older data on resistance due to numerous environmental and clinical surveys lacking positive cultures while utilizing conventional culture methodology. Azole resistance in *A. fumigatus* became evident in the late 1990s, with rates gradually increasing in the years since. (Bueid et al., 2010; Howard et al., 2009). In 2009, data from Manchester, United Kingdom indicated a 20 percent resistance rate, which was almost entirely driven by treatment, said Denning. Patterns of resistance indicate variation, with some cases of pan-azole resistance found. Data from the Netherlands indicate rates of resistance lower than 5 percent during the 1990s, but gradually increasing in recent decades (Buil et al., 2019). Denning noted that almost all of the *A. fumigatus* azole resistance found in that study was driven by environmental resistance.

The rise of azole resistance has led experts to question whether azoles can be retained for clinical practice, said Denning (Verweij et al., 2016). Jennifer Shelton, doctoral student at Imperial College London, conducted a community science project throughout the United Kingdom and found that 14 percent of isolates sampled were resistant to tebuconazole (see

Chapter 4) (Shelton et al., 2022). This constitutes a higher rate than earlier research, indicating a gradual increase in resistance. Research conducted in the Mekong Delta in Vietnam used a screening assay to identify azole resistance in 95 percent of environmental isolates, with persistent azole residues detected in the soil (Duong et al., 2021). Research conducted in China suggests resistance rates of approximately 80 percent in some agricultural areas (Zhou et al., 2021). Denning stated that given the need for azoles, these rates of resistance suggest a crisis situation.

THE EMERGING THREAT OF ANTIFUNGAL-RESISTANT *CANDIDA* SPECIES

Jackson provided an overview of the prevalence and risk factors for *Candida* infections. He discussed increasing *Candida* resistance to antifungals, the emergence and characteristics of *C. auris,* challenges of addressing resistant fungi, and hypotheses about why resistance is increasing.

Candida Infection Prevalence and Risk Factors

Candida species are a leading cause of health care-associated bloodstream infections in the United States and likely worldwide, said Jackson, who presented a hypothetical case to illustrate the threat *Candida* can pose to hospitalized patients (see Box 3-1). The COVID-19 pandemic further increased these infections with the associated rise in patients requiring ICU and central line treatment. He emphasized that *Candida* infections are not rare pathogens. A study found that in 2020, 28 percent of central line-associated bloodstream infections (CLABSI) in U.S. ICUs were caused by *Candida* (Weiner-Lastinger et al., 2022). Additionally, *Candida* caused 13 percent of CLABSIs in adult hospital wards. This fungus was more common than any single type of bacteria in both ICU and adult ward settings (Magill et al., 2018; Weiner-Lastinger et al., 2022).

Data from an ongoing 10-site surveillance study suggests that *Candida* bloodstream infections are associated with a mortality rate of 25-30 percent, a significant portion of which is attributable mortality (Toda et al., 2019). Risk factors include broad spectrum antibiotic use—due to the associated disruption of the microbiomes—as well as central lines, immunocompromisation, prolonged ICU stay, and abdominal surgery. *Candida* infections can occur when the intestinal barrier is disrupted. In such cases, conventional wisdom largely holds that *Candida* infection stems from autoinfection when the host flora enters the bloodstream. Deep-seated *Candida* bloodstream infections can affect specific internal organs. However, the frequency of these deep-seated infections is unknown due to the difficulty in detecting and monitoring them.

BOX 3-1
Hypothetical Case Study of Healthcare-Associated *Candida* Infection

A 61-year-old man with hypertension and pre-diabetes presents to the emergency department in late 2020 with a cough, fever, shortness of breath, and recent exposure to COVID-19. In this hypothetical scenario, the hospital admits him due to low oxygen levels and confirms SARS-CoV-2 infection. He is treated with remdesivir, the steroid dexamethasone, and tocilizumab, a potent immunosuppressive interleukin-6 inhibitor. Additionally, empiric antibiotics are administered for possible simultaneous community-acquired pneumonia. The following day, he rapidly worsens and requires intubation and admission to the ICU. He then develops septic shock and his blood pressure plummets. Vasopressors are administered through a central line to address the low blood pressure. Over the next few days, the man improves. On day 6, his respiratory status is improving, oxygen requirements are decreasing, and the fever is resolving. However, on day 10, he develops a new fever and his blood pressure once again drops. Care providers think he may have contracted a health care-associated bacterial infection and prescribe broader antibiotics. Two days later, blood culture results indicate *Candida* species with small white colonies having developed on the culture plate. At that point, he is still doing poorly, and fluconazole is added. On day 13, the yeast species is identified as azole-resistant *C. glabrata*. Therefore, his fluconazole treatment is changed to echinocandin. In this scenario, the individual's likelihood of survival is approximately 50 percent.

SOURCE: Presented by Brendan Jackson on June 21, 2022.

Jackson stated that non-invasive candidiasis should not be ignored. Types of non-invasive candidiasis include vulvovaginal infections ("yeast infections"), oral infections ("thrush"), esophageal infections, and skin infections. Esophageal *Candida* infections can be associated with morbidity. Although most cases of non-invasive candidiasis are not lethal, they require substantial medical care. In 2017, more than 3.6 million outpatient visits were due to these infections, creating over $2 billion in direct medical costs (Benedict et al., 2019). Furthermore, these infections likely account for the vast majority of systemic azole therapy, said Jackson (Benedict et al., 2022a).

Antifungal Resistance Among *Candida* Species

Jackson explained that *Candida albicans* was once the dominant *Candida* species for both invasive and non-invasive infections, accounting for approximately 90 percent of invasive human infections. Currently,

two-thirds of invasive *Candida* infections are non-*albicans* (CDC, 2021c). In the United States, *C. glabrata* is almost as common as *C. albicans*, and *C. parapsilosis, C. tropicalis,* and *C. krusei* cause a significant portion of infections. In some countries, *C. parapsilosis* and *C. tropicalis* have become more common than *C. glabrata*. Jackson remarked that the rise of other *Candida* species is significant due to antifungal resistance rates being higher in non-*albicans* species. To this point, CDC labeled drug-resistant *Candida* species as a serious threat in 2019 (CDC, 2019).

The CDC Emerging Infections Program conducts sentinel surveillance in the United States, said Jackson. In testing bloodstream samples, they found that 6-10 percent of *C. glabrata* isolates were resistant to azoles, 1–4 percent were resistant to echinocandins, and less than 1 percent were resistant to polyenes (CDC, 2021c). He noted that efforts are made to avoid treating *Candida* with polyenes, given the complications outlined by Spec in his presentation. Increasing resistance can be found in other species of *Candida*, including *C. parapsilosis* and *C. tropicalis*, which have up to 10 percent and 6 percent fluconazole resistance, respectively, in the United States (Toda et al., 2019). Moreover, increasing emergence of resistant clones has been found worldwide for both species (Fan et al., 2017; Govender et al., 2016; Pristov and Ghannoum, 2019). Jackson stated that some strains are resistant to both fluconazole and echinocandins, and *C. krusei*—the fifth most common species—is typically intrinsically resistant to fluconazole.

Increasing resistance to fluconazole contributed to the Infectious Disease Society of America recommending echinocandins as first-line treatment for most forms of candidiasis in 2016, said Jackson (Pappas et al., 2016). However, the guidelines have yet to be fully adopted in clinical practice; a recent study found that up to 30 percent of patients were receiving fluconazole as initial treatment (Gold et al., 2021). Furthermore, 56 percent of patients were found to have a non-*albicans* species, reducing the likelihood that fluconazole would be the most effective treatment. Growing concern regarding antifungal resistance prompted the World Health Organization (WHO) to include *Candida* in their Global Antimicrobial Resistance and Use Surveillance System, which should help increase the volume of data collected worldwide on this type of resistance.[2]

The Rise of *Candida auris*

C. auris was basically unknown in the scientific community before 2009, when it was detected in an ear specimen and was thus named "*auris*,"

[2] More information on the Global Antimicrobial Resistance and Use Surveillance System (GLASS) is available here: https://www.who.int/initiatives/glass (accessed February 15, 2023).

Jackson explained. By the early 2010s, global reports of invasive *C. auris* infections began to emerge. In 2015 and 2016, outbreaks occurred in the United Kingdom and cases were detected in the United States, garnering media attention (Richtel and Jacobs, 2019; Smothers, 2016). He described *C. auris* as behaving like multidrug-resistant bacteria, sharing similarities with *Staphlococcus aureus, Clostridioides difficile,* and *Acinetobacter.* This yeast can spread rapidly in health care facilities, and long-term care facilities are fertile ground for outbreaks in the United States. *C. auris* colonizes patients, especially skin, and appears to exploit a disrupted microbiome. Jackson noted that health care employees do not tend to be colonized for very long, if at all, indicating that a disrupted microbiome may be key in the colonization process. Moreover, *C. auris* causes invasive infections in 5–10 percent of people colonized with the yeast (Southwick et al., 2018). The fungus survives disinfectants, particularly quaternary ammonium compounds, used to decontaminate health care facilities. Special disinfectants are often required to kill *C. auris*, Jackson added.

In comparison with *C. glabrata, C. auris* resistance to antifungals is more prevalent, said Jackson. Approximately 90 percent of *C. auris* isolates are resistant to azoles and 33 percent are resistant to polyenes. Echinocandin-resistance is lower, at less than 5 percent, but cases of resistance have occurred during echinocandin treatment. Pan-resistant *C. auris*—resistant to all three drug classes of antifungals—has also emerged (Lyman et al., 2021). Furthermore, resistance is not only developing in patients receiving echinocandin therapy, but also in patients in health care facilities who never received antifungal therapy and contracted transmitted strains that are already resistant. If the type of outbreaks that have occurred in Texas and in Washington, D.C., begin to occur more frequently, echinocandins may become far less useful, creating a lack of treatment options.

Jackson stated that *C. auris* is perplexing in that at least four, and possibly five, clades developed in different parts of the world at approximately the same time (Chow et al., 2018). These clades were first detected in South Asia, southern Africa, east Asia, and South America. Within the United States, the clade that first appeared in South Asia emerged in New York and New Jersey and spread to other northeastern states. The clade that was first detected in South America emerged in Chicago, and the clade that was first detected in Africa appeared in Indiana. In some cases, CDC was able to contact trace the spread to an incoming traveler from a country where a clade of *C. auris* was present, but in other cases, the initial introduction remains unknown. He described fungal taxonomy as complicated and in flux. According to this taxonomy, *C. glabrata* and *C. albicans*—the two most common species of *Candida*—are as different from each other as humans are from fish (Gabaldón and Carreté, 2016). Jackson added that the *Candida* taxonomy includes *Saccharomyces*, the

yeast used in making bread and beer, which was not named *Candida* due to its common uses. The species of *Candida* not yet discovered likely number in the hundreds of thousands or even millions.

Although *C. auris* was discovered relatively recently, it is not new and has had lengthy time to evolve, said Jackson. Improved detection methods may have played a role in its discovery, but a variety of causes may be responsible for its emergence and proliferation in human health care settings. Specifically, modern health care settings have facilitated its spread and it is probable that multiple spillover events from the environment have occurred. Relatives of *C. auris* have recently been isolated from the environment, and people have collected samples of it from flowers in Southeast Asia, rubber tree sap, bugs, fish, and dolphins. In 2021, *C. auris* was located for the first time outside of health care and clinical settings in the Indian Ocean (Cunningham, 2021). Multiple theories for the rise of *C. auris* have been proposed, including (1) effects of climate change, (2) intrusion of humans into its natural habitat via deforestation, (3) human activities that may amplify the pathogen's reservoir, such as shrimp aquaculture in which antibiotics and fungal probiotics are added to water, (4) the use of environmental fungicides and antifungals, and (5) changes to the human host's microbiome (Casadevall et al., 2019; Jackson et al., 2019; Steffen et al., 2015). Jackson noted increasing evidence that *Candida,* including *C. glabrata* and *C. albicans*, are common colonizers that can occupy trees and migratory birds that then play a role in circulating the fungi (Al-Yasiri et al., 2016; Bensasson et al., 2019).

The extent of the linkages between *Candida* and the environment are not yet known, added Jackson. However, there has been a marked increase in the use of azole fungicides over the last decade (Toda et al., 2021). This increase of fungicides has taken place in different parts of the world at different rates; its use in the United States is driven by corn, wheat, and soy. Research on links between *Candida* resistance and agricultural fungicides is in the early stages (Brilhante et al., 2019). Jackson cautioned that the full effect of azole fungicides on the yeast species remains to be seen. Because the environment appears to play a stronger role in the emergence of resistance than once thought, resistance stewardship will be a key pillar in addressing *Candida*.

CURRENT RESEARCH ON PATHOGENESIS AND HOST IMMUNITY TOWARD TREATMENT OPTIONS

Lionakis discussed how understanding interactions between the host and fungi at the research level may enable hematologists and oncologists to develop targeted immune-based therapies to treat severe fungal infectious diseases. Robust immune factors protect humans from the

commensals—such as *Candida*—and fungi that they are exposed to on a daily basis. The occurrence of immune perturbations increases the risk of fungal infections. Therefore, a deeper understanding of these events could facilitate efforts to boost immune responses, cultivate a more thorough knowledge of patients at risk, and develop personalized therapies.

Pathways for Fungal Infections in Immunocompromised Patients

Some individuals have genetic or acquired iatrogenic risk factors that increase their likelihood of developing fungal infections, said Lionakis. Susceptibility to infection can emerge when patients are given certain medications or when congenital genetic diseases are inadvertently triggered or exacerbated in the hospital setting. Understanding these pathways could enable diseases to be researched in mouse models to discern what helps the immune system control fungal disease, which in turn would inform the development of treatments. Research in mice thus far has demonstrated the ability of neutrophils and macrophages to attack and destroy fungi. However, despite medical therapy, *Candida* and *Aspergillus* cause over 20,000 deaths in the United States each year. This mortality is greater than that of *Staphylococcal* infections or HIV/AIDS and highlights the need for new treatments.

Lionakis recalled that until the introduction of imatinib in 2001, no personalized approach to treat oncologic patients was available outside of toxic chemotherapy. The use of imatinib ushered in a wave of biologics that have transformed the treatment of cancers (Wu et al., 2016). In 2018, Jim Allison won the Nobel Prize for Physiology or Medicine in response to his discovery of the immune checkpoint inhibitor, which utilizes the immune system to combat cancer. Lionakis posited that fungal treatment can follow a similar trajectory to that taken by cancer treatment. Transplantation, HIV, and chemotherapy are conventional risk factors for fungal infection related to the immune system. He predicted that the number of patients prescribed biologics will expand into a significant subset of individuals at risk of developing fungal infections. Furthermore, physicians prescribing biologics are not always aware of this risk.[3]

[3] Warnings of increased risk of serious and life-threatening meningococcal infections are included in the medication guides for some biologics. An example is available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/125166Orig1s427s428MedGuide.pdf (accessed July 30, 2022).

Complement Component 5 Pathway

Lionakis provided examples of how biologics used in an expanding population of already-immunocompromised patients can create pathways for severe fungal infections. Complement is an ancient protein network utilized within the immune system to defend against bacterial disease. The advent of medical treatment that neutralizes complement component 5 (C5) led to an eruption of fungal disease. In response, the U.S. Food and Drug Administration (FDA) updated the drug's package insert to caution that patients on the drug may develop fungal disease. People on complement C5 inhibitors are at an increased risk of developing infections from *Aspergillus* or *Candida,* with mortality rates that can exceed 70–80 percent. Lionakis described that this information is being used to develop predictors for poor outcomes in patients. Researchers have found that mice who lack complement C5a die, whereas their wildtype counterparts do not (Mullick et al., 2004). With ICU patients, measuring low activation of the C5a pathway due to the introduction of biologics enables the development of independent predictors of patient outcomes.

Although invasive candidiasis has become a common disease in acutely ill patients in the ICU, both disease severity and mortality vary among this group of patients. Research has examined the relationship of genetic variation in certain genes with resistance and immunopathology levels (Collar et al., 2018; Kumar et al., 2014; Lionakis et al., 2017a; Lionakis et al., 2012; Smeekens et al., 2013; Swamydas et al., 2016). Certain studies indicated that some genetic variations will not make a person prone to disease until they are in a hospital setting, at which point they can be up to 20 times more likely to develop fungal infections (Kumar et al., 2014). Lionakis stated that this has important implications for envisioning the future of developing treatments and personalized approaches to patient care.

SYKCARD9 Pathway

Lionakis presented another example of the relationship between biologic drugs and fungal infections. Humans have a pathway of C-type lectin receptors that utilizes spleen tyrosine kinase (SYK) and caspase recruitment domain family member 9 (CARD9) protein-coding genes to eliminate fungi. Deficiencies in the SYK-CARD9 pathway increase the likelihood of severe fungal infections (Drummond et al., 2019). In 2018, a drug called fostamatinib gained FDA approval for treatment of chronic immune thrombocytopenia (FDA, 2018). Fostamatinib is an SYK-inhibitor, yet many physicians who prescribe it are unaware that the drug has the capacity to predispos patients to fungal disease, said Lionakis.

Providing a case example, Lionakis described a Colombian child that

came under his care due to infection by *Corynespora*, a plant pathogen that is responsible for target leaf spot on cucumbers and tomatoes and is presumed not to infect humans. Moreover, it is not supposed to grow in temperatures over 30 degrees Celsius, he added. However, in a susceptible human host, *Corynespora* has developed the ability to grow at 37 degrees. Lionakis remarked that this demonstrates the ability of fungi to evolve and to develop azole resistance within the human brain to the point of killing the patient despite bone marrow transplant. Ten percent of patients treated with fostamatinib have developed severe fungal disease, indicating that expansive populations of patients are susceptible (Zarakas et al., 2019). Lionakis added that this susceptibility puts individuals at risk of developing resistance or capturing fungi with intrinsic resistance during the process of receiving medical treatment.

Ibrutinib and Fungal Infections

Ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, is an example of a small molecule drug that transformed the treatment of hematological malignancies, said Lionakis. Ibrutinib treatment may be associated with the later development of fungal infections (Ahn et al., 2016; Lionakis et al., 2017b; Messina et al., 2017). He described a series of patients seen at the National Institutes of Health (NIH) who had refractory central nervous system lymphoma that historically caused death within 2 to 3 months. Ibrutinib was effective in significantly reducing the size of brain tumors (Lionakis et al., 2017b). However, while the lymphoma moved into remission, the incidence rate of *Aspergillus* infections increased to approximately 30 percent among these patients. This high rate of fungal infection is in the context of a treatment that caused years of almost complete remission for nearly 70 percent of patients, who otherwise would have died within months (Roschewski et al., 2018). These simultaneous high rates of infection and near-remission led researchers to examine how to provide patients with life-extending therapy without the high risk of fungal disease. In this case, targeted prophylaxis with isavuconazole enabled over 100 patients to receive ibrutinib treatment without developing invasive aspergillosis.[4] Lionakis noted that this example demonstrates how understanding immunity can inform a targeted prophylactic approach to safely delivering a potentially toxic therapy. Exploring host-pathogen interactions can lead to identification of potential prognostic biomarkers, which in turn inform patient risk stratification. This understanding can also create awareness in clinicians regarding the infections their patients on biologics are prone to contracting.

[4] More information about this ongoing study is available at https://clinicaltrials.gov/ct2/show/NCT02203526 (accessed July 30, 2022).

Developing Immune-Based Treatment Options

Lionakis also shared that research could potentially lead to the development of immune-based therapies that co-opt the immune system to boost the effect of antifungal drugs. NIAID research on ibrutinib found that the drug hinders the ability of neutrophils to fight *Aspergillus* within three days of initial administration. Healthy neutrophils produce oxidative burst products that kill *Aspergillus* by damaging the hyphae of fungi. Ibrutinib disrupts this process, inducing a reactive oxygen species (ROS) defect in neutrophils. By gaining an understanding of the molecular underpinnings of that process, researchers found that utilization of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF)—an FDA-approved drug—restores the ability of neutrophils to generate oxidative bursts. Researchers have successfully overcome the BTK-inhibition-induced ROS defect by using GM-CSF in the laboratory setting, and GM-CSF also rescues the ROS defect in individuals who are BTK-deficient. GM-CSF could potentially be used in patients receiving ibrutinib treatment in the future to restore their ability to fight *Aspergillus*. He noted that GM-CSF actually boosts the immune system's ability to kill *Aspergillus* above normal levels.

Antibiotics are a common risk factor for systemic candidiasis in both humans and mice, said Lionakis. Mice models indicate that in addition to the role of microbiota, antibiotics cripple the immune system's ability to kill fungi through lymphocytes and GM-CSF mechanisms (Drummond et al., 2022). Part of this susceptibility can be rescued by giving mice GM-CSF. Furthermore, examination of a large dataset of over 10,000 U.S. patients with candidemia suggests that this observation may translate into humans. This is another example of the potential for future immune-based interventions that can be added to antifungals in treating patients prone to fungal infections. In the 1990s, interferon gamma was the first (and remains the only) FDA-approved immune-based therapy for infectious disease (International Chronic Granulomatous Disease Cooperative Study, 1991). Twenty years ago, no FDA-approved targeted biologics for hematology oncology were available, Lionakis noted. Just as immune therapies have advanced for infectious diseases and oncology, further developing the fungal-host interaction knowledge base will increase the ability to treat difficult fungal disease in patients with immune defects and in patients with resistant fungal infections.

Therapeutic strategies that dampen the immune system to combat fungal disease are also being explored, said Lionakis. COVID-19 illustrated how some infections can trigger an excess immune response, leading doctors to treat COVID-19 patients with steroids and Janus kinase (JAK) inhibitors. Chronic mucocutaneous candidiasis is a severe, non-lethal infection that can cause significant morbidity and resistance. Approximately 60 percent of

these patients develop resistant *Candida*. Researchers have discovered that susceptibility to *Candida* is not necessarily due to an inability of the immune system to address the fungi; it can also be attributed to an exacerbated immune response known as autoimmune regulator (AIRE) deficiency (Break et al., 2021). They found that by inhibiting the interferon gamma immune response in mice with AIRE deficiency and uncontrolled *Candida*, the mice became able to control the infection. A phase II clinical trial at NIH are currently testing this intervention, and Lionakis shared anecdotal evidence based on a study (manuscript in preparation) of patients with multidrug-resistant *Candida* and candidiasis going into remission within one week of treatment with an immune modulator such as a JAK inhibitor. He added that this type of research can inform approaches to more common diseases, including HIV, in which CD4 decline is unlikely to be solely responsible for *Candida* infections, as other patients with CD4 deficiency such as those with idiopathic CD4 lymphocytopenia are not at risk for developing mucosal candidiasis. A better understanding of host-fungal interactions could fuel the development of personalized risk stratification and prognostication strategies, targeted prophylaxis strategies, and immune-based adjunct therapies for treating patients with resistant and difficult-to-treat infections. Given that the development of multiple new classes of antifungals is unlikely, this area of research holds particular promise in improving patient outcomes.

DISCUSSION

Diagnostic and Data Challenges

Noting challenges in fungal infection diagnosis and data on disease burden and resistance, Chiller asked about the factors that contribute to these challenges and their connection to stewardship efforts. Denning replied that antigen and antibody tests are the most sensitive and rapid tests for fungal disease not occurring on the skin, but a culture is required for identifying resistance. However, culture tests are relatively insensitive for fungal infections: the sensitivities of *Candida* blood culture and of *Aspergillus* sputum culture are approximately 40 percent and 30 percent, respectively. This low sensitivity poses a problem for diagnosis. Moreover, in low- and middle-income countries, diagnostics are often unavailable. For instance, many countries in Africa only have microscopy and simple skin culture diagnostics. Thus, global health issues contribute to the challenges that the insensitivity of diagnostics present. Spec added that fungi are ubiquitous, which makes it difficult to differentiate between fungal infection, colonization, contamination, and presence from a culture. The combination of this ubiquity and the insensitivity of cultures makes accurate diagnosis, and especially detection of resistance, challenging.

Aspergillosis Incubation and Dosing

Chiller asked about the route of transmission and infectious dose range for aspergillosis. Denning stated that the incubation period is variable, from 2 to 90 days. Lungs are not sterile, and autopsy research has demonstrated that *Aspergillus* can grow in human lungs (Lass-Florl et al., 1999). Macrophages, neutrophils, and epithelia in the lungs play an important role in eradicating *Aspergillus*, but they are not fully effective, leading to ongoing colonization. Denning added that the infective dose for *Aspergillus* has not yet been fully determined, although more progress has been made on determining the infective dose for *Histoplasma*.

Cornyespora Infections and Resistance Types

Given that the young patient suffering with *Corynespora* had an azole-resistant fungal isolate, Chiller asked whether the resistant type at play was inherent or acquired due to the medical use of azoles, and—if the latter—whether the resistance mechanisms were investigated. Lionakis replied that the child arrived at NIAID with a fully susceptible strain for azoles. During the 15 months of treatment, the brain isolate became resistant, while the skin isolate did not. He posited that the lower levels of azoles within the brain tissue may have facilitated the development of secondary resistance. The strains are currently in the process of whole genome sequencing; thus, the mechanism of acquired resistance has not yet been determined. Cases have occurred in which patients are susceptible and happen to contract an intrinsically resistant strain. Lionakis stated that as more individuals become immunocompromised due to medical treatment, both patterns will occur. Some patients will contract inherently resistant strains; others patients who require intensive therapy for difficult-to-treat infection sites will have strains that acquire resistance during treatment.

The discussion continued at the end of the day. Marin Brewer, associate professor of Mycology and Plant Pathology at the University of Georgia, co-moderated this portion of the discussion with Chiller. Spec stated that a fascinating aspect of this case is that *Corynespora* is a plant pathogen. Historically, plant pathogens have rarely caused human disease. However, in recent years, he has seen several cases at Washington University involving plant pathogens, including *Thyronectria austroamericana*, a pathogen of honey locust trees. These trees have long thorns that can cause injuries leading to infections. He co-authored a case study about a patient in Missouri who developed *Thyronectria austroamericana* septic arthritis, and shortly after publication he was contacted by a physician in Kentucky who developed a tenosynovitis after receiving a wound from a honey locust tree (Rutjanawech et al., 2021). Spec emphasized that this pathogen had never before been

described as causing human disease, yet in two years, two cases of human infection from the pathogen were identified within the same ecological area. Recently, he has seen a case of mycorrhizal fungi that caused brain disease in a patient who was exposed via his work on tractors and tilling equipment. Spec remarked that it is not yet known whether these cases can be attributed to improved detection efforts, or if new pathogens are now infecting humans.

Antifungal Drug Development

Chiller asked whether pharmaceutical companies are investing in antifungal therapies for medical use. Spec replied that an uptick in the development of antifungals has taken place in recent years. No new major class of drugs has been developed for invasive mycoses since the echinocandins in 2002. Currently, several antifungal drugs are in clinical trials with the possibility of being marketed in the next few years. For instance, phase 3 clinical trials for ibrexafungerp was approved with a limited indication for vulvovaginal candidiasis, but it has not been approved for invasive disease. Olorofim is another new antifungal drug in phase 3 clinical trials and belongs to the orotomide class of antifungals. Spec remarked that the number of clinically available antibiotics far outnumber the number of antifungal drugs. He highlighted systematic disinvestment in antifungals for financial purposes, stating that the lack of financial incentives plays a large role in the limited antifungal options available. Denning commented that small biotechnology companies are often responsible for the development of antifungal drugs, and once a drug looks promising, large pharmaceutical companies become involved to make marketing deals. Antifungal research is often funded by nondilutive grants followed by venture capital, which tends to invest more heavily in antibacterials than in antifungals. Thus, a lack of funding persists in both the nondilutive funding space and with large pharmaceutical companies.

Denning added that three companies are developing inhaled azole antifungals for the management of patients with severe asthma, cystic fibrosis, and for prophylactic use in patient with leukemia and transplants. These forthcoming drugs could be rendered ineffective should rates of resistance become high. Thus, minimizing azole resistance in the environment plays a role in maintaining the effectiveness of existing and newly developed antifungal drugs.

Aspergillus flavus

Noting that *Aspergillus flavus* (*A. flavus*) is less relevant from a clinical perspective than *A. fumigatus*, Brewer asked whether azole resistance is also present in *A. flavus*. Denning stated that low levels of *A. flavus* resistance to

voriconazole as a sole compound have been found, but the mechanisms for this resistance have not yet been determined. The researchers that looked for *A. fumigatus* in Vietnam did find high rates of *A. flavus* with resistance in the environment, at approximately 50 percent (Duong et al., 2020). *A. flavus* is a bigger issue in some countries that in others—for instance, it is particularly common in India. *A. flavus* is more related to cutaneous infection, superficial infection, and sinus infection for reasons not fully understood, said Denning.

Preventative Measures for People at Risk

Chiller asked whether any preventative measures related to lifestyle factors such as diet, travel, or employment can be taken to decrease the likelihood of fungal infections. Lionakis replied that keeping one's body healthy can help to some extent; he highlighted the value of patients with neutropenia controlling diabetes and staying vigilant in their health and lifestyle decisions. However, fungi are ubiquitous and mold outbreaks can occur in most locations. Denning added that immunocompromised patients should avoid any significant amount of gardening and composting. For instance, pruning flowers may be safe, but heavy garden work is not advised. Wood chips and mulch contain huge quantities of spores, and therefore these immunocompromised people should avoid mulching entirely. Additionally, people with compromised immune systems should avoid any kitchens or bathrooms that are in the process of being remodeled and should not sort through dusty old photographs or papers, said Denning. He noted that this guidance is a bit vague and acknowledged that although immunocompromised people are advised to avoid large fungal inocula, the effectiveness of these preventative measures is unknown. Spec echoed that although the inoculum levels are not known, highly susceptible individuals should avoid situations known to expose them to a large inocula. Mulching poses a documented risk of pneumonitis from fungi for patients with inherent immunodeficiencies but quantifying the exact amount of outdoor exposure that poses a significant health risk is difficult. Spec recalled that during a recent trip to Houston, he saw a construction site and imagined that the combination of humid weather and activity that potentially disperses more fungi spores into the environment could synergize to increase the risk of infection. However, quantifying such scenarios into real risk for patients is complicated.

Risk Assessment for Fungicide Resistance

Fungicides are evaluated for their potential to develop resistance in plant pathogens, and *A. fumigatus* is not a plant pathogen, Chiller noted (see Chapter 4 for further detail). Given that antifungal resistance in

A. fumigatus is a downstream effect of fungicide use in the environment that ultimately affects human health, he asked how the evaluation or risk assessment process can help assess the potential for resistant fungi. Denning stated that this question is difficult to answer from a clinical perspective. One method involves sampling of the environment for resistance. European guidelines on *Aspergillus* recommend annual sampling of at least 100 strains from a locality's environment to determine the likelihood of a resistant strain (Ullmann et al., 2018). In cases where 10 percent or more of the captured fungal isolates exhibit resistance, patients who are critically ill or severely immune-compromised should receive combination dual antifungal therapy rather than monotherapy, which is the standard recommendation. Denning noted that these guidelines do not specify the strains or quantity to sample, nor the location from which to collect the sample. Collaborative generation of better data on rates of environmental resistance could inform guidance on a standard approach and sampling methods and locations (e.g., whether to test only for *A. fumigatus*, itraconazole, or tebuconazole in a certain scenario). Developing the knowledge base to provide a more specific set of guidelines would be a valuable joint exercise, said Denning.

Infection by Fungi Ingestion

Chiller asked whether it is possible to become infected by ingested fungi spores via the gastrointestinal tract. Denning replied that this can occur in leukemia patients with profound neutropenia. He has seen cases of intestinal *Aspergillus*, and he stated near certainty that this is contracted through the intestines. Although such cases of infection via ingestion are exceedingly rare, food preparation can serve as a transmission route. For example, during the grinding and sprinkling processes, pepper mills can aerosolize *Aspergillus* that are then inhaled. Similarly, loose tea can release spores into the air that can be inhaled and lead to infection.

Differentiating Colonization and Infection

Chiller asked whether colonization can be differentiated from infection in patients with COPD. Additionally, he queried whether susceptible hosts are treated for all fungi. Denning stated that this question goes to the heart of the difficulty of distinguishing an infection from a colonizing organism. In the context of *Candida*, the presence of the fungi in the respiratory tract is rarely treated because *Candida* pneumonia and bronchitis are uncommon. *Aspergillus* is more complicated in that its yield is often higher in COPD patients than in the general population. This is due to the depression of the ability of epithelial cells and macrophages to kill *Aspergillus* in people with COPD. Furthermore, inhaled steroids are often prescribed

to people with COPD, further depressing their ability to kill *Aspergillus* in the airways. Denning remarked that these factors increase the likelihood that colonization, rather than infection, is taking place, although diagnosis is difficult. He added that traditionally, *Aspergillus* was regularly dismissed as colonization in patients who did not have leukemia or transplants. Awareness of allergic aspergillosis and *Aspergillus* bronchitis has increased, and clinicians need to be better educated about factors affecting susceptible hosts in determining treatment, said Denning.

Variations in Azole Resistance

Given that azoles have been used in agriculture since the 1970s, but resistance with *A. fumigatus* did not appear until the twenty-first century, Brewer asked whether evidence indicates that some azoles are more inclined to promote resistance than others. Denning replied that in the agriculture environment, five of the more modern azoles, such as the triazoles, seem to have produced a slightly higher rate of resistance than older azoles, such as the imidazoles. In the clinical environment, itraconazole likely has a slightly higher rate of resistance than voriconazole and posaconazole. He added that not much is yet known about isavuconazole. Lionakis stated that fungi do not have a uniform evolutionary response to azoles; different fungi can have varied responses. He emphasized the importance of examining how humans are changing the microbial community of the environment. In addition to concerns about resistant *A. fumigatus,* the killing of some types of fungi potentially creates opportunities for other fungi to grow, such as *Lomentospora* or *Scopulariopsis*, which Lionakis described as "nightmare fungi." The use of azoles that increase the relative fitness of fungi in the environment could be increasing the likelihood of a fungus that is ubiquitous, spreads through the air, and cannot be treated.

Standardized Assays to determine resistance emergence

During the discussion at the end of the second day of the workshop, Denning was asked whether having a standardized assay to measure the effect of exposure to sublethal concentrations and minimum inhibitory concentrations (MIC) on the potential for bystander species to develop resistance would be beneficial. He agreed that this type of assay is beneficial, explaining that the current process to develop antifungal includes repetitively exposing a set of known fungal strains to MIC and sub-MIC doses over multiple generations to measure the emergence and levels of resistance. Establishing this assay is essential because a drug for which resistance emerges in the fungus after two or three generations would not be fit for clinical practice. Furthermore, this research is relatively inexpensive.

4

Fungicide Resistance in Plant Protection Use

The third panel of the workshop explored the development of antifungal resistance in response to the agricultural use of fungicides. Marin Brewer, associate professor of Mycology and Plant Pathology at the University of Georgia, and Tom Chiller, chief of the Mycotic Diseases Branch at the Centers for Disease Control and Prevention, co-moderated the session. Matthew Fisher, professor of Fungal Disease Epidemiology at Imperial College London, discussed the history, genetics, and mechanisms of antifungal resistance. He described the prevalence of resilience in *Aspergillus fumigatus (A. fumigatus)* and challenges in developing antifungal treatments. Kevin Doughty, senior stewardship manager at Bayer AG Crop Science division, outlined the conditions capable of promoting the selection and amplification of resistant isolates of *A. fumigatus,* as well as the role these settings can play in the distribution and frequency of azole-resistant fungal strains.

GENETICS AND MECHANISMS OF FUNGICIDE RESISTANCE

Fisher discussed the history of antifungals, the distinction between resistance and tolerance, the polymorphism processes involved in resistance, the prevalence of azole-resistant *A. fumigatus* (ARAf), and the development of new antifungal clinical treatments.

The Proliferation of Antifungals, Tolerance, and Resistance

Humans have been battling blights and molds since agricultural practices began, said Fisher. Over the past 400 years, methods of combating

fungal infections have ranged from urine and brining in the seventeenth century, arsenic and copper sulphate in the eighteenth century, and a Bordeaux mixture of grapes in the nineteenth century. The era of modern fungicides began in the twentieth century with the introduction of a panoply of fungicides. During the current century, new methods such as RNA interference are being developed. Although numerous fungicides are used in agriculture, there are far fewer classes of antifungals available for animal use, due to their toxicity. At present, only four classes of antifungal drugs have been developed for use on animals.

Fungi are eukaryotes with flexible genomes that have the ability to respond adeptly to natural selection, Fisher explained. A large and comprehensive literature review found that fungi develop resistance to all classes of drugs used against plant and animal fungal infections (Fisher et al., 2018) (see Figure 4-1). Fungi utilize a spectrum of mechanisms to avoid the toxic effects of antifungals. Resistance mechanisms that have been identified thus far include (1) changing the structure of the target site, (2) overexpressing genes to produce more copies of the target site, (3) deleting the target site or molecule, (4) removing the drugs out of the organism through efflux pumps, (5) regulating stress response pathways that counteract the drug effects, and (6) utilizing genomic plasticity to alter regions of the genome (e.g., aneuploidy, hypermutation).

Fisher drew a distinction between resistance and tolerance, noting that the term "resistance" is often inaccurately used in cases of tolerance. Resistance is a hardwired trait—heritable and often genetically encoded—that is acquired due to changes that directly or indirectly affect the drug-target interaction (Fisher et al., 2022). Tolerance describes the ability of a fungus to grow at drug concentrations above a set point minimum inhibitory concentration of a target fungal pathway (Berman and Krysan, 2020). This more complex trait involves a wide range of epigenetic and/or general stress response pathways. A fungus growing in the presence of an antifungal drug does not necessarily indicate resistance, as a tolerance mechanism could be at play.

The relatively limited number of mode-of-action targets available in fungi has led agriculturalists to iterate on the same class of chemicals used in clinics, said Fisher. Azoles best exemplify this dual use. Azoles have been used as fungicides since the 1970s, with at least 20 varieties of azoles in use today. He remarked that large quantities of azoles are utilized in the environment (e.g., in agricultural products, paints, antifouling agents, and others) and that they may constitute the most widely used chemicals on the planet. These include both first-generation and newer generations of azoles. Pulmazole and opelconazole are examples of newly available antifungals.

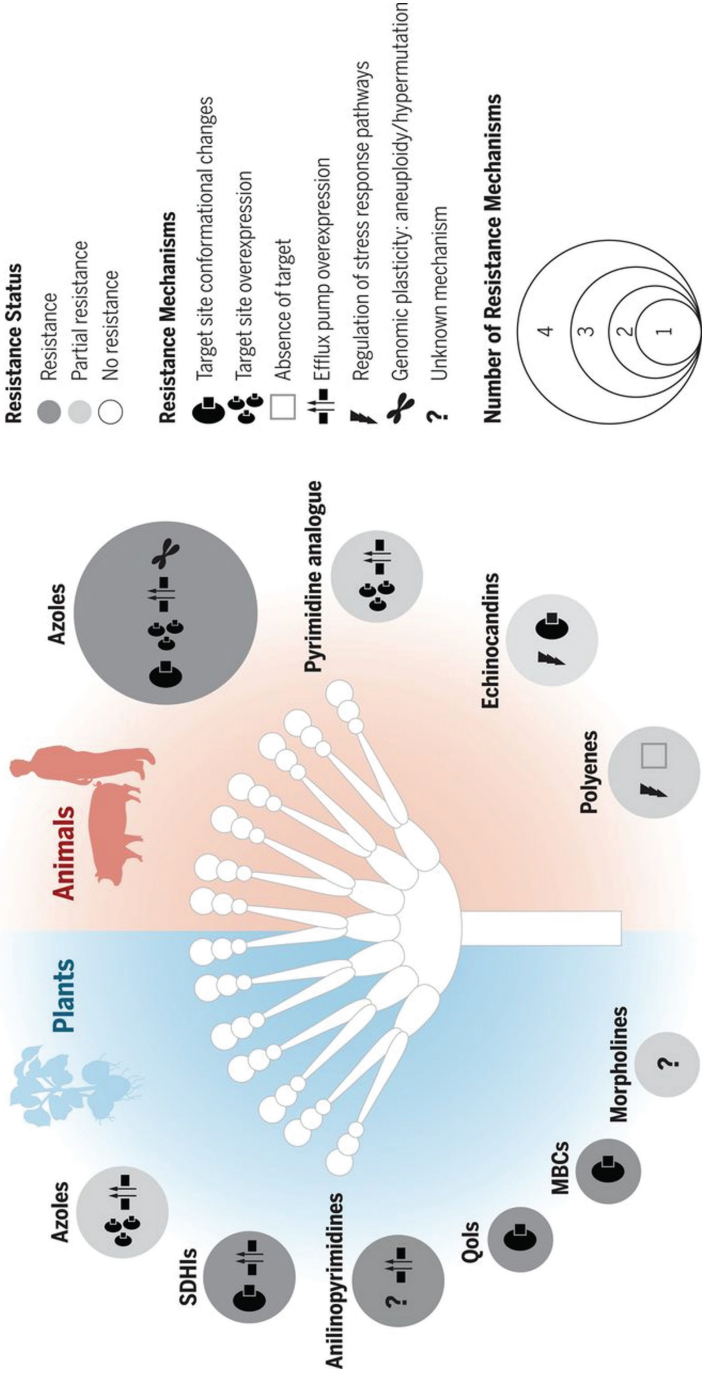

FIGURE 4-1 Resistance occurs to all classes of drugs used against plant and animal fungal infections.
SOURCE: From Fisher et al., 2018. Reprinted with permission from AAAS.

Azole Resistance in *Aspergillus*

Farmers defend crops against a wide spectrum of blights using azole fungicides that target the ergosterol biosynthetic pathway, said Fisher. Azoles disrupt the pathway by inducing steric inhibition of the sterol 14-alpha-demethylase (CYP51), an enzyme within the cytochrome P450 superfamily (Bhattacharya et al., 2018). Polymorphisms in CYP51 take place within a number of plant pathogens, leading to resistant pathogens that affect a broad range of crops essential for the human food supply (Price et al., 2015). As these plant pathogens evolve resistance, farmers must respond by shifting to new formulations of azoles or modifying the crop through trait breeding or genetic modification to increase its resilience to the evolved fungal pathogens.

Fisher's literature review revealed a sizable increase in azole resistance in both the human and plant spheres (Fisher et al., 2018), which raises the question of how much of the increasing resistance seen in patients is acquired through fungal adaptations to fungicides. Multiple research groups have focused on answering that question, Fisher noted. For instance, *A. fumigatus* is a ubiquitous thermophilic fungus found throughout the planet. Patients with chronic fungal infections are typically prescribed long-term azole treatment, and *A. fumigatus* evolves in response to become resistant to repeat exposures of the azole drugs over time (Snelders et al., 2011). Fisher stated, however, the polymorphisms that develop in these clinical isolates in response to long-term azole treatment tend to be single nucleotide polymorphisms in the *cyp51A* locus (Gsaller et al., 2016). These differ from a set of mutations that occurs in ARAf in the wild, in which tandem repeats occur in a promoter region that is the domain for the cap-binding complex (CBC) transcription factor binding. These tandem repeats increase the transcription of *cyp51A*. When these tandem repeats occur with polymorphisms that induce conformational changes that confer resistance, the result are the widely distributed alleles—TR34 L98H and TR46 Y121F T289A—found in the environment (Verweij et al., 2009).

Fisher described a study in which he and colleagues performed whole genome sequencing of *A. fumigatus* samples from across the United Kingdom (Rhodes et al., 2022). This population genetic analysis confirmed acquisition of azole resistance in *Aspergillus* environmental and clinical isolates throughout the U.K. The dense clustering on the phylogenetic analysis suggests that azole resistance in the *Aspergillus* population analyzed arose through a relatively recent origin instead of spreading via recombination throughout the entire phylogeny. The genomes featured strong signs of selection on the *cyp51A* locus. Fisher noted that the resistant isolates from the environment were nearly identical to resistant clinical isolates, suggesting that patients are being infected by ARAf from the environment.

The Prevalence of Resistant *Aspergillus fumigatus*

Fisher remarked on the outstanding question of how patients were becoming infected with *A. fumigatus* from the environment. This fungus is ubiquitous in the soil and air. Jennifer Shelton, an applications scientist at Oxford Nanopore Technologies, developed a community science (also known as "citizen science") approach to examine community exposure to ARAf during her doctoral research at Imperial College London (Brackin et al., 2020; Shelton, 2021; Shelton et al., 2020). Generating interest through social media, Shelton enlisted participants from across the United Kingdom. Sterile sticky films and envelopes were mailed to participants who then exposed the adhesive films to the open air for an 8-hour period, then mailed the samples back to the research team. Shelton received 1,894 returned samples from which she cultivated 2,366 *A. fumigatus* samples collected from the open air and screened these samples for resistance to a commonly used azole fungicide, tebuconazole (Brackin et al., 2020; Shelton, 2021; Shelton et al., 2020). She found that, of the samples screened, 1 in 20 were resistant to tebuconazole, 1 in 25 to itraconazole, 1 in 40 to voriconazole, 1 in 25 isavuconazole, and 1 in 150 were resistant to all tested medical azoles (Shelton, 2021). Fisher described that these findings constitute a broad country-wide exposure to multidrug-resistant *A. fumigatus* bioaerosols. Shelton then sequenced the *cyp51A* locus and found that the majority of the azole-resistant varieties contained the TR34/L98H allele, which is found broadly across Europe and the rest of the world, said Fisher (Shelton, 2021). He stated that by his own quick calculations, a person in the United Kingdom is on average exposed to 83,000 *A. fumigatus* within an 8-hour period, and nearly 4,000 of these are azole-resistant. Fisher pointed out that even if his calculations are incorrect by two orders of magnitude, an average person would be exposed to 40 azole-resistant *A. fumigatus* spores across an 8-hour period.

New Antifungal Drug Development

The prevalence of resistant fungi requires new antifungals, and phase II and phase III trials are currently testing promising clinical antifungals with novel modes of action, Fisher noted (Fisher et al., 2022). These include fosmanogepix, an inhibitor of the Gwt1 enzyme (an acyltransferase necessary in glycosyl-phosphatidylinositol biosynthesis); ibrexafungerp, rezafungin, and opelconazole, which are antifungal triterpenoids; and olorofim, a dihydroorotate dehydrogenase (DOHDH) inhibitor. However, agricultural fungicides are also being developed that potentially share the same target site as these clinical antifungals, and thus concerns of developing cross-resistance persist (EPA, 2022; Fungicide Resistance Action Committee, 2022). For example,

the U.S. Environmental Protection Agency has proposed registration for ipflufenoquin, a DHODH inhibitor fungicide (EPA, 2022; Fungicide Resistance Action Committee, 2022). Ipflufenoquin may or may not share the same target as the clinical DOHDH inhibitor, olorofim. Fisher remarked that if the clinical and agricultural antifungals have different targets, they can both be safely used. In cases where antifungals share the same target, this overlap in use cases may impede the ability of the next generation, novel mode-of-action clinical antifungals to effectively treat the broad range of patients in need of new treatment options.

AZOLE-RESISTANT *ASPERGILLUS FUMIGATUS* IN AGRONOMIC SETTINGS: HOTSPOTS AND COLDSPOTS

Doughty discussed the agricultural uses of azoles, the processes and ideal conditions for the development of ARAf, the features of agronomic settings that can contribute to ARAf selection and amplification, in particular the importance of plant waste management in addressing ARAf. Representing CropLife International, the industry association of the major crop protection research and development companies, Doughty described the agronomic context as including broad acre crops, horticultural crops, and plantation crops. Given the link between environmentally derived *A. fumigatus* isolates found in patients and the use of azole fungicides in agricultural and agronomic settings, the development of mitigation strategies depends upon understanding the locations where selection and amplification of resistance are taking place.

Azole-Resistant *Aspergillus fumigatus* in Context

Doughty echoed previous speakers that ARAf presents a significant, widespread hindrance to treatment of invasive aspergillosis with medical azoles. In addition to medical use, azoles (known as demethylation inhibitors or DMI fungicides) are used in the environment in the fields of agriculture/horticulture, material protection, and veterinary medicine. Within agriculture, azoles are the backbone of crop protection strategies that have been used for decades to ensure stable and reliable food security. He described azoles as an essential tool in avoiding resistance to fungicides with other modes of action and the primary tool for fighting mycotoxins that can be produced by *Fusarium* species in wheat and maize. Thus, their use mitigates health issues mycotoxins can cause in humans. He noted that azoles used in agriculture can be—but are not necessarily—active against *A. fumigatus*. While agricultural azole fungicides that are active against *A. fumigatus* can foster cross-resistance with medical azole fungicides, Doughty specified that *A. fumigatus* is not a

target pathogen in the crop protection use of agricultural azoles. However, collateral exposure of the fungus within agronomic settings can occur, particularly in the form of residues that remain after agricultural azoles are applied. He added that the selection and amplification of pre-existing resistance genotypes in these settings is most likely attributable to *A. fumigatus* coming into contact with residues in plant waste piles.

Agronomic "Hotspots" for Azole-Resistant *Aspergillus fumigatus*

The potential medical ramifications of ARAf underscores the value of determining the characteristics of agronomic settings that contribute to ARAf selection and amplification processes, said Doughty. He defined a "hotspot" for ARAf as an agronomic setting in which the following criteria are fulfilled: (1) favorable conditions for growth and multiplication of *A. fumigatus*, (2) exposure of *A. fumigatus* to residual concentrations of demethylation inhibitor (DMI) fungicides that are selective for resistant genotypes, and (3) mass release of airborne spores of *A. fumigatus* into the environment. Selection and amplification cannot take place in the absence of residues of a DMI fungicide that is active against *A. fumigatus*. For *A. fumigatus* to develop resistance, the DMI fungicide must be effective against the fungus at concentrations that add selective pressure to the organism. Furthermore, sufficiently high concentrations of DMI residues (in relation to the minimum inhibitory concentration for wild-type *A. fumigatus*) are required to increase the proportion of the resistant genotypes at the expense of the wildtype. The result is an amplification of the resistant portion of the *A. fumigatus* population. A hotspot then needs a method for mass release of airborne, predominantly spores from ARAf. According to current understanding of the process, this mass release of predominantly resistant spores is a precondition for the link between the hotspot and the patient.

Patterns of ARAf Distribution and Frequency in Agronomic Settings

Doughty and colleagues conducted a literature review to examine which agronomic settings might contribute to ARAf selection and amplification (Doughty et al., 2021). The existence of a background proportion of ARAf within the environmental *A. fumigatus* population (including agronomic settings) has been reported, although the proportion of resistant isolates varied between settings. No distinction was detected between the frequency of ARAf in agronomic settings and in samples from urban settings. Furthermore, researchers found no clear distinction in the recovery of ARAf between azole-treated crops and organic soils and crops. Doughty noted that these comparative studies are particularly useful in understanding hotspots

because much of the research on ARAf has been conducted by sampling soils withoutwithout a comparative element in the survey. Given that *A. fumigatus* has a background level of resistance that is presumably reflected in the air spora, and thus deposition of spores onto agronomic settings, comparative work sheds more light on the role of different agronomic settings.

The growing crop itself is not a particularly conducive habitat for *A. fumigatus*, said Doughty. The fungus has been found and recovered from tomato plants, but it does not thrive on the plant surface and does not attack the plant. However, because *A. fumigatus* is a saprobic fungus, the waste derived from crops that do not necessarily harbor ARAf can become hotspots for ARAf. A growing crop and surrounding soil may harbor limited proportions of *A. fumigatus*, but a large increase in the incidence of resistant isolates can occur in the waste created when the crops are harvested. Doughty stated that waste piles can fulfill all of the requirements of a hotspot and warrant focused attention in efforts to address ARAf.

Flower bulb waste is a hotspot example that has been extensively investigated. Research indicates that stockpiling plant waste can create a hotspot, depending on how the waste is managed (Zhang et al., 2021d). In addition to supporting large populations of *A. fumigatus*, plant waste piles also provide the conditions for sexual reproduction of the fungus, making them an ideal environment for the generation of new genotypes. Doughty noted that wide variety of *cyp51A* mutations of ARAf isolates have been identified in flower bulb waste piles, representing genetic variability (Zhang et al., 2021b).

In contrast to flower waste hotspots, Doughty described cereal crops—one of the main targets of azole fungicide use—as ARAf "coldspots." Large populations of *A. fumigatus* or ARAf are not found in the cereal crop, soil, grain, or straw. The most frequent environmental ARAf genotypes that were detected are TR_{34}/L98H and TR_{46}/Y121F/T289A, which have been found in soils for cereal crops, but in low quantities. The frequencies of ARAf genotypes present in azole-treated cereal crop soils are similar to frequencies found in urban air. Additionally, a U.K. comparative trial examining the relative frequency of ARAf in long-term treated soils and in soils untreated with azoles found similar low proportions of resistant isolates in both treated and untreated soils (Fraaije et al., 2020). A German study compared the relatively low frequency of *Aspergillus*-resistant isolates in cereal fields and apple orchards in both organic and azole-treated fields (Barber et al., 2020). Once again, both settings were found to be a cold spot.

Approaches to Addressing Azole-Resistant *Aspergillus fumigatus*

Doughty stated that waste management is a major area of concern due to the large numbers of *A. fumigatus* spores that can be generated in plant

waste. Although ARAf is the central target for investigation, mitigation, and avoidance measures, reducing the release of *A. fumigatus* could generally be a supplemental aim. In positioning fungicides in agronomic settings, the crop protection industry should consider the risk of selecting resistance in human pathogenic fungi, he suggested. Informed by the expanding knowledge base around azole resistance, the industry could apply lessons learned to avoid potential additional selection and amplification of resistant isolates. Similar measures can be taken with fungicides with new modes of action in agronomic settings. He added that the integrated disease management approach taken in addressing fungicide resistance for target pathogens in the crop itself is unlikely to address hotspots in agricultural waste. For example, the alternation of using fungicides with different modes of action against target pathogens in crops will not necessarily extend to effective management of resistance in waste piles of non-target organisms. Doughty maintained that understanding crop waste management processes and options will be foundational in developing prevention and mitigation strategies and in informing future decisions of how fungicides are positioned in agronomic settings.

DISCUSSION

Overlap in Agriculture and Clinical Use Considerations

Brewer asked whether the use of antifungals in the clinic and environment can be safe under specific conditions. Fisher replied that only a small change in the binding site can confer resistance. Therefore, it is essential to identify where the fungicide is binding to in comparison to the clinical antifungal drug. For example, cross-resistance problems can occur if a DHODH inhibitor in both the clinical and agricultural product is affecting the exact same space on the enzyme. However, any difference in the drug-target binding could potentially avoid issues that can arise with this overlap in use. Binding studies are critical in understanding the drug-target interaction, and experimental evolution can be used to proactively learn about potential cross-resistance, said Fisher. In cases where cross-resistance is not observed, the overlapping use in agricultural and clinical settings can be safe. Doughty noted the dilemma of achieving twin aims of human health protection and food security. New modes of action are pressing needs in both medicine and agriculture to maintain resistance management strategies and to have effective tools to address infection. Doughty stated that if a DHODH inhibitor is introduced into the fungicides market, it will be important to know whether it is active against *A. fumigatus* and whether the specific intended use of the DHODH fungicide will create residues to which *A. fumigatus* will be exposed. Understanding both the resistance

profile of the human pathogenic fungi and exposure scenarios are the starting point for avoiding problematic use cases, he added.

Mutations in *Aspergillus fumigatus*

Given that TR mutations have not been described for plant pathogens occurring in crops regularly treated with azole fungicides, Brewer asked for an explanation about why *A. fumigatus* have the TR_{34} and TR_{46} mutations that lead to pan-azole resistance. Fisher acknowledged that he does not have an explanation, but that it is too soon to say that no tandem repeats are occurring in plant pathogens afflicting crops. Far more genome sequencing has been performed on human fungal pathogens than on agricultural ones, thus tandem repeats may exist in crops but have not yet been discovered. He noted that *A. fumigatus* has a wide array of tandem repeats in environmental settings, indicating a proclivity for this activity, yet this does not seem to occur in *de novo* evolution in the lungs during infection. The reason for this is not yet understood, Fisher added.

Fungicide Risk Assessment for Resistance

Given that *A. fumigatus* is not a plant pathogen and that issues of *A. fumigatus* resistance are a downstream effect of the introduction of fungicide to the environment, Chiller asked how fungicides can be evaluated for risk of resistance and effects that ultimately affect human health. Doughty replied that the regulation of new agricultural tools should be based on understanding of the intrinsic activity of a new molecule and the risk of exposure of human patients. The European Commission has issued a mandate to review this situation for azole fungicides with regard to ARAf. In coming years, discussions about how policy will approach the regulation of pesticides may include consideration of the risk of selection of resistance for human pathogens, said Doughty. Chiller commented on the importance of these discussions in light of the balance needed in safeguarding human health and food supply. He added that experts in human health who are concerned about the potential for new fungicides to cross-react with new antifungals—or even antibacterials—need to take the critical role of fungicides in agriculture into account.

Routes of Transmission of Azole-Resistant *Aspergillus fumigatus*

Brewer asked about the relationship between hotspots and transmission—e.g., whether people working or living near resistance hotspots are at an increased risk of contracting a resistant fungal infection. Doughty replied that he is not aware of direct transmission to humans

working in tulip fields (see Chapter 8 for further detail). Fisher stated that Shelton's community science survey results were used to build numerous statistical models with environmental variables to use in identifying associations (Shelton, 2021). The only variable that demonstrated significance in the risk of potential transmission was industrial composters. Green waste recycling is now taking place on a large scale, with approximately 350 industrial composters in the United Kingdom. However, no association was found in terms of the amount of ARAf. Fisher said this may be due to the low number of azole-resistant isolates, which warrants further research given the association of industrial composters with high burdens of *A. fumigatus*.

Brewer asked about the routes of transmission of ARAf to humans, whether these routes include sporulation occurring on food, and whether azole-resistant isolates are associated with different types of food. Fisher stated that since agricultural waste piles have been identified as hotspots, he is curious about whether small countertop compost bins in household kitchens could also become hotspots. Relatively high fungicide residues could be expected in a bin containing fruit and vegetable peelings. Given that many people spend up to 90 percent of their lives indoors, Fisher and colleagues conducted a small community science indoor surveillance and found more azole resistance indoors than outdoors (Shelton et al., 2022). The numbers were small, but the finding suggests more research on indoor air is warranted. The relative abundance of spores was very similar in indoor and outdoor air, with the former heavily biased to molds and the latter biased to yeasts. Thus, moving from outdoors to indoors involves exposure to both yeast-dominated and mold-dominated aerobiomes. Fisher noted that this surveillance was conducted in the United Kingdom, a damp climate that promotes mold in house. During a recent trip to Singapore, a humid climate, he noticed that indoor air filtration is common. In the United Kingdom, air cleaning filtration systems were uncommon until they grew in popularity in response to the COVID-19 pandemic. Although air filtration systems can remove spores from the air, filters that are not regularly cleaned can act as growth surfaces; the systems then pump new spores into the air.

Doughty stated that *Aspergillus*—including resistant genotypes—is found on food commodities. For instance, resistant *Aspergillus* can be found on fruits in the supermarket. Moreover, *Aspergillus* can be found in coffee samples in spite of the coffee having been roasted at several hundred degrees. This link is possibly disconnected from fungicide use in the field, as food commodities could come into contact with *Aspergillus* during storage, transport, or processing. For example, coffee is particularly susceptible to *Aspergillus* contamination during sea transport. Doughty remarked on the importance of understanding the relation between the use of azole

fungicides in crops and the appearance of resistant isolates on food commodities at point of sale.

Chiller asked whether the development of resistant strains in humans is related to locations where azoles have been applied in the environment and whether foods that have been treated with azole during production carry resistant strains through the supply chain. Fisher responded that research on aerosols indicates that dispersal from hotspots happens rapidly, and sampling in the United Kingdom indicates broad, low levels across the country. Precise epidemiology is required to locate hotspots, although certain crops such as bulbs are well-known for heavy azole use and *Aspergillus* presence between their layers. A huge global industry ships bulbs around the world, with the Netherlands exporting billions of bulbs annually. International trade enables resistant strains to become globalized easily; *Aspergillus* spores then become aerosolized and spread throughout the importing countries. Doughty also described research on strains isolated from individuals and from their immediate surroundings that found no correlation between genotypes from patients and from nearby fields in terms of tandem repeat and single nucleotide polymorphism profile (Rocchi et al., 2014). However, a clear link was found between the genotypes recovered from patients and from their garden compost heaps. This carries the implication that the compost heap is a potential source of infection, whereas the surrounding fields of maize, wheat, and barley are not.

Coldspot Crop Management

Chiller asked how the cereal crop cold spots in the United Kingdom or other testing locations managed crops in terms of crop residue and whether strategies such as no-till were used. Doughty recalled research in which samples from the long-term Rothamsted field trials—which began in the 1800s—were compared with various types of treatment on cereal plots (Fraaije et al., 2020). The comparison examined samples from plots treated with azoles since their market introduction, plots that had not been treated, and grassland. With regard to whether no-till strategy was used with straw crops, Doughty was uncertain.

Factors Promoting Azole Resistance

Brewer asked whether some azoles are more inclined to promote resistance than others, given that agricultural azole use began in the 1970s, but ARAf has increased significantly in the past two decades. Fisher remarked that fungicide azoles vary widely: their chemical structure can be long-tailed or short-tailed; they can have slightly different modes of action; and their resistance profiles vary in similarity to clinical azoles. In addition to these

factors, azoles are used in much higher quantities now than they were in the 1990s and decades prior. Thus, the force of selection is greater and more widely applied. Doughty referenced a study examining a group of azoles most structurally similar to medical triazoles (Snelders et al., 2012). The study found that homogeneity in the mode of action at the active site seemed to indicate that this group was particularly relevant to the selection of resistant isolates in the environment. Individual azoles vary greatly in terms of *Aspergillus* activity, thus the properties of each individual compound should be examined, said Doughty.

REFLECTIONS ON DAY ONE

Paige Waterman, interim chair of medicine and vice chair for clinical research at the F. Edward Herbert School of Medicine at the Uniformed Services University of the Health Sciences, Bethesda, and Jeff LeJeune, food safety officer in the Food Systems and Food Safety Division of the Food and Agriculture Organization of the United Nations, offered reflections on the first day of the workshop. The workshop began with an overview of fungal diseases within humans and the effect of rising environmental temperatures—in conjunction with lower human temperatures—as a natural driver of the increasingly adapted fungal pathogens with the potential to wreak havoc. Efforts of the global Quadripartite Joint Secretariat on Antimicrobial Resistance (AMR) to connect work, and in particular surveillance, in the One Health, AMR, and antifungal resistance sphere were highlighted. Existing mechanisms for fungicide tracking, use, and resistance within the United States were described. Meaningful responses to antifungal use and resistance—whether coordinated or not—are lagging in connection with the human tendency to focus on familiar or recent threats rather than on emerging ones. Speakers offered examples of deadly consequences of fungal infections, and highlighted the extent to which fungi strains resistant to antifungals remain problematic to diagnose and treat.

The second panel discussed the complexities inherent in developing new antifungal drugs, including the "antihuman" properties of antifungals due to the similarities between the fungal and animal kingdoms, said Waterman. Increasingly, fungal pathogens cause systemic infections with high morbidity and mortality that can affect people who are not immunocompromised, fueling the need for new drugs. Antifungal resistance is also rising, driven in some countries by the environment. The reliance on azole fungicide for crops such as corn, soy, and wheat and the spread of resistance are factors to balance. Additionally, challenges in diagnosis factor into stewardship efforts in both the agricultural and medical settings. Speakers discussed the evolving nature of fungi and specific fungal pathogens, including the somewhat unexpected emergence of *Candida auris*.

Azole resistance in general and *A. fumigatus* were the focus of the third panel of the workshop. Community science research and compost studies have demonstrated the ubiquity of aerosolized exposure of *Aspergillus*. Waterman remarked that the large number of settings meeting the requirements of resistance hotspots warrants further study. Fungal pathogens are ubiquitous in nature and commonly develop resistance—driven by both environmental factors as well as the frequent use of azoles—with implications for both food security and human health. The fungal pathogen transfer from plants to humans may not be as uncommon as previously thought, further fueling the need to coordinate and develop surveillance, detection, diagnosis, risk assessment, mitigation, and therapeutics efforts.

LeJeune commented on the disease triad involving effects of pathogens, humans, and the environment on disease manifestation. The first day of the workshop discussed fungal diseases in humans, fungal agents, the ramifications of aspergillosis and candidiasis, and modern molecular epidemiology and medicine used to characterize and treat these infections. Changes in human susceptibility, immunity, and even temperature factor into increasing infection rates. Environmental hotspots may be generating fungal pathogens, and the United States is currently monitoring agricultural use of antifungal agents, accumulation of residues, and crude commodities. Speakers explored the interplay of the environment, climate crisis, and the manifestation of fungal diseases, as well as the underrepresentation of fungal diseases in research and public awareness.

5

Role of Fungicide Use in Food Safety and Security

The fourth session of the workshop explored the role of fungicides in producing an adequate and safe food supply. Philip Taylor, training manager for Plantwise at the Centre for Agriculture and Bioscience International, moderated the session. Tim Brenneman, professor of plant pathology at the University of Georgia, discussed the role of fungicides in the peanut industry, the history of fungicide use and associated development of resistance, and the rise of resistant strains of *Aspergillus*. Pierce Paul, professor, plant disease epidemiologist, and state extension specialist in the Department of Plant Pathology at The Ohio State University, discussed the role of azole fungicides in disease control for field crops and factors that have affected fungicide use patterns in corn, wheat, and soybean production.

ROLE OF AZOLE FUNGICIDES IN SAFEGUARDING FOOD SAFETY AND SECURITY

Brenneman reviewed challenges in producing adequate food supply, the role that fungicides play in meeting some of those challenges, and the different classes of agricultural fungicides. Within the context of the peanut industry, he discussed various products that have been introduced over the past 50 years, their efficacy against peanut leaf spot, and the resistance that has developed in response to most of these chemicals—particularly in *Aspergillus*.

Role of Fungicides in Food Production

Fungicides are necessary tools, among many others, that are needed to feed a growing world, said Brenneman. Food production must increase substantially to meet the needs of an expanding global population, with multiple analyses predicting that the volume of global food production may even need to double by 2050 to meet the burgeoning demand. Unfortunately, maintaining food production levels is becoming increasingly difficult, making the goal of doubling the global food supply all the more daunting, he remarked. Among the host of barriers to increasing food production include soil degradation, urbanization, climate change, water depletion, scarcity of inputs such as fertilizers, and the effects of politics and armed conflicts. In addition to affecting food supply, some of these factors are influencing fungicide use. For example, many crops are grown in Texas and New Mexico, where fungicides are of lesser importance due to the dry climate. However, water supply in those states is decreasing, leading production to shift to the Southeast region of the United States where groundwater and rainfall are more abundant. Fungal diseases thrive under wetter conditions, so as production moves to more humid regions, the need for fungal disease control increases.

Fungicides are essential in keeping crops alive, Brenneman stated. In the absence of fungicide seed treatment, soil-borne diseases can prevent plants from sprouting (see Figure 5-1). Despite utilization of integrated pest management practices such as crop rotation, fungicides remain critical to food production, he remarked. Over the past century, the yield for peanut production per hectare in the United States has increased dramatically

FIGURE 5-1 Comparative effect of fungicide seed treatment versus no treatment on peanut plant sprouting.
NOTES: Sprayed with fungicides 5-8 times annually, even with integrated pest management (e.g., crop rotation).
SOURCE: Brenneman presentation, June 22, 2022.

to become the highest in the world. Although multiple factors have contributed toward this increase, the modern era of fungicide use has played a large role. Azoles have been instrumental in improving fungal disease control. Prior to their development, terraclor was used in the 1980s to address *Athelia rolfsii* (i.e., "white mold"). A granular material, terraclor was applied at 112 kilograms per hectare and resulted in 25–30 percent fungal disease control. In 1994, terraclor was replaced with tebuconazole, a liquid spray applied in quantities of 0.2 kilograms per hectare that achieved 75 percent control or better. In addition to treating *Athelia rolfsii,* tebuconazole controlled leaf spot and foliar diseases. The advent of azoles signified a new era of plant disease management.

Sterol Biosynthesis Inhibitors

Brenneman described sterol biosynthesis inhibitors (SBI) as having a broad spectrum of activity and reducing some toxins such as deoxynivalenol (DON), a toxin that causes enormous losses in small grains. Featuring varying degrees of systemicity and post-infection activity, SBIs have several advantages over previous protectant fungicides. Some SBIs are relatively inexpensive, particularly older products such as tebuconazole that are available in generic versions. However, he noted that the low price of SBIs could potentially contribute to overuse. Labeled for use on a wide variety of crops, SBIs represent approximately one third of the global fungicide market. Thus, this class of fungicides plays a significant role in disease control in the food production system. Four subclasses of SBIs include demethylation inhibitors (DMI), amines or morpholines, hydroxyanilides, and squalene-epoxidase inhibitors. The largest subclass, DMIs include 36 different fungicides, including azoles. Resistance to DMIs is not uncommon, although it seldom causes a complete loss of efficacy, as has happened with other fungicides such as quinone outside inhibitors (QoI) or the benzimidazoles. A gradual loss of efficacy can occur that is associated with the quantitative or stepwise polygenic resistance inherent with DMIs. This class can also experience backshifts due to fitness loss, which can be mediated to some degree by temporarily pulling the fungicide from use. Multiple documented mechanisms of resistance create a scenario that complicates the ability to determine which mechanism is at play. A newer generation of DMIs is often more active than older fungicides due to the quantitative shift. Therefore, despite these newer DMIs being subject to the same resistance mechanisms, they have a higher level of inherent activity. Brenneman described DMIs as a critical fungicide class for many production systems that features long-lived chemistry.

The Rise and Fall of Fungicides for Peanut Leaf Spots

Brenneman provided an overview of the waves of fungicides used to treat peanut leaf spots over the past 50 years. The 1970s saw the introduction of benzimidazoles and chlorothalonil. Benomyl, a single-site mode-of-action benzimidazole, was only effective for approximately 3 years before widespread use and single-site mutations led to virtual immunity in leaf spot. In contrast, chlorothalonil has been in use for 50 years with no cases of resistance. This multisite fungicide has maintained integrity and has helped address resistance in other fungicide classes. In the 1980s, several DMIs became available such as propiconazole, tebuconazole, and fenbuconazole. These single-site fungicides worked for about a decade before resistance began appearing. Brenneman highlighted that the introduction of tebuconazole was a game changer in eliminating leaf spot almost entirely. However, within 10 years, resistance levels were such that some peanut crops treated with tebuconazole were still nearly defoliated from the disease. He noted that tebuconazole continues to be effective against some soil-borne pathogens. In the 1990s, single-site QoIs such as azoxystrobin and kresoxim-methyl became available. Once again, resistance developed within about 10 years, and efficacy of this class was essentially lost for leaf spot. Succinate dehydrogenase inhibitor (SDHI) fungicides were introduced 10–15 years ago, and some signs of resistance are beginning to show within this class as well. Brenneman added that chlorothalonil has been used in combination with all the other classes in an attempt to manage the resistance, yet resistance has developed nonetheless.

Brenneman highlighted data that indicate that adding micronized sulfur to DMI fungicides results in synergistic effects on peach scab and peanut leaf spot (Culbreath et al., 2019; Schnabel and Layne, 2004). In the study, untreated peanut crops experienced nearly 100 percent defoliation. Tebuconazole, once highly effective before resistance diminished its effect, only decreased defoliation to 70–80 percent. Micronized sulfur used independently reduced defoliation to 40–50 percent. When tebuconazole and micronized sulfur were used in conjunction, a synergy took effect and achieved a defoliation rate of less than 10 percent. Thus, adding micronized sulfur to the fungicide helps overcome the effects of resistance. He added that similar synergistic effects have been demonstrated with QoIs and SDHIs.

Aspergillus Resistance in Peanuts

Climate change-related heat and drought have contributed to a rise in *Aspergillus* in peanut crops, as the fungus appears to thrive in these conditions, said Brenneman. *Aspergillus flavus (A. flavus)* and *Aspergillus niger* are the seed-borne species that pose the most substantial problems

in the peanut industry. Both species have developed resistance to QoI fungicides, requiring farmers to change seed treatment regimes. Previously, azoxystrobin was a main component of seed treatment, but in 2020, the peanut industry shifted to an azole-based regime comprised of ipconazole, SDHIs, and prothioconazole to compensate for QoI resistance.

The QoI fungicides inhibit cellular respiration through cytochrome b, Brenneman explained (Fernández-Ortuño et al., 2010). They have a known high-risk single-site mode of action, and multiple point mutations confer different levels of resistance from partial to immunity. He and his colleagues have identified two mutations in the mitochondrial cytochrome b gene that confer *A. flavus* resistance (Ali et al., 2021). A wildtype allele that has not mutated has an average EC50 value (i.e., the concentration of a drug that gives half-maximal response) close to 0, signifying high sensitivity to QoI chemistry. In contrast, alleles associated with the F129L mutation phenotype had EC50 values of 50 and the G143A mutation phenotype had EC50 values well over 100. Brenneman emphasized the importance of phenotyping for DMI resistance due to the large range of phenotypes and the potential combinations of resistance mechanisms in response to DMIs. Resistance factors can vary substantially, thus phenotyping is required to fully capture resistance levels.

Brenneman remarked that while DMI fungicides are losing effectiveness, they continue to make valuable contributions to food production. Resistance is a pressing threat to all fungicide chemistries: that is, each time a class of fungicide is lost to resistance the difficulty in preserving remaining classes increases. Thus, the more modes of action the agriculture industry can utilize, the more effective antifungal efforts are likely to be. The loss of older multisite products to resistance could dramatically accelerate the lack of remaining fungicide tools. Acknowledging the scrutiny placed on chlorothalonil, he stated that losing this product and using single-site modes-of-action fungicides alone would increase challenges in addressing fungicide resistance. Therefore, new approaches to plant disease management are needed, with molecular approaches like RNA interference holding promise in reducing selection pressure on fungicides and prolonging the life of existing fungicides.

AZOLE USE FOR FOOD SAFETY AND SECURITY

Paul provided a field crops perspective on the use and importance of azole fungicides for disease control and, consequently, for food safety and security. He discussed the factors that created significant shifts in fungicide use patterns in field crops over the past 20 years and described the role of azoles in addressing Fusarium head blight. The varied fungicide formulations are classified into three chemical groups according to mode

of action: QoI, DMI, and SDHI.[1] DMI fungicides (i.e., azoles) and QoIs are the predominant products in current use. Most field crop fungicides are used for above-ground diseases—specifically foliar diseases—although some products also offer seed and seedling disease control capability. Paul noted that fungicides are particularly important in controlling rust diseases in wheat, corn, and soybeans. These products offer effective control of several diseases that affect both yield and quality, making them an integral part of disease management programs for field crops.

Recent Shifts in Field Crop Fungicide Use Patterns

A major shift in fungicide application and use patterns in field crops occurred in the mid-2000s. At that time, fungicide use became widespread in response to multiple factors, said Paul. Claims that plant health benefited from use of fungicide—even when disease was absent or at low levels—contributed to a change in product labels. When limited control environment studies indicated that QoIs affect crop physiology such that yields could increase even in the absence of disease, some labels began listing a plant health benefit. This led some people to believe that spraying fungicide on healthy plants would increase yield, thus fueling the widespread use of fungicides.

Additionally, grain prices increased and modern hybrids of corn saw higher yield potential during this period. Historically, fungicide use in field crops was cost prohibitive because prices were not high enough to offset application costs. With the increase in both prices and yield potential, expenditures on fungicides became more appealing. This particularly contributed to fungicide use pattern changes within the corn belt. Furthermore, major disease outbreaks and threats occurred during this time, such as soybean rust. Several major azole fungicides were limited to certain crops in their labeling, but the soybean rust outbreak led to section 18 exemptions under the *Federal Insecticide, Fungicide and Rodenticide Act*,[2] which authorizes the U.S. Environmental Protection Agency to allow unregistered uses of pesticides to address emergency conditions. Several azoles attained full labels and registration via this avenue. Paul noted that azoles also gained labeling for other field crops in response to farmers stockpiling fungicides for soybean rust and then needing ways to use their surplus. Thus, the soybean rust outbreak led to increased azole use in field crops other than soybeans.

[1] More information about fungicides and efficacy levels is available at https://cropprotectionnetwork.org/publications?collections=%5B%22Publications%22%5D (accessed August 7, 2022).

[2] *Federal Insecticide, Fungicide, and Rodenticide Act of 1947*, Public Law 80-104, 80th Cong., 1st sess. (June 25, 1947).

The mid-2000s also saw a boom in the ethanol industry with the establishment of numerous ethanol plants. The demand generated by ethanol plants led farmers to plant more acres of corn. Paul described that the production of continuous corn also increased in an effort to meet demand; this shift from crop rotation caused more disease. This time period coincided with another change in production practices that also contributed to fungicide use: the practice of conservation tillage to help reduce erosion. These various factors combined to cause a sizeable increase in fungicide use in field crops in the mid-2000s. Paul added that in the years since, fungicide use has dropped considerably overall, but remain widely used in field crops.

Despite the considerable use of fungicides in field crops, the production of corn, wheat, and soybeans does not rely as heavily on these chemicals as do other agricultural production systems, said Paul. Typically, a single application is sufficient for effective disease control for field crops. He noted that recommendations for a second application have been made for some cases of southern rust and tar spot diseases. However, in most cases—provided the application is timed correctly—a second application is not required. Moreover, the value of the crop often does not justify a second application, as the benefit is insufficient to offset the application cost. Label restrictions also contribute to lower use of fungicides in field crops than in other agricultural industries. The majority of field crop diseases develop in the early reproductive stages but applying a second application often infringes on legal pre-harvest intervals. Paul stated that these factors combine to prevent excessive use of fungicides in field crops in comparison to other production systems.

Role of Azoles in Field Crop Production

Paul explained that azoles are the main ingredient in most types of field crop fungicides. Of the 24 fungicides used on corn, 19 contain an azole active ingredient. In soybeans, 26 of the 34 fungicides are azole-based, and the proportion is even higher for wheat, with 15 of 18 fungicides containing azoles.[3] Across these three crops, seven core primary azoles are featured in the various azole-based fungicides: flutriafol, propiconazole, prothioconazole, tebuconazole, tetraconazole, cyproconazole, and metconazole. Paul clarified that the 26 azole-based fungicides used on soybeans comprise 26 different combinations of 7 azoles, *not* 26 different azole active ingredients. Among the most effective fungicides against economically important diseases, azoles are effective against leaf spots, blotches, and rusts, the latter being some of

[3] More information about azole-based fungicides used in field crops is available at https://cropprotectionnetwork.org/publications?collections=%5B%22Publications%22%5D (accessed August 7, 2022).

the most damaging diseases to field crops. Azoles are used in rotation or in combination with QoIs and SDHIs as a fungicide resistance management strategy and to increase the spectrum of activity. Additionally, azoles are the only control option for some diseases such as FHB (Fusarium head blight) and *Gibberella* ear rot and the mycotoxins associated with these diseases.

Fusarium Head Blight

Caused by *Fusarium graminearum*, FHB causes bleached, discolored spikes in wheat that lead to mycotoxin grain contamination. Paul stated that this fungal disease poses a major food safety concern due to features of the mycotoxins. FHB is associated with several toxins, including DON. Both water soluble and heat stable, DON spreads easily and persists after being cooked or baked. Moreover, DON can conjugate with other compounds, enabling it to be masked and hide undetected until it enters an animal system, at which point it can be unconjugated to release the active DON toxin again. Azoles are one of the most effective fungicides for reducing both FHB and associated mycotoxins.

Integrated management guidelines for FHB include using the most resistant variety of grain, crop rotation, tillage, and fungicide. Using genetically resistant grain varieties is not effective independent of other measures, because no variety is immune and some of the most resistant varieties offer lower yields. Tillage and crop rotation are inadequate in and of themselves due to the ability of spores to travel easily. Paul stated that fungicide is a necessary component of an integrated management program that effectively reduces FHB and the mycotoxin contamination of grain. Several azoles are considered industry standards for FHB, including triazole, tebuconazole, prothioconazole, and metconazole. Proline and Caramba are products that use individual azoles, and Prosaro and Sphaerex utilize azole combinations. Increasing fungicide resistance has led to combination products comprised of azoles and SDHIs. Efficacy data indicate that azole-based fungicides generate more than a 50 percent reduction in both disease and mycotoxin contamination of grain (Edwards and Godley, 2010). Newer combination of fungicides utilizing azoles and SBHIs achieve similar results, although it is notable that the new active ingredients have not been found to be more effective than existing active ingredients.

Azole-based fungicides remain the most effective tool in reducing FHB and DON in wheat, said Paul. In fact, QoIs used in isolation on field crops have been found to increase DON levels in grain rather than reduce them. Five different QoI products were found to increase DON levels from 6-18 percent above untreated wheat. In contrast, azoles maintain grain yield and quality, reduce food safety concerns, and benefit numerous industries including livestock, milling, baking, brewing, and ethanol.

DISCUSSION

Quinone Outside Inhibitors and Mycotoxin Levels

Given that a fungicide with even limited activity would be expected to reduce mycotoxin production of the fungus, Taylor asked Paul to expound on how QoIs were found to increase DON production. Paul affirmed that plants treated with QoIs did have higher average levels of DON than plants that are untreated or that are treated with azoles. In some cases, the QoIs reduce visual symptoms and disease levels of FHB, yet the mycotoxin contamination levels in grain simultaneously increase. A hypothesis for this phenomenon is that QoIs selectively control non-mycotoxin-producing fungi, thus leaving the mycotoxin-producing fungi to thrive in the wheat spikes and produce higher levels of mycotoxin. Additionally, QoI fungicides keep plants greener for longer, which increases the likelihood of higher levels of mycotoxin accumulating over time.

Fungicide-Related Fitness Cost

Taylor asked whether a pathogen experiences any fitness costs when it becomes resistant to fungicides. For instance, does the pathogen become less virulent or sporulate less? Paul replied that he was unaware of any reported fitness cost associated with resistance. Brenneman stated that fitness cost varies with fungicide class: fungi resistant to benlate experienced little if any fitness cost; resistance to triazoles is known to have some fitness cost; and fungi resistant to triphenyltin has shown clear fitness cost, with high levels of resistance observed one year and resistance levels subsiding considerably the following year. This exemplifies how pulling a fungicide out of the system can reset the timetable on efficacy loss.

Micronized Sulfur

Replying to a query about whether sulfur must be micronized in order to cause the synergistic effect of reducing resistance, Brenneman remarked that research conducted by Albert Culbreath, his colleague at the University of Georgia, indicated that traditional, larger-particle size sulfurs do not achieve the same effect (Culbreath et al., 2019). He added that micronized sulfur's ability to boost fungicide efficacy is significant in helping to maintain efficacy of some at-risk chemistries; this effect was seen in QoIs, SDHIs, and the DMIs.

Resistance to Recent Azoles

Taylor asked whether any resistance has been identified in the newer azoles—namely, prothioconazole and mefentrifluconazole—and whether they are expected to remain effective for a longer period given that resistance is already in the system. Paul replied that some gradual reduction in sensitivity has been found for FHB in the wheat system, but he would not necessarily characterize this as resistance. However, he noted that evidence of resistance in FHB as well as other diseases has been found in Europe, where these products are used more frequently than in the United States. A gradual eroding of sensitivity to these fungicides is evident, especially in comparison to tebuconazole and metconazole, which have older active ingredients than prothioconazole.

Considerations in Fungicide Blends and Concentration Rates

In terms of utilizing multiple fungicides, Taylor asked whether mixing fungicide blends or rotating the fungicide used between seasons or applications is more likely to prevent resistance. Brenneman remarked that this topic is debated among plant pathologists. He stated his view that the best method varies with the system. In small grains that receive only one or two applications, blended combinations play a substantial role in prevention. In peanuts or pecans, a season-long strategy that utilizes a single available active ingredient at a time provides flexibility; if one of the products meets resistance, other options remain available, whereas a blend might involve all available options in the first application. Brenneman added that the process of mixing fungicides can be complicated. If all the fungicides are used at full rate, this approach becomes expensive. Using high rates can also induce a gradual shift in sensitivity, and selection for higher and higher rates may take place. On the other hand, if rates are reduced to sublethal levels and the product becomes ineffective, that too causes problems. The ideal mixture cuts the rate while maintaining efficacy via the combined modes of action.[4]

Paul noted the role of pre-mixes, in which chemical companies create the mixtures. These pre-mixes use lower rates of the two active ingredients in comparison to the rate of each ingredient in a single-active-ingredient product. Assuming the companies have carried out adequate research in establishing rates for these mixtures, the products should still be effective at these lower rates. He added that most of the blends used are pre-mixed, but on infrequent occasions, products have been mixed in the field.

[4] This topic was further discussed at the end of the day; see the discussion section at the end of Chapter 6.

Noting that limited literacy and numeracy skills among some agricultural workers can lead to inadvertent divergence from fungicide label recommendations, LeJeune asked about the relationship between the misuse of fungicide concentration levels and excess application. Paul replied that the general understanding of sublethal doses is that they increase fungicide resistance by exposing organisms without killing them. The surviving subpopulations then develop resistance, which is passed on and increases with each subsequent generation. When fungicides are developed, various concentration rates are tested, informing recommendations about the rate that is lethal to the fungus or spores. Paul stated that he does not know what effect higher-than-recommended concentrations have on resistance. Models have indicated that the general understanding of exposure to sublethal doses does not hold with every pathogen or system. For example, research reveals that in some situations higher dose rates of fungicide can be more problematic than half rates (van den Bosch et al., 2018).

LeJeune asked whether the use of fungicides at lower-than-recommended concentrations, due to cost considerations, is known to affect resistance. Paul remarked that he does not have specific data on this dynamic, but use patterns suggest waves of outbreaks associated with people veering from recommended doses as a cost-saving measure and then returning to label dosing guidelines when disease occurs. For example, in the mid-2000s, fungicide was commonly used at the half dose rate during earlier growth stages, based on the assumption that a half dose would provide a smaller plant with adequate coverage. Once plants grew in size, the full dose rate was used. After several years of this practice, it fell out of favor not because of resistance, but because it was found to be ineffective at increasing disease control and yield. Paul added that research of such practices in real-world situations is insufficient to determine exact repercussions of dosing deviations, but simulation models demonstrate which effects are likely or unlikely.

6

Dimensions of Fungicide Regulations, Use Management, and Risk Assessment

The fifth session of the workshop explored efforts to assess the extent and causes of antifungal resistance and the role of regulatory bodies in addressing it. Lynn Goldman, Dean and Professor of Environmental and Occupational Health at the Milken Institute School of Public Health at George Washington University, moderated the session. Philip Taylor, training manager at the Centre for Agriculture and Bioscience International (CABI), reviewed data collected from plant care consultation clinics located in low- and middle-income countries (LMIC) around the world and described fungicide usage patterns in various global regions. Nathan Mellor, product manager in the fungicide branch of the Environmental Protection Agency (EPA) Office of Pesticide Programs, Registration Division, outlined the EPA's process and requirements for registering new or updated fungicides. Magdalini Sachana, policy analyst at the Environment Health and Safety Division of the Organisation for Economic Co-operation and Development (OECD), provided an overview of OECD's testing guidelines, tools, and data sharing efforts relevant to fungicide registration. Raquel Sabino, mycologist at the Portuguese National Institute of Health Dr. Ricardo Jorge, Lisbon School of Medicine, discussed occupational exposure to *Aspergillus*.

Goldman recounted that health risks related to fungal infections have increased in response to a larger proportion of the population being treated with immunosuppressant therapies, coupled with the emergence of new pathogens such as *Candida auris*. Utilization of antifungal agricultural products has increased, possibly in response to effects of climate change and a shift in demand for specific crops. Resistance is emerging in both medical

and agricultural antifungals, yet the development of new drugs is slow. Goldman stated that these trends pose challenges to regulatory systems on the national and global levels. Striking a balance between addressing public health issues and meeting food supply needs requires dual focus, however. To that end, regulators have tools that can spur data reporting, insights into antifungal use volume and patterns, data production, and additional research.

USE OF FUNGICIDES IN LOW- AND MIDDLE-INCOME COUNTRIES

Taylor provided an overview of Plantwise, a CABI program designed to improve food and financial security through consultation services to small farms. These services are offered at plant clinics, and data from these clinic visits are collected to gain a better understanding of farming practices and challenges in LMICs. He reviewed patterns in fungicide recommendations across four different global regions.

Plantwise Plant Clinics

As a non-profit, science-based development and information organization, CABI specializes in agricultural development, biosecurity, biocontrol, and publishing. Nearly 50 countries are members of this worldwide organization, which has 26 regional offices in six continents. Membership at a modest fee provides consultation services for biosecurity and phytosanitary issues. Plantwise is a CABI global program intended to increase food security by reducing crop losses, thereby improving rural livelihoods. Plantwise offers plant clinics pioneered by CABI to bolster extension services in nations around the globe. Although information is available about practices that improve food security, not all farmers have access to it, noted Taylor. Plantwise clinics address this gap by offering advice tailored to local circumstances. These clinics are temporarily set up in public places, such as markets, village squares, and human health centers. Farmers can discuss a diseased crop or other plant issue, show a sample to an extension worker—referred to as a "plant doctor"—and receive a diagnosis and recommendation for treatment. Plant doctors also give advice on how to prevent the problem from reoccurring in the future. Taylor emphasized that Plantwise clinics are very basic. They do not have scientific equipment such as microscopes, culture media, or autoclaves; plant doctors are only equipped with magnifying glasses, literature, and experience and expertise. Taylor acknowledged that the program's tagline—"any crop and any problem"—is ambitious, but even if a visit to the plant

clinic does not result in a solution, farmers will at least come away with a better idea of what is causing the problem.

Plantwise clinics have reported benefits, with 79 percent of farmers responding that yield increased after visiting a plant clinic and 70 percent indicating increased income. Taylor remarked that Plantwise has a pragmatic view toward chemical application: if chemicals are needed, farmers should use them. However, Plantwise does not recommend indiscriminate or inappropriate use of chemicals, or the use of more toxic products. Over half of the plant clinic prescriptions recommend non-chemical inputs. He added that 25 percent of all Plantwise plant doctors are female. The program relies on 70 private sector organizations and on government partners for funding contributions and donations of staff time toward Plantwise activities.

Plantwise Data Collection

Plant doctors collect data from these interactions, which are recorded on a form received by both the farmer and CABI. The form includes a diagnosis—which may be accurate and precise or may be imprecise—and treatment instructions to address the issue. Taylor noted that Plantwise is gradually moving away from paper forms to electronic forms completed on tablets, which are then sent to the farmer's phone by an SMS message. If a plant doctor is unsure of a remedy, and they have access to the internet, they can consult the online Plantwise Knowledge Bank.[1] This open access data source is available to everyone. Plantwise also has an online management system with restricted access that houses all the data collected at clinic visits. These data can have a political dimension because governments may not always want to disclose that certain pests or diseases are present in their countries. Thus, CABI protects these data and assures governments that data are held securely and will not be shared without permission. He emphasized that these data are utilized without sharing identifying information.

Plantwise Fungicide Use Study

Plantwise conducted data analysis on usage of commercially available fungicides. Taylor noted that botanicals, household products, or other non-commercial agricultural fungicides were not included in the study. Additionally, the study did not distinguish between blends or alternatives (i.e., the data collection tool included blends and alternatives within the

[1] The Plantwise Knowledge Bank is available at https://www.plantwise.org/KnowledgeBank (accessed August 9, 2022).

same response option). Language issues posed a challenge to data collection, as many of the plant doctors do not speak English as a first language. Some forms completed in English were unclear, while other forms completed in the plant doctors' first languages generated translation issues. Thus, the meaning of individual responses was not always straightforward. Additionally, spelling inconsistencies related to tradenames and chemical names prevented automated data analysis. For example, over 300 spelling versions of mancozeb and mancozeb products were included in responses. Taylor cautioned that Plantwise data may not accurately reflect agricultural problems in a country, nor the treatments actually applied. The data are generated from plant doctor recommendations, not from verified farmer practices. He added that farmers generally do follow plant doctor advice, but that is not guaranteed.

To anonymize data, responses were grouped by global region rather than by country (see Figure 6-1). The regions included Latin America and the Caribbean (LAC), South Asia (SA), Southeast Asia (SEA), and Sub-Saharan Africa (SSA). Record analysis examined fungicide recommendations and percentage of microbial pathogen diagnoses. Taylor specified that the working definition of microbial pathogen that was used included fungi, oomycetes (i.e., water molds), and bacteria; viruses and nematodes were not included. The analysis found that plant doctors in LAC and SA had an approximate ratio of microbial diseases to fungicide recommendations of 1:1 and 1:1.2,

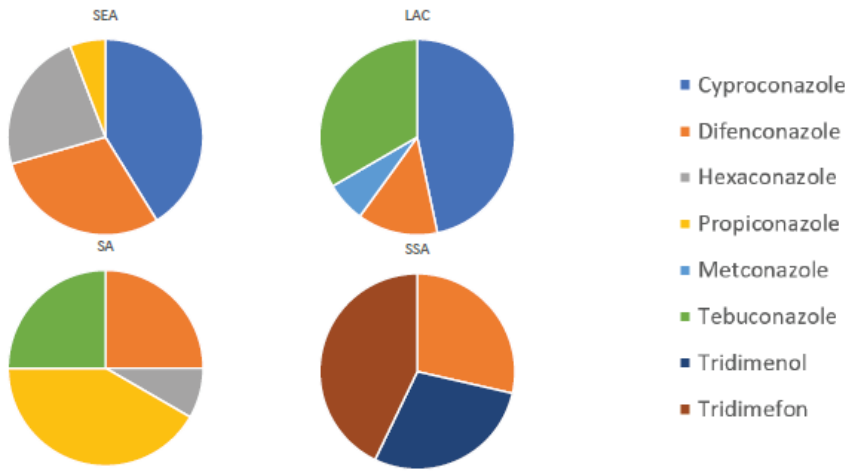

FIGURE 6-1 Regional breakdown of azole fungicide use based on Plantwise data analysis.
NOTES: LAC = Latin America and Caribbean; SA = South Asia; SEA = Southeast Asia; SSA = Sub-Saharan Africa.
SOURCE: Taylor presentation, June 22, 2022.

respectively. In SEA and SSA, the ratio was approximately 1:0.5, indicating that plant doctors in these regions were less likely to recommend fungicides.

Taylor explained that in cases where fungicides were recommended, the diagnoses spurring the recommendations were analyzed. Not surprisingly, "fungus" was the most cited response across regions, accounting for 45–62 percent of recommendations. Oomycetes was common, associated with 7–20 percent of recommendations, depending on region. Bacteria and arthropods were the only other categories that comprised more than 4 percent of responses in all four regions. Less precise categories such as "symptom," "deficiency," "environmental," "disease," and "unknown" featured less prominently across regions but had substantial responses in one or more regions. He highlighted a finding in SA that a sizeable proportion of fungicide recommendations were made for diseases that are not caused by microbial pathogens. Taylor remarked that this indicates prophylactic spraying. For instance, a farmer might present an insect problem to the clinic and be advised to use a mix containing fungicide.

The analysis also categorized the fungicides used according to whether the active ingredient is found in the Fungicide Resistance Action Committee (FRAC) 3 group of fungicides, which is comprised of demethylation inhibitors (DMI). Taylor emphasized that while the FRAC 3 group is predominantly composed of azoles, not every azole belongs to this group. He noted an unexpected data finding that veterinary products albendazole and clotrimazole are being used in rice fields in SEA against rice blast. Across all regions, plant clinics recommended fungicides with 87 active ingredients from 30 FRAC groups. Of the 37 fungicide active ingredients with the FRAC 3 code, 17 were recommended by Plantwise clinics. Taylor specified that in order to simplify data, a "1 percent rule" was applied, in which an active ingredient was excluded from a region's analysis if it did not feature in more than 1 percent of that particular region's responses recommending fungicide. Application of this rule simplified analysis; for example, SSA had 66 active ingredients represented in regional data, but only 15 were featured in more than 1 percent of responses. Across all regions, 34 active ingredients met the 1 percent rule criteria. In comparing the regional fungicide usage rates by FRAC group, variations in regional tendencies became apparent. For instance, 17 percent of all fungicides recommended in SEA were FRAC 3 azoles, compared to 12 percent in SA, 10 percent in LAC, and 7 percent in SSA.

Taylor highlighted that chlorothalonil is an important component of disease control strategies in many countries, yet it was seldom used LMICs. Five FRAC codes—1, 3, 4, MO1, and MO3—make up more than 84 percent of fungicide records in SA, SEA, and SSA; these codes comprise 61 percent of records in LAC. He noted substantial regional differences in the specific azoles being used in each of the four regions, with difenoconazole being the only

azole used in all four regions. SSA appears to be using older azoles, whereas the other three regions are using more modern ones. The study revealed differences in treating ascomycetes and basidiomycetes—the two fungi phyla to which most plant pathogens belong—and FRAC 3 triazoles were recommended more frequently against ascomycetes than for basidiomycetes across all regions. Additionally, a combination of FRAC 3 and FRAC 11 chemicals was very popular in LAC against both phyla, representing a significant aspect of the disease prevention strategy in that region, but it was not common in the other three regions.

Analysis of the number of active ingredients per recommendation revealed that over 50 percent of recommendations in all regions contained a single active ingredient. Two active ingredients per recommendation were indicated in 25-40 percent of responses for each of the four regions. However, because blends were not differentiated from alternatives, the recommendations indicating two active ingredients may actually signify alternatives rather than blends, Taylor explained. Therefore, blends are not recommended often. When blends were advised, the most popular FRAC 3 blends in LAC were FRAC 3 triazoles and FRAC 11 strobilurins, and in SEA, FRAC 3 triazoles and MO3 mancozeb were most common. In SA and SSA, FRAC 3 blends represent a minor portion of recommended blends. Taylor added that within commercial FRAC 3 blends available to farmers, the other active ingredients are most often FRAC 11 strobilurins. Less frequently, FRAC 5 or FRAC 8 ingredients are used and, in some cases, azoles are mixed with insecticides or even with other azoles.

U.S. FUNGICIDE REGULATION AND REGISTRATION

Mellor outlined the process by which the EPA Office of Pesticide Programs registers fungicides via its fungicide branch. He reviewed conventional pesticides, labeling considerations and procedures under the *Federal Insecticide, Fungicide, and Rodenticide Act* (FIFRA),[2] and future concerns and initiatives.

Labeling Data Requirements

Conventional pesticides are typically synthetic chemicals that are produced to prevent, mitigate, destroy, or repel any pest or to act as a plant growth regulator, desiccant, or defoliant. These differ from biological pesticides and antimicrobial pesticides, Mellor noted. Conventional pesticides are designed for various use sites including agricultural, turf, and

[2] *Federal Insecticide, Fungicide, and Rodenticide Act of 1947*, Public Law 80-104, 80th Cong., 1st sess. (June 25, 1947).

ornamental. Use patterns extend beyond agricultural products to include aquatic, greenhouse, forestry, residential, and indoor spaces. The products come in a range of formulations such as liquids, flowable, granules, pellets, dust, and wettable powders; they can be applied via ground, aerial, chemigation, and fogger methods.

The process for registering a pesticide begins with the registrant, who initiates the process by drafting a label and submitting it to the EPA for approval, said Mellor. EPA risk managers review the labels, request revisions as necessary, and review any new chemicals involved. Once the risk manager is able to ensure compliance with EPA guidelines and regulations, they grant approval for the product to become usable on the ground. The FIFRA pesticide data requirements for chemical active ingredients include product chemistry, product performance, chemical-specific and product-specific toxicology, ecological effects, exposure and exposure use studies, pesticide spray drift exposure, environmental fate, and residue chemistry. Thus, risk managers have substantial amounts of data to examine during the review process, some of which is generic to the active chemical ingredient and some of which is product-specific.

Product Reviews and Risk Assessments

The product-specific review includes an assessment of acute toxicity, which involves approximately six studies' worth of data, Mellor explained. Risk managers examine first aid and precautionary statements and listed personal protective equipment (PPE) required to safely handle the product. Conducting a parallel review to risk managers, the product chemistry group reviews all components of the product, generates statements for ingredients and for physical-chemical hazards, and determines whether any present impurities are of concern. Any potential problematic issues are then examined by the toxicology group. Assessments are conducted for human health risks, environmental risks, and benefits. The human health risks assessment includes residue chemistry, dietary exposure, and occupational and residential exposure. Mellor specified that residential exposure does pertain only to chemicals used in the home—i.e., for general lawn care or flower gardens—but it also includes their use in playgrounds, athletic fields, and other areas where people may be exposed during leisure activities. Environmental risk is assessed for effects on drinking water and ecological fate. This assessment considers factors such as effects on non-target organisms, chemical travel patterns, whether chemicals will contaminate drinking water, whether chemicals will persist in the ground or will travel, and what happens to the chemical in the long term. A benefits analysis assesses crop and disease levels and beneficial effects of the chemical to determine whether any risks to human health and the environment are outweighed by any benefits.

Product Directions and Restrictions

Once all risk assessments are complete, risk managers review the product label to determine compliance, said Mellor. The directions of use must include the product's agricultural uses, spray drift, mixing instructions, tank mix prohibitions, resistance management, precautions, and restrictions. An established minimum set of restrictions requires a product to list (1) the single maximum application of active ingredient per acre, (2) the annual maximum application rate per acre, (3) the maximum number of applications per year, and (4) the minimum re-treatment interval. Mellor noted that these restrictions constitute an absolute minimum and additional restrictions may be appropriate and applicable for a chemical. For instance, chemicals labeled for multiple crops may have reentry levels, plant back intervals, and preharvest intervals listed in addition to the aforementioned restrictions. Directions for use must also include storage and disposal instructions for end users. These vary depending on the product and on the container in which the chemical is sold or housed. Risk managers also review the warranty statement to ensure it is not false or misleading. For example, manufacturing products—which are designed to be used in creating other products—are typically highly concentrated and are not to be used in the field. Therefore, the warranty for a manufacturing product should not refer to field use, Mellor explained.

Conclusions, Mitigations, and Implementation

All aspects of labeling must adhere to FIFRA and to the *Food Quality Protection Act*,[3] said Mellor. Risk managers ensure that all data requirements are fulfilled, leaving no gaps in the data, and that studies have been performed correctly. If a data gap is identified, the review process halts until receipt of the necessary data. Often, an original submission indicates that more data are needed, and conditionally-required data from secondary studies may come into play in these cases. In an effort to register only products that pose no adverse effects on human health or the environment, label mitigations are applied as appropriate. Chemical-specific or generic data may indicate no adverse effects, but mitigations such as buffers, aquatic restrictions and PPE can minimize potential adverse effects of spray drift, run off, and worker exposure. Other mitigations can also be applied during the review process, including reduced application rate and maximum number of applications per year.

[3] *Food Quality Protection Act of 1996*, Public Law 104-170, 104th Cong., 2d sess. (August 3, 1996).

Any labeling mitigations obtained from EPA risk assessments must be integrated into the label prior to approval. Mellor explained that chemicals are re-reviewed every 15 years to ensure that data meet the standards of the day. Risk managers must ensure that any new learnings are implemented for products in this process, including adding mitigations as appropriate. Furthermore, the label review manual is updated periodically to incorporate generalized learnings, such as new types of PPE that are helpful in preventing adverse effects. Any updates in the manual must be reflected in the product label. Risk managers are also responsible for ensuring that all items on a label check list are complete. Mellor remarked that "the label is the law": once a label is reviewed by the product manager or branch chief and is stamped as approved, it becomes legally enforceable.

Several working groups within EPA's pesticide office work together to ensure that the labeling is clear and concise for users in the commercial and consumer markets, said Mellor. These include the Product Manager Workgroup, the Label Consistency Committee, and the State Label Issues Tracking System. Registrants and users can also contact the risk manager or program manager regarding any concerns about a label's content. He noted that such direct inquiries are fairly common.

Future Considerations

Mellor stated that the most significant challenge currently facing the EPA label registration process is resource limitations. The recently released EPA workplan, *Balancing Wildlife Protection and Responsible Pesticide Use*,[4] to address the challenge of protecting endangered species from pesticides has generated numerous requests for actions to be added to the workplan that are not currently included. However, the limited workforce will be fully directed at implementing measures already in the 7-year workplan and EPA cannot guarantee additional work can be completed unless mandated, said Mellor. Multiple resistance management efforts are underway for plant pathogens, particularly for fungicides that have a high risk of developing resistance. The agency is discussing a stewardship management plan and a potential resistance assessment, which would focus on antibiotics and involve a modified version of the U.S. Food and Drug Administration's (FDA) Guidance for Industry #152 on evaluating antimicrobial animal drugs.[5] Furthermore, EPA is considering ways to strengthen risk assessments. For instance, the agency recently updated the

[4] The workplan is available from https://www.epa.gov/system/files/documents/2022-04/balancing-wildlife-protection-and-responsible-pesticide-use_final.pdf (accessed August 25, 2022).

[5] This guidance is available at https://www.fda.gov/media/83488/download (accessed August 10, 2022).

exposure model for seed treatment occupational exposure. Mellor noted that periodic updates are part of overall efforts toward making chemical use as safe as possible.

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT PESTICIDES PROGRAMME

In a prerecorded presentation, Sachana provided an overview of OECD and the organization's Pesticides Programme, including its testing guidelines, tools, and data sharing efforts relevant to fungicide registration that do not differ from the ones applied for all conventional pesticides used as plant protection products.

Organization for Economic Co-operation and Development

"OECD is a forum in which governments work together to coordinate and harmonize policies," said Sachana. Government members use this forum to discuss and share their experience on issues of mutual concern and work together to respond to international issues by adopting consensus-based decisions. For example, members could determine that resistance to fungicides within agricultural systems is a mutual problem of concern, then they could convene to discuss the issue and develop consensus-driven solutions. OECD also provides comparative statistic, economic, scientific, and social data in more than 250 publications per year, which are housed in the OECD iLibrary.[6] Additionally, the organization develops tools and maintains a database. OECD currently has 38 member countries covering the whole of North America, most of the European Union, Japan and Korea in eastern Asia, Australia and New Zealand, and Chile, Colombia, and Costa Rica in South America. Sachana noted that in addition to member countries, OECD works with Brazil, China, India, Indonesia, and South Africa as key partners.

With a focus on issues relevant to the economy and development, OECD has long been active in agriculture sustainability, Sachana stated. In 1992, the OECD Pesticides Programme was established to streamline pesticide approval processes. One of the program's objectives is to develop practical and harmonized tools that countries can use to implement legislation regarding safe pesticide use. These tools include test guidelines, standardized formats for data submission, and risk assessment methodologies. The Pesticides Programme focuses on sustainable approaches toward plant protection, such as new technologies for pesticide design and application that reduce risks to humans and wildlife. She added that many OECD countries are shifting away from using conventional pesticides.

[6] The OECD iLibrary is available at www.oecd-ilibrary.org (accessed on August 10, 2022).

Pesticides Programme Data Generation and Management Tools

Testing Guidelines, Principles, and Practices

Generating the data required to register a product is a major challenge in pesticide safety, said Sachana. OECD develops standardized and internationally agreed *in vitro* and *in vivo* methods to test the potential adverse effects of chemicals. The OECD Guidelines for the Testing of Chemicals are organized into five sections: physical-chemical properties, effects on biotic systems, environmental fate and behavior, effects on human health, and pesticide residue chemistry. For example, the human health effects series includes standardized methods for assessing carcinogenicity, developmental neurotoxicity, endocrine disruption, and other effects. These standardized methods enable the various data requirements embedded in national regulations to be addressed. Furthermore, these test guidelines are regularly updated to keep pace with progress in science, animal welfare, and cost effectiveness. The guidelines adhere to the OECD Principles of Good Laboratory Practice (GLP), a quality control system for the process and conditions of health and environmental study planning, performance, monitoring, recording, and reporting.

The OECD test guidelines and GLP form the requirements of the Mutual Acceptance of Data, a legal agreement among all OECD member and adherent countries "that share a common data requirement to accept data generated by one another." Thus, toxicity data generated under OECD test guidelines and GLP contribute to data sharing efforts. Maintaining the Mutual Acceptance of Data system offers several benefits, Sachana noted. It keeps safety testing and assessment costs manageable for countries and industries by avoiding or reducing duplicative testing. It also prevents unnecessary animal testing. The system maintains a level playing field across countries, allowing them to claim the same standards and exchange data.

Data Sharing

This reciprocal arrangement requires facilitation and management of data sharing once data are collected, said Sachana. To this end, the Pesticides Programme developed common data reporting formats that can be used across numerous countries.[7] OECD dossier guidance compares specific data requirements and numbering systems between countries.[8] The

[7] More information about OECD formats for data submissions and reviews is available at https://www.oecd.org/chemicalsafety/pesticides-biocides/agricultural-chemical-pesticide-registration.htm (accessed August 11, 2022).

[8] More information about OECD dossier guidance is available at https://www.oecd.org/env/ehs/pesticides-biocides/oecdguidancedocumentsforpesticideregistration.htm#dossier (accessed August 11, 2022).

guidance lists OECD data points and links each data point to a specific test method and to the corresponding data guidelines or code requirements in the various member countries. Sachana noted the challenge of keeping pace with the rapid rate at which regulations across the globe are evolving. The International Uniform Chemical Information Database (IUCLID) is an OECD tool that some countries use to submit dossiers (OECD, 2019). OECD-harmonized templates are standard formats for reporting information used in the risk assessment of chemicals. Countries complete these templates for dossiers that are then submitted through IUCLID.

Initially, the OECD-harmonized templates were developed for industrial chemicals, but they were recently adapted to address reporting needs for pesticides and biopesticides. Sachana remarked that biopesticides are safer technologies for use in plant protection products. OECD is working intensively to improve test methods for microorganisms in particular. For instance, in September 2022, OECD hosted a conference on innovating microbial pesticide testing. The organization also publishes works that address barriers to biopesticides regulation. OECD organizes seminars that generate reports or recommendations that can then become the basis for guidance documents. For example, OECD has published guidance for risk assessment of secondary metabolites of microbial biocontrol agents and for the technical evaluation of microbial strains (OECD, 2018a, 2018b). Sachana highlighted an upcoming document on potential antimicrobial resistance (AMR) related to microbial pesticides that will be published on the OECD website. This publication will address considerations regarding the evaluation of plant protection products within the context of resistance, featuring OECD member countries' approaches to assessing AMR in microorganisms used as biopesticides.

All of these efforts are part of a comprehensive program to streamline pesticide registration and to reduce the risks of pesticides through improved testing. Pesticide practices continue to evolve with innovative products, but new products can bring new challenges, said Sachana. OECD aims to provide tools to address these challenges to enhance the protection of humans and the environment, integrate green growth strategies, and facilitate cooperation and cost savings for industry and regulatory authorities.

OCCUPATIONAL EXPOSURE TO ENVIRONMENTAL RESISTANT FUNGI AND POSSIBLE IMPLICATIONS IN HUMAN HEALTH

In a prerecorded presentation, Sabino discussed occupational sources of fungal exposure. She described *Aspergillus* exposure and infection, resistance mechanisms, associated health implications of resistance, and sources of environmental *Aspergillus* resistance.

Occupational Exposure and Development of Fungal Disease

Exposure to fungi in a work setting can occur via oral intake of contaminated food, dermal contact with contaminated materials, and, most commonly, inhalation of aerosols, Sabino stated. During and after occupational exposure to fungi, numerous adverse health effects may occur (see Figure 6-2). These effects range from mild symptoms—such as runny nose, sneezing, coughing, sore throat and itchy eyes—to severe conditions, such as asthma, pneumonitis, cancer, and even death. The severity of adverse effects depends on the environment's fungal load, fungal species or strains, temperature, humidity, ventilation, and presence of large amounts of dust and particles. Symptom severity also depends on physical features of the exposed worker, particularly immune system functions and history of respiratory conditions. She noted that the most deleterious occupational fungal exposures tend to involve *Cladosporium, Alternaria, Stachybotrys, Penicillium* and *Aspergillus*.

Aspergillus Features and Health Risks

Several features of *Aspergillus* enable it to grow in occupational environments and increase the likelihood that workers will be exposed to it, said Sabino. *Aspergillus* produces environmentally resilient conidia in large amounts that easily disperse into the air and these airborne spores can be inhaled. Furthermore, *Aspergillus* tolerates a wide range of temperatures and has high nutritional versatility. With the ability to grow on a variety of construction materials—such as concrete, acrylic paints, and wood-based materials—this fungus is associated with occupational exposure. Thriving in moist environments, *Aspergillus* is often found in decomposing organic matter.

Sabino stated that more than 90 percent of *Aspergillus*-related conditions are caused by *Aspergillus fumigatus* (*A. fumigatus*) (Latgé, 1999). The small diameter of *A. fumigatus* conidia enables them to reach the pulmonary alveoli once inhaled. The impact of exposure on human health depends on the host immune system, previous pulmonary lesions, the concentration of conidia in the air, and strain virulence in terms of features including mycotoxin production, antifungal resistance, and thermotolerance. A range of health effects are associated with occupational exposure to *A. fumigatus*. These include respiratory disorders—with hypersensitivity responses such as allergies and fungal induced asthma—as well as mycotoxicosis and irritant effects caused by mold exposure. Additionally, serious opportunistic infections can occur. For instance, invasive pulmonary aspergillosis poses health risks to more than 30 million people who are susceptible to this disease due to corticosteroid and other

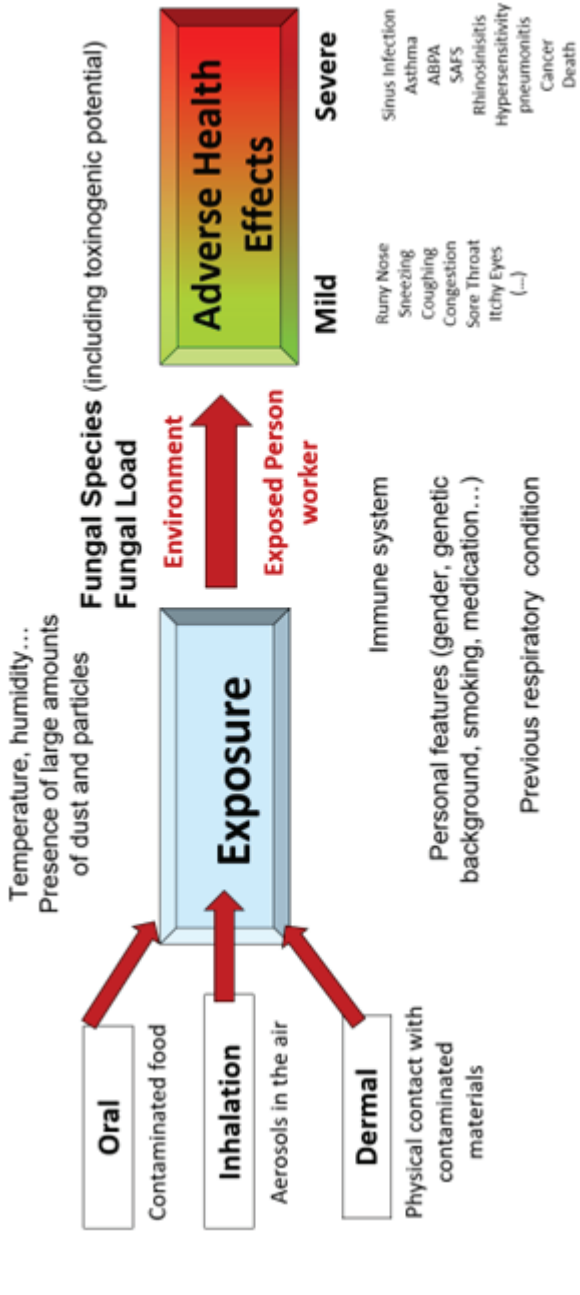

FIGURE 6-2 Occupational exposure versus development of fungal disease.
SOURCE: Sabino presentation, June 22, 2022.

immunosuppressant treatments.[9] Incidence in the United States and Europe is between 1.1 and 4.6 in 100,000, and trends show an increase in recent years (WHO, 2020). The mortality rate is approximately 50 percent if treated and greater than 99 percent if untreated. Resistant *A. fumigatus* is identified in 1.5–13 percent of all cases of invasive pulmonary aspergillosis (WHO, 2020).

Occupational Exposure to Resistant *Aspergillus*

Exposure to airborne *Aspergillus* spores can occur in the home, hospital, and workplace environments, said Sabino. A study comparing different occupational settings with high *Aspergillus* spp. loads found that wastewater plants, waste treatment plants, and poultry and swine farms had higher amounts of *Aspergillus* than other settings included in the study (i.e., cork industry, slaughterhouses, animal feed industry) (Viegas et al., 2017). Furthermore, the waste treatment plants were the most likely setting for fungal contamination to occur. In addition, agricultural settings can expose workers to high amounts of *Aspergillus* fungal spores, including those from antifungal-resistant isolates. Azole resistance in *A. fumigatus* has been emerging since 2007, with the presence of pan-azole-resistant isolates being detected in an ever-greater number of countries (Bueid et al., 2010). Concern regarding azole-resistant *A. fumigatus* has been increasing to the extent that the U.S. Centers for Disease Control and Prevention (CDC) included it on the watch list in their 2019 report on antibiotic resistant threats (CDC, 2019). Additionally, the World Health Organization (WHO) Antifungal Expert Group highlighted *A. fumigatus* as a priority fungal pathogen (WHO, 2020).

Sabino explained that antifungal resistance in *Aspergillus* can be classified as either primary or secondary. In primary (i.e., intrinsic) resistance, all organisms of the same species are resistant to a specific antifungal. Secondary (i.e., acquired) resistance occurs when only some isolates of a species are resistant to a specific antifungal. This type of resistance occurs in response to prolonged therapy and prophylaxis with clinical azoles and the usage of agricultural fungicides (Beardsley et al., 2018; Van Der Linden et al., 2011). Thus, workers in agricultural and sawmill settings can be exposed to high levels of environmental azole-resistant isolates. Triazole DMI pesticides are used to protect crops and preserve materials from fungal decay. Although azole fungicides are not used to target *A. fumigatus*, many DMI fungicides are active against this fungus, which has led to the emergence of resistance. DMIs and clinical antifungals have very similar chemical structures (Kelly

[9] More information about invasive pulmonary aspergillosis is available from http://www.life-worldwide.org/fungal-diseases/invasive-aspergillosis (accessed August 25, 2022).

and Kelly, 2013). This similarity and the ability of fungal spores to disperse easily from crops to other locations via circulating airflows have generated concerns that azole resistance could become a global public health threat.

Resistance Mechanisms in *Aspergillus*

Azole resistance in *A. fumigatus* is associated with mutations in the *cyp51A* gene, said Sabino. This gene encodes an enzyme that is responsible for biosynthesis of ergosterol, which is essential for the viability of the fungal cell. Resistance mechanisms associated with prolonged antifungal therapy and prophylaxis are often identified as point mutations inside the *cyp51A* gene (Zhang et al., 2017a). These point mutations can be coupled with tandem repeat (TR) integrations—TR_{34} and TR_{46}—in the gene promotor. These TRs are associated with environmental acquired resistance. Although the environmental link for resistance has not been fully uncovered, there is evidence for a relationship, Sabino stated. This includes the presence of azole-resistant *Aspergillus* isolates in patients that have not previously been treated with azole antifungals (Verweij, Mellado, and Melchers, 2007; Snelders et al., 2012). Also, the presence of a TR in the promotor of the *cyp51A* gene is not found in any *A. fumigatus* isolates that became resistant through patient therapy, but it has been found in azole-resistant phytopathogenic molds. Furthermore, *A. fumigatus* isolates recovered from the environment are genetically clustered to *A. fumigatus* isolates featuring TRs found in patients. These are different from wildtype susceptible *A. fumigatus* isolates. Additionally, medical azole-resistant *A. fumigatus* isolates are cross-resistant to five triazole fungicides used as pesticides.

Development of Resistant *Aspergillus* in Agricultural Settings

Sabino explained that inhalation of *Aspergillus* spores from resistant isolates can automatically render antifungal triazole treatment ineffective and poses the risk of severe health ramifications (Garcia-Rubio et al., 2017) (see Figure 6-3). This gives rise to the question of whether agricultural fungicides induce resistance to clinical azoles. Several studies found this to be the case by demonstrating that isolates exposed to agricultural fungicide develop resistance to clinical antifungals, and those resistant isolates carry the TR_{34} and TR_{46} mutations (Faria-Ramos et al., 2014; Ren et al., 2017; Zhang et al., 2017b). An analysis of 52 published studies that detected azole-resistant *A. fumigatus* in the environment found that the majority of resistant isolates came from agricultural and developed settings (Burks et al., 2021). A large proportion of azole-resistant isolates originated from flowers and specific crops including rice, some cereals, and certain berries. Soil, compost, hair, or plant debris yielded more resistant isolates than other

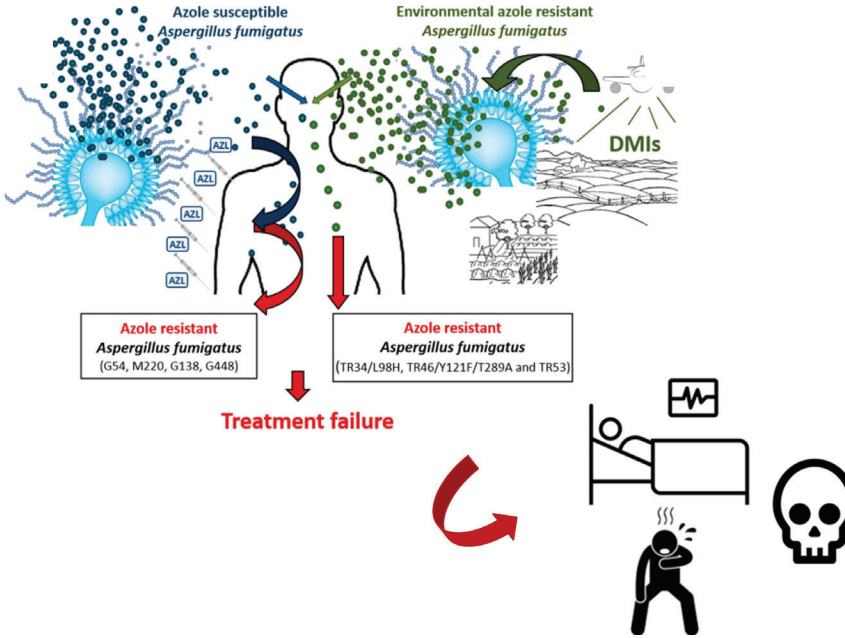

FIGURE 6-3 Clinical implications of resistant *Aspergillus* spores.
SOURCES: Sabino presentation, June 22, 2022; adapted from (Garcia-Rubio et al., 2017), reprinted with permission from Springer Nature.

substrates. Molecular analysis identified the presence of TR_{34} and TR_{46} mutations in 75 percent of these isolates (Burks et al., 2021).

Ecological Resistance Hotspots

Data obtained thus far have brought forth the concept of ecological resistance hotspots, said Sabino (Burks et al., 2021; Schoustra et al., 2019b). These locations feature the physical, biotic, and abiotic conditions to facilitate fungal growth and spread over prolonged time periods, thus allowing the fungi to complete all stages of the growth cycle. Additionally, fungal growth occurs in contact with different azole concentrations sufficient for selection in populations. High quantities of azole-resistant *A. fumigatus* in agricultural environments, flower gardens, and hospitals could indicate that these settings are potential hotspots, she noted. Studies report the detection of azole-resistant isolates in agricultural environments (Chen et al., 2020), waste sorting plants (Goncalves et al., 2020), and sawmills (Viegas et al., 2022). She described sawmills as an occupational

environment of concern, given that azole fungicides are used to protect spruce and pine fields and are used on milled resinous woods to prevent deterioration due to phytopathogenic fungi.

Sabino stated that fungicide treatments remain essential for maintaining healthy crops with reliable, high-quality yields. Therefore, monitoring is vital in determining whether resistance related to agricultural use is causing challenges in human disease control and whether resistance management strategies are effective. Recognizing potential exposure to antifungal resistance within occupational settings is important in facilitating appropriate health interventions, she added.

DISCUSSION

Extending the Life of Fungicides

Given that antifungal resistance often develops about a decade after market introduction, Goldman asked how this relates to resistance management efforts and whether any tools are available to prolong intervals of efficacy. She added that developments in the field of chemistry are not meeting the pace of resistance. Taylor replied that chemical blending and chemical rotation are vital to maintaining the effectiveness of antifungals. The inclusion of both multi-site and single-site chemicals also plays a role. He remarked that mancozeb and chlorothalonil are mainstays of fungal control around the world, yet these multi-site antifungals are being withdrawn in many countries. Although DMIs are under pressure from resistance, they have not been rendered completely ineffective. Judicious use that involves blending or rotation with other chemicals can prolong their utility. He added that many farmers who attend Plantwise plant clinics do not want to stray from a practice that is working well. Plantwise works to educate farmers that unless they do change their plant protection treatment plans, these plans will stop working. Furthermore, the effects of antifungal resistance extend beyond the farmer who is not taking preventative measures. Due to the widespread nature of fungi, spores from resistant fungi can travel hundreds or thousands of miles away, affecting other farmers who are using preventative measures. Goldman remarked that individual practices anywhere can affect people everywhere.

Prophylactic Antifungal Usage

Goldman noted her surprise in hearing the extent to which farmers in LMICs are using prophylactic fungicide treatment in the absence of a documented fungus problem. She asked whether it would be possible to have a low-technology tool that could be used to verify the presence of

fungus on samples brought to the plant clinics. Taylor responded that few fungicides have any restorative activity; almost all of them prevent further infection sites but do not address the fungus that has already established itself on plants. Thus, most fungicides are essentially prophylactic in that they work best when placed on clean leaves rather than waiting until a fungus takes hold.

Cost Considerations

Given that pesticides can be expensive, Goldman asked about their widespread use in LMICs. Taylor stated that farmers in LMICs tend to seek the least expensive option. He said he has seen cases of agrochemical dealers selling fake products side-by-side with real products while openly admitting that the cheaper products are fake, and yet they still manage to sell their product. This highlights the importance of price in this market, he added.

U.S. Fungicide Regulation Classification

Goldman remarked that fungicides are regulated as conventional pesticides, even though some pesticides are considered to be antimicrobials. Fungi are microbes; therefore, fungicides can be considered as antimicrobials. She asked Mellor about the implications of the EPA's approach and whether the benefits analysis includes consideration of public health benefits in terms of controlling mycotoxins. Furthermore, she requested he speak to the overlap of a microbe being both a plant pest and a potential pathogen. Mellor replied that the issue of fungicides is emerging at EPA. Most antibiotics for agricultural use are treated as conventional chemicals at EPA, and in-depth analysis is included in the original review for these products. Each time one of these antibiotics is registered for a new use, any changes are reviewed closely. These determinations can be complicated, as future human uses can be difficult to predict. Furthermore, requiring additional data from registrants without these requirements specifically included in the regulations can be problematic. Mellor added that the EPA division that processes antimicrobial registrations has the same FIFRA data requirements as the fungicide branch.

Given that public health issues require the attention of the EPA, CDC, and FDA, Goldman asked whether any cross-agency approaches to this issue are being taken. Mellor stated that partner discussions take place for antibiotics involve EPA, CDC, FDA and the U.S. Department of Agriculture. The possibility of convening similar discussions for fungicides is under consideration. He is unsure as to how far that process has moved forward. A determination to replicate efforts regarding antibiotics for

issues around fungicides is possible or an entirely new process may be developed.

Resistance Management Regulation Considerations

In response to a question about the processes that the EPA uses to evaluate and manage the role of fungicides in the development of antifungal resistance, Mellor remarked that EPA issued a pesticide registration notice in 2008 that requires certain language, reviews, and monitoring information to be included on every fungicide label.[10] In addition to language requirements, EPA emphasizes the role of rotating fungicides usage based on FRAC groups and modes of action by including this practice in the benefit analysis for new uses, and the agency encourages integrated pest management practices. These factors are all considered during the processes for determining the benefits of a new chemical and reducing risk. A reduced risk classification is applied to certain chemicals, and if a product features a new mode of action, site, or FRAC group, this indicates that it could help resistance management efforts and increases the likelihood of being classified as reduced risk.

Seed Treatments and Genetically Modified Seeds

Goldman asked about the possibility of seed treatment leading to occupational exposure and whether this relates to the use of dust formulations. Furthermore, could genetically modified seeds serve as an alternative to fungicide use on seeds? Mellor replied that seed treatments often do use a dust-type formulation and thereby lead to inhalation exposures for agricultural workers. New scenarios of occupational exposure from seed treatment were presented to EPA; in response, the agency added this factor into risk calculations. EPA adjusts practices when any route of exposure not previously considered comes to the agency's attention. Mellor specified that this does not necessarily mean that dust formulations will be regulated differently. However, EPA works to ensure that determinations are inclusive of exposures and that exposures are not underestimated. Thus, the agency strives to limit any effects of any product that may contribute to unanticipated health problems. At times, mitigations can reduce exposure risk without prohibiting the seed treatment. For example, requirements can stipulate respirator use or that the product be applied as a broadcast instead

[10] More information about PRN 2008-1: Notice to Manufacturers, Producers, Formulators, and Registrants of Pesticide Products is available at https://www.epa.gov/pesticide-registration/prn-2008-1-notice-manufacturers-producers-formulators-and-registrants (accessed August 12, 2022).

of in seed treatments. Each determination is chemical- and product-specific based on data on exposures and the actual risk to human health. Mellor remarked that he is unsure whether a regulation is in place for genetically modified seeds and, if so, whether EPA would be the regulating agency.

Induction Versus Selection

During the discussion at the end of the day, Sabino was asked how resistance induction is defined and how to differentiate between induction and resistance selection. She replied that a selection case involves a group of genetically different isolates: resistant isolates and susceptible isolates. Under antifungal pressure, positive selection of resistant isolates can occur. In the case of induction, susceptible isolates are exposed to antifungal pressure with sublethal doses. As the fungus tries to survive under those conditions and successfully reproduces, its genetic variability improves, with some isolates presenting mutations that allow the fungus to survive under those conditions.

7

Innovations in Antimicrobial Resistance Surveillance Tools and Technologies

The sixth session of the workshop explored nontraditional approaches to antimicrobial resistance (AMR) research, including community science, simulation modeling, and network modeling. Jeff LeJeune, food safety officer in the Food Systems and Food Safety Division of the Food and Agriculture Organization of the United Nations, moderated the session. Wieland Meyer, deputy head of the Curtin Medical School and associate dean of Biomedical Science, Pharmacy, and Medical Radiation Science at Curtin University, Perth, Australia, described community science research currently being conducted on *Aspergillus fumigatus* azole resistance in Latin America. Brian Bailey, associate professor of plant sciences at University of California, Davis, discussed how simulation models can increase the pace of response to emergent threats and optimize current resistance management practices. Karen Garrett, Preeminent Professor of plant pathology and the Institute for Sustainable Food Systems and Emerging Pathogens Institute at the University of Florida, provided an overview of dynamic network models, impact network analysis, and applications for antifungal resistance research.

ASPERGILLUS FUMIGATUS AZOLE RESISTANCE SURVEY BY AIR SAMPLING THROUGH A COMMUNITY SCIENCE APPROACH

Meyer described the impetus and methodology of a study that he and colleagues are currently conducting on *A. fumigatus* azole resistance in Latin America utilizing a community science approach. Azole resistance research is not geographically uniform, and data from Latin America are limited, he said. A Brazilian study examined 199 isolates in a clinical collection

and located two azole-resistant isolates with mutation in the *cyp51A* gene (Pontes et al., 2020). A study in Peru also examined a clinical collection and among 143 *A. fumigatus* samples, 3 were azole-resistant (Bustamante et al., 2020). Mexican research on *Aspergillus* spp. detected 49 *A. fumigatus* isolates, 6–10 percent of which were azole-resistant (Trevino-Rangel et al., 2021). A larger study compared samples from Mexico, Paraguay, and Peru with those from African countries, and researchers found a variable resistance rate between 7–10 percent in the Latin American countries (Resendiz-Sharpe et al., 2021).

Resistance Survey Project Objectives

Meyer remarked that he and his colleagues responded to a call by the U.S. Centers for Disease Control and Prevention for data on the prevalence of antifungal resistance in *A. fumigatus* in low- and middle-income countries (LMIC). He acknowledged Matthew Fisher, professor of Fungal Disease Epidemiology at Imperial College London, for pointing him toward this opportunity. His team developed a project to "evaluate the presence of azole-resistant *A. fumigatus* isolates in air samples in Latin America." He noted that the ease with which air samples can be collected factored into the project design. Once collected, azole-resistant *A. fumigatus* isolates from the samples will be assessed for the presence of mutations in the *cyp51A* gene. Researchers will compare genetic relationships between resistant strains found in different Latin American countries. Additionally, they will examine environmental sampling and azole agricultural usage data to determine whether a correlation exists between resistant isolates detected and azole fungicide use. The project is also an opportunity to establish a network of medical mycologists in Latin America to monitor resistant *Aspergillus* and conduct future studies, he added.

Latin American Medical Mycology Network Laboratories

During previous research on *Cryptococcus* isolates in Latin America, Meyer and colleagues created a loose network of researchers with expertise in medical mycology, infectious diseases, environmental sampling, strain isolation, and molecular characterization of isolates and epidemiology. The group served as a basis for the Latin American Medical Mycology Network (LAMMN), a network of laboratories established for the *Aspergillus* project. Comprised of 26 laboratories, LAMMN spans across Mexico, Colombia, Venezuela, Brazil, Paraguay, Uruguay, Argentina, Chile, Peru, Ecuador, Costa Rica, and Guatemala. Meyer noted organizational challenges in working with such a large network in which three languages—Spanish, Portuguese, and English—are spoken. Although English serves as

the common language, many of the researchers have limited English skills. Moreover, considerable organizational difficulties arose from the varied regulations and postal systems among the countries involved. In addition to the Latin American partners, the project aims to include isolates collected from Antarctica. Meyer remarked that Antarctica is one of the most pristine environments on earth in terms of limited human populations. Thus, knowing whether *Aspergillus* is found there would add to data on the reach of these fungi. He added that Australia and Brazil are contributing molecular genotyping expertise to the project.

Among the LAMMN laboratories, four were selected to serve as reference laboratories responsible for collecting samples from four regions determined by the historical strength of networking between institutions, Meyer explained. The State University of Campinas (Universidade Estadual de Campinas) in Brazil is the LAMMN reference laboratory for institutions in Brazil, Argentina, and Venezuela. Pontifical Javierian University (Pontificia Universidad Javeriana), Colombia, serves as the reference laboratory for Colombia, Ecuador, Paraguay, and Uruguay. Cayetano Heredia University (Universidad Peruana Cayetano Heredia) in Peru is the reference laboratory for Peru and Chile, and the National Autonomous University of Mexico (Universidad Nacional Autónoma de México) represents Mexico, Costa Rica, and Guatemala.

Project Design

Meyer and his team based their project on the community science approach utilized in Jennifer Shelton's[1] research described by Fisher in Chapter 4 (Shelton et al., 2020), recognizing this simple method would enable coverage of a large part of Latin America. Structured into two levels, the first half of the project involves collecting 200 samples from each participating country four times in one year. Two samples are collected from each participant—taken at a distance of approximately 1 kilometer from one another—necessitating 100 participants per country. All samples are sent to one of the four above specified reference laboratories, which is then responsible for identifying resistant isolates via microbiological culture and MIC testing. All resistant isolates are then sent to Fundação Oswaldo Cruz laboratories for further genotyping.

Participant recruitment for sampling efforts began in November 2021. Meyer acknowledged this process proved to be more challenging than expected. Specific social media feeds were used to encourage participants

[1] As described in Chapter 4, Jennifer Shelton, applications scientist at Oxford Nanopore Technologies, developed a citizen science approach to collecting data samples during her doctoral research at Imperial College London.

to register via Twitter, Facebook, and Instagram, but he found that word-of-mouth appears to have contributed more to participant recruitment than the social media campaign. A website was created for registration, a challenging endeavor due to the need to translate the content from English to Spanish and Portuguese in order to make it available in three languages.[2] When a participant registers online, they are first directed to complete a registration form that collects contact information. Next, they are given instructions on how to receive the sampling kit, with options to have it mailed to a personal address or pick a kit up in person from one of the participating institutions. The form collects location information where the participant will be collecting a sample and includes a field to indicate whether it is a rural or urban setting.

As of June 21, 2022, approximately 1,600 participants had registered, with roughly half recruited via the webpage and social media and the other half recruited directly by participating laboratories. Registered participants represent all 12 countries in the survey, constituting a substantial percentage of coverage of Latin America. Not surprisingly, areas such as the Amazon rainforest are under-sampled, so efforts are being made to increase coverage of these locations. Meyer noted that baseline data generated in this project will largely cover the entire South American continent. During the summer of 2022, State University of Campinas is preparing sampling kits to ship to the laboratories that correspond geographically to the participants. The kits are pre-labeled with the names and home addresses of those participants receiving them by mail or with the names and institutions of those picking them up in person.

Meyer described that each kit contains supplies for two samples, which participants will use and send them back to the appropriate national laboratory, which in turn will send the samples to the corresponding reference laboratory for isolation (see above). Given the 1,600 participants and four testing periods over the course of a year, 12,800 sampling kit must be prepared and distributed. Each sampling kit includes an information leaflet with the objectives of the study, sampling instructions, a form for recording the sampling conditions, two air sampling adhesive films, envelopes to place the samples in, and a larger prepaid envelope for returning both samples to the laboratory. Organizational challenges required rescheduling the collection dates several times. In addition, the sample strips used in both a preliminary study and in Shelton's research were determined to be cost prohibitive and in short supply due to supply chain and shipping issues in South America. Thus, a locally sourced sticky film tape was evaluated and selected as a substitute. Meyer and colleagues confirmed that this less

[2] The Latin American *Aspergillus fumigatus* Azole-Resistance Survey website is available at https://www.latasp.com/ (accessed August 15, 2022).

expensive material has the same performance as polymerase chain reaction (PCR) plate covers used as the adhesive collection film in Shelton's study. The first round of sampling kits was mailed to participants in June 2022, with collection taking place during the second week of July 2022. The collection process will repeat every three months, concluding in April 2023.

Sampling and Analysis Processes

The materials for sample collection feature a circle the size of a petri dish with a sticky arm on each side. The participant peels the supply paper off the back of the arms and uses them to adhere the samples to windowsills in two different locations for an exposure of 10-12 hours, Meyer noted. The participants then recover the sampling film and mail the samples in sterile envelopes to the laboratories. Each of the four reference laboratories will culture the collected material to look for *A. fumigatus*, and all isolates that look morphologically similar to *A. fumigatus* will be confirmed via matrix-assisted laser desorption/ionization-time of flight (i.e., MALDI-TOF) or beta tubulin (i.e., β-TUB) sequencing, depending on the reference laboratory's facilities. The *A. fumigatus* isolates will then be screened for resistance against itraconazole and voriconazole. The reference laboratories will send resistant isolates to either the Federal University of Mato Grosso do Sul (Universidade Federal de Mato Grosso do Sul) or Adolfo Lutz Institute (Instituto Adolfo Lutz), both in Brazil, where minimum inhibitory concentration (MIC) testing will be conducted against a wider range of azoles. Resistance isolates showing high MICs will be sent to State University of Campinas or Nantes University in France for amplification of the *cyp51A* gene regions. Depending on the number of isolates available, microsatellite analysis or whole genome sequencing will be carried out at Nantes University (Nantes Université), the Oswaldo Cruz Foundation (Fundação Oswaldo Cruz) in Brazil, or Curtin University in Australia. These data will enable comparisons between isolates found in Latin America and those in other parts of the world. In a parallel process, the LAMMN laboratories are collecting agricultural azole usage data to determine whether a correlation exists between agricultural azole usage and the azole-resistant isolates found in the same region.

Proposed Project Impact

Meyer emphasized that as of June 2022, the project has only been underway for six months, and no data are yet available to present. However, preliminary studies indicated *A. fumigatus* isolates in a number of locations using the proposed methodology. The project has several aims.

- Provide baseline data on environmental *A. fumigatus* resistance throughout Latin America.
- Provide Latin American governments with data to identify links between agricultural antifungal usage and emerging resistance in environmental *A. fumigatus* isolates.
- Provide evidence to governments to influence the usage of antifungals in agriculture.
- Provide the World Health Organization with baseline information on *A. fumigatus* azole resistance to develop better treatment guidelines.
- Enable decision making for clinical treatment of invasive aspergillosis between monotherapy with a first-line triazole or a combination therapy of a triazole with an echinocandin or liposomal amphotericin B in cases with environmental triazole-resistance prevalence greater than 10 percent, as recommended by earlier studies (Verweij et al., 2015).

Although the study is at an early stage, Meyer shared his optimism about this project, given that is has already overcome organizational challenges regarding varying government regulations, work in remote areas, and complications with the different postal systems involved. Furthermore, the number of participants enrolled—from government employees to community scientists learning of the project online—is encouraging, he said. A growing global knowledge of resistance in the environment will inform whether a link between resistance and agricultural azole usage is at play.

INNOVATION IN DATA COLLECTION TOOLS FOR SURVEILLANCE AND MITIGATION: CROP AND ENVIRONMENT SIMULATION TOOLS

Bailey discussed simulation models that could aid in creating more efficient fungicide management programs to reduce the development of resistance and respond more rapidly to novel fungal pathogen threats. He noted that changing conditions can transform a pathogen that historically has not posed problems to humans into both a food security and human health threat. Rapid response to changing pathogens can minimize these negative effects.

Trial-and-Error Versus Simulation Modeling

Various mitigation and management strategies are used to disrupt the fungal disease cycle in agricultural settings, said Bailey (see Figure 7-1). Most of these strategies center around disrupting pathogen development

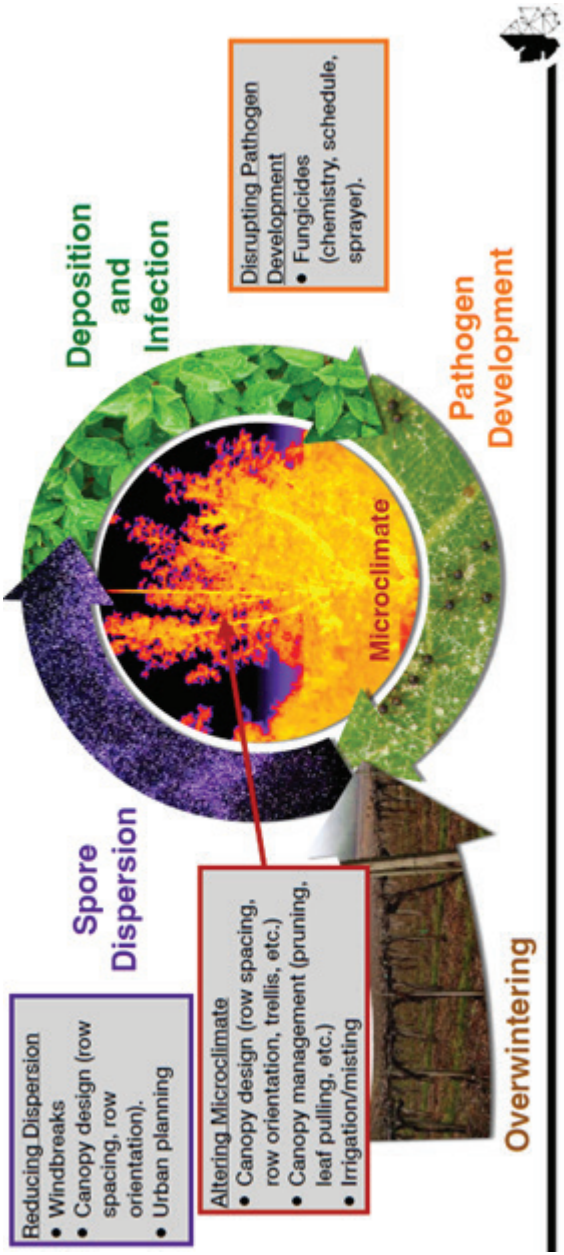

FIGURE 7-1 Mitigation and management strategies across the fungal pathogen life cycle.
SOURCE: Bailey presentation, June 22, 2022.

and include (1) applying fungicide, (2) alternating the chemicals used, (3) rotating crops, (4) shifting to a different mode of action, and (5) altering the schedule application method. A single change in the system carries the potential to affect the entire fungal life cycle. Traditionally, determining the most effective management practices has been a process of trial and error. Prior knowledge about existing diseases is utilized as a foundation in developing modified practices for new threats. Experiments and approaches are conducted and evaluated under changing field conditions, though some experiments may first take place in the laboratory before moving into field trials. An iteration process continues until an acceptable outcome (e.g., efficacy of crop protection) is achieved. Bailey remarked on the time-consuming nature of this traditional experimentation approach. Testing a method under various weather conditions and other pertinent factors can require several seasons to establish sufficient confidence in the method to translate into production. Additionally, the efforts may converge toward a local optimum rather than to a global one.

In contrast, industries outside of agriculture are using simulation and modeling approaches to accelerate discovery and innovation, Bailey stated. For example, a design engineer creating a component of a jumbo jet would likely use a simulated environment where all variables can be controlled, thus enabling rapid iteration through various designs. These designs would be tested through realistic, physically based models to determine the critical variables. Only a satisfactory prototype would be built to test in a wind tunnel, and then progressing to build a full-scale aircraft. Models and simulations have accelerated the pace at which many industries can optimize designs and bring products to market. Furthermore, simulations can test variables that are not readily measurable in the physical world.

Utilizing simulation technology in agriculture—and specifically in fungicide resistance—could bypass much of the trial-and-error process, said Bailey. In silico iteration could enable all the various parameters to be tested simultaneously, thus narrowing down to the most promising methods to test in the field. This simulation loop could potentially decrease experimentation time, control for different conditions, and increase the speed of response to an identified problem. An ideal scenario would include a field experiment, but in situations requiring immediate response, the method could potentially move directly from simulation to roll out.

Resistance Modeling Considerations and Model Types

Bailey acknowledged challenges inherent in simulating a system of AMR, given that it entails numerous variables that all interact with one another. This system must consider many potentially significant processes and inputs, a number of which are not readily acquirable for a given site

or under certain conditions. Additionally, researchers may want to investigate multiple mitigation strategies. AMR modeling needs to be capable of incorporating relevant inputs, determining how the mitigation strategies interact with each other, and predicting outputs ranging from fungicide resistance to efficacy in controlling disease to human pathogen exposure. Moreover, model parameterization requires experimental data in which simultaneous measurements of important sub-processes are collected (see Figure 7-2). Bailey remarked that a potentially beneficial initial approach to this complex task might be selecting a few prioritized inputs and conducting a limited number of experiments to evaluate them.

Empirical, Mechanistic, and Heuristic Models

The level of detail needed to create a helpful model can vary depending on specific needs. Bailey explained that determining which variables to include depends on the particular problem being investigated and the end goal. Different types of models can be utilized based on specific needs. Empirical models use observed data trends to infer relationships between variables. An advantage of empirical models is that all of the mechanisms at play do not necessarily need to be understood in order to use them. These models only require that variables can be measured as they change within a data set, and that a set of mathematical equations can be determined that describe the observed trends. However, empirical models typically require substantial amounts of data; more variables require larger amounts of data to cover the parameter space.

Mechanistic models, guided by laws of nature rather than trends in datasets, are used to quantify relationships between variables. Bailey noted that no model is fully mechanistic because empirical parameterizations are needed at some point. In many cases, mechanistic models offer the advantage of requiring less data, given that the laws of nature constrain some of the relationships between variables. In theory, these models are more robust when conditions change because they do not require complete datasets in order to represent every relationship. Mechanistic models also have the potential to be more robust when applied to future events because they do not necessarily require data on conditions that have not yet occurred. A downside to these models is the need for theoretical understanding of how systems work, which can be challenging to achieve in life sciences, biological systems, and agriculture. For this reason, researchers in life science tend to use models that are more empirical in nature rather than mechanistic ones, said Bailey.

Heuristic models describe relationships more generally between variables, with trends typically reproducible. Such models may be utilized when data are insufficient to develop an empirical model—for example,

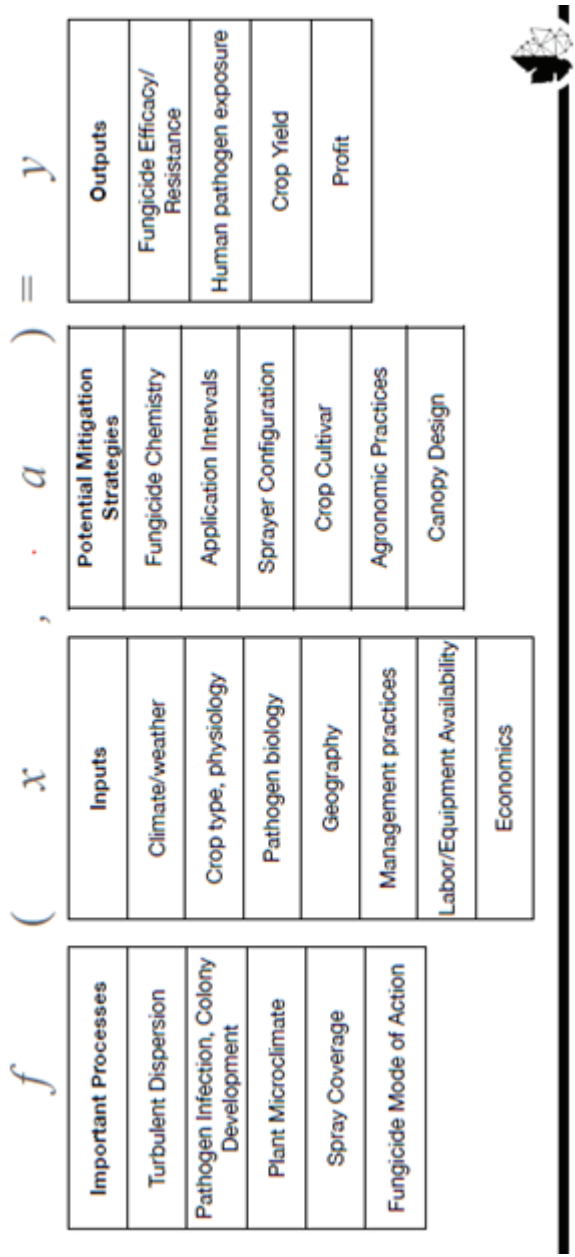

FIGURE 7-2 Example of modeling antimicrobial resistance.
SOURCE: Bailey presentation, June 22, 2022.

limited data may be available about an epidemic. Bailey remarked that disease data are often sparse in comparison to other fields. However, automated and community science approaches to data collection are efforts to address gaps in disease data.

Helios Modeling Framework

Most models for antifungal resistance target one or two phases of the fungal pathogen cycle (see Figure 7-1), said Bailey. For instance, the host is generally not addressed due to the assumption that susceptible host tissue is abundant and does not limit disease development. The environmental effects of microclimate are often ignored; even in cases where ambient weather data are considered, the differences between weather and the actual microclimate that the pathogen is experiencing are often neglected, Bailey stated. He and colleagues have been working in recent years to develop Helios, a scalable, three-dimensional (3D) simulation framework for plant and environmental modeling. Helios combines all processes together in a systems level model with a mechanistic approach. For example, the model is able to predict the local microclimate at the pathogen and tissue level and the evolution of the host based on the local microenvironment. The model is also able to resolve impacts of changing the canopy, geometry, or architecture, and it can determine effects at the local pathogen scale in terms of colony development or spread across tissue. Bailey said that work is underway to incorporate fungicide aspects to enable simulated fungicide application. This could make it possible to predict the effect of ultraviolet exposure on chemicals, the trajectory of efficacy decline over time, expected levels of fungicide resistance by genotype, and the likelihood of resistance evolving throughout and beyond the agricultural system to potentially affect human health.

Artificial Intelligence Modeling

Bailey stated that machine learning and other types of artificial intelligence (AI) models are steadily gaining in popularity. He described machine learning as the "ultimate empirical model," requiring massive amounts of data to extract trends and make predictions for the future. Agricultural settings—and plant pathology specifically—present substantial challenges in applying machine learning models; collecting adequate data for simplified empirical models is already difficult and AI-based models require significantly larger quantities of data. Bailey clarified that AI uses in agricultural modeling are possible, depending on the data source and desired output. This modeling is commonly used for high throughput screening. Multispectral, thermal, lidar, and RGB imaging techniques can be used to detect

plant stress caused by disease (Oerke et al., 2014). Therefore, AI modeling could use these images to infer what is taking place within the plant images as various conditions change. Bailey noted that such models are not diagnostic per se: for example, they may indicate stress in the plant without providing a diagnosis. Practitioners would then need to conduct more intensive sampling to determine which pathogen is affecting the plant. Furthermore, AI may not be able to detect problems until the signature symptoms of the disease present, at which point it could be too late to treatment.

Simulated Data

When sufficient data are lacking, simulated data can be used as a supplement in developing AI-based models, said Bailey. For example, machine learning models have been used to test self-driving cars in environments created with simulated data. These detailed simulated environments enable researchers to vary a wide range of parameters. A simulated data approach can also be applied to agricultural environments. Bailey and colleagues developed an initial test case for fruit detection in which they generated a 3D simulation that contained numerous images of the crop in various conditions (Fei et al., 2021). The simulation allows for every pixel within an image to be auto-labeled and used to train the machine learning model. Bailey is hopeful that simulated data modeling will lead to applications relevant to disease detection and management. For instance, generated 3D models could predict multiple factors affecting the system, simulate an intervention—such as changing the way that plants interact with light—and generate large amounts of data, which would then train a machine learning model that would be applied to agricultural practices in the real world. He added that this technology could potentially extend beyond detecting disease to inferring risk or areas likely to have fungicide resistance. Scaling this technology could enable the prediction of human exposure to resistant fungi. Bailey emphasized that field-level disease data are often sparse in agricultural environments, and alternative modeling methods are needed to accelerate the pace of response to emergent threats and for optimization of current practices.

IMPACT NETWORK ANALYSIS FOR GUIDING SURVEILLANCE AND MITIGATION

In a prerecorded presentation, Garrett introduced the general concept of dynamic network models and described impact network analysis and applications for antifungal resistance research. Garrett remarked that "scenario analysis allows users to consider likely outcomes from potential mitigation strategies." For instance, a manager weighing various options

can utilize scenario analysis to determine the costs and benefits of different approaches. A socioecological approach integrates the process of human decision making into a platform to assess human influence on the management of factors such as potential pesticide resistance.

Dynamic Network Models

Network models consist of nodes and links that connect them, Garrett explained. The links between pairs of nodes can be weighted in relation to the level of interaction between the nodes. In many systems, these link weights are of a dynamic nature in that they can change over time. Possibilities for network model structures are infinite. The nodes can be locations in spatial networks, such as counties, fields, plants, leaves, cells, organelles, or microbes. Nodes can also be entities like individual people, species, genes, or molecules. The links between the nodes can indicate the strength or likelihood of interaction; these links are dependent on environmental variables. In the context of potential movement of pesticide resistance genes, nodes could include individual human hosts and farms, while the links could indicate the probability of movement of pesticide resistance genes between the people and farms (e.g., through spread of resistance microbes from one location to another).

A network has several traits and features that, once characterized, can be used to draw conclusions about the importance of different nodes in the network, said Garrett. The structure of a network can be socioeconomic or biophysical. A socioeconomic network contains nodes that represent people or human institutions. In the context of antifungal resistance, a socioeconomic network could contain nodes representing farmers, extension agents, and scientists. A biophysical network features nodes that represent geographic locations, such as individual plants, farms, storage facilities, and wildlands. Once the structure of a network is established, conclusions can be drawn about the nodes from several aspects related to centrality. Degree centrality is the number of links connected to a node. All else being equal, the more links a node has in the network, the greater the likelihood that the node is important, she remarked. Closeness centrality measures how readily other nodes can be reached. Betweenness centrality refers to how often a node serves as a bridge between other nodes. Centrality of neighbors ranks nodes as more important when they have proximity to neighboring nodes of importance.

Garrett described how she and colleagues used networks to characterize potential movement of pesticide resistance and other traits in grain storage (Hernandez Nopsa et al., 2015). Managers of grain storage facilities make decisions that can affect the likelihood that fungi using the grain as a substrate will select for resistance. Once pesticide resistance is established

at one location, it can spread to other facilities. A characteristic network of wheat movement by U.S. railroads aids in identifying locations that are prime for transmitting spread. The network used in this study includes nodes for each U.S. state, proportionally sized to wheat production in that state. Links between nodes represent grain volume transported between locations; the thicker the weight of the link, the greater the volume of grain moving between two states. The network model revealed that Illinois—a state that is not one of the top grain producers—serves as a key hub in wheat transportation. Thus, managing pesticide resistance in Illinois locations connected to this transport system is important in preventing spread, said Garrett.

Networks can also be used in the context of agricultural lands. Garrett was involved in research on cropland connectivity that used gravity models to predict the probability of a pest or pathogen moving from one location to another (Xing et al., 2020). She noted that these models could also be used to predict spread of pesticide resistance genes. The gravity model factored in the abundance of host crop available at both locations, the distance between them, and the dispersal patterns of the pathogen species that is potentially spreading. This example demonstrates that networks can be used to predict risk of movement, including risk of movement through landscapes. These types of data can be used in the context of impact network analysis (INA).

Impact Network Analysis

Garrett noted her work on INA and the associated R program in various contexts including problems, such as pathogen movement, and benefits, such as the spread of improved crop varieties.[3] The networks can be characterized based on observational data or in terms of potential scenarios. She described using INA to evaluate management technologies for stored grain that are currently available for adoption, including strategies that are less likely to lead to fungal selection for pesticide resistance but may have potential trade-offs in cost (Garrett, 2021). Managers are faced with a decision of whether a lowered risk of pesticide resistance is worth the additional cost. She created a socioeconomic network to represent the exchange of information among decision makers, with each node representing people forming an opinion about this tradeoff of extra cost for decreased resistance risk. A biophysical network was created to represent the dispersal of a focus bioentity—in this case, pathogens with resistance genes—and how

[3] The R program is an open-source software suite used for data manipulation, calculation, and graphical display in statistical computing and graphics. More information about the R program is available at https://www.r-project.org/about.html (accessed August 18, 2022).

management adoption influences the establishment of the bioentity. In order to define a management scenario, INA enables various components of these networks to be adjusted to arrive at potential outcomes. For example, INA can be used to predict the likelihood that a policy to subsidize better management strategies will be successful in decreasing the spread of pesticide resistance.

Scenario analysis with INA technology facilitates exploration of questions of interest, said Garrett. For example, it can provide insight on key locations of focus for antibiotic resistance management efforts. INA can also indicate the influence of subsidies and policies on system outcomes. For instance, INA could develop scenarios for both a policy that subsidizes improved management systems and one that imposes a penalty if management strategies are not implemented. The outcomes of these approaches could then be compared to see which is more likely to decrease the risk of pesticide resistance. INA can also be used in informing surveillance strategy. Garrett created networks to represent the potential spread of an invasive bioentity, such as pesticide-resistant genes (Garrett, 2021). The nodes in these networks represent individual points of potential spread of pesticide resistance. INA generated various scenarios, with nodes shaded by color to represent their potential value in surveillance efforts. These scenarios then inform which points of potential spread should be prioritized for surveillance and management efforts. Garrett acknowledged that small systems may not require INA to achieve these insights. However, INA can be helpful in representing scenarios for systems that are too large to visualize in traditional ways. INA can also be used to determine target management adoption rates in the event of increased probability of the establishment of resistant genes (Garrett, 2021). For each percentage of increased probability of establishment, INA can generate the mean adoption rate required to keep the risk of resistance from increasing. This information can inform the level of intervention needed to maintain the sustainability of a system when one factor is disrupting the balance.

Garrett emphasized that complete analysis via modeling methods demands large volumes of data. When specific data are insufficient, more general questions can inform the process of characterizing the system. For example, researchers can consider how a change in the impact network components can maintain system stability and account for increased risk (Garrett, 2021). As additional data are collected, models can become more realistic. At this point, researchers can consider how changes in network traits, including mechanisms of influence between actors, affect system outcomes. Once more information is known, questions can become more precise, such as identifying specific decision makers or location nodes as "key control points for successful management."

DISCUSSION

Data Considerations in Simulation Modeling

Given that real world data may be lacking, LeJeune asked how models can be validated under that circumstance. Bailey replied that the data requirement is often substantially lower for validation than it is for developing an empirical model from the start. He acknowledged that the more available data will support stronger validation. However, validation can be performed by targeting specific inputs and outputs of different scenarios, without necessarily measuring every subprocess. In instances where data are lacking but physical relationships or theory are represented, direct validation may not be possible. In these cases, focus would be placed on input mapping to various outputs. Statistical approaches can also be used in which certain parameters or variables are treated as unknown, and the importance of random variables are assessed using sensitivity analyses.

LeJeune asked how robust these models are in terms of application—i.e., whether a new model needs to be developed for each pathogen and for each commodity. Bailey stated that fundamental inputs are needed in terms of different pathogens and crops, in addition to pests and microclimate features. His team worked to build the Helios model to be as modular as possible. Directly representing the 3D geometry of the system allows for a more generalizable framework. Different and more specific data are needed when moving from system to system. For example, data from fungal pathogen isolates may be required without needing other data from a full-scale field experiment. The model uses the specific pathogen-related data and essentially scales up to field scale. In other words, not all the components need to be remeasured each time a new model is developed. Bailey noted that the development of crop models has been limited by the amount of data on crop growth and yield needed to parameterize the model, as this can equate to decades' worth of work. He and his colleagues are working to create generalizable models that facilitate the ability to move between systems and to predict future occurrences.

LeJeune asked how data gaps are identified and prioritized, given the number of variables involved in modeling fungal spread—including crop production, fungal growth, fungicide usage, pathogen emergence, and fungicide resistance. Bailey remarked that an advantage of this modeling approach is that a model can be initially formulated based on well-understood environmental interactions, and then used to determine the key variables. This determination enables the identification of narrowed and system-specific data needs. He added that current data gaps include grower management practices, both in terms of fungicide records and other practices. Complementary measurements on the local microclimate and crop

performance would also contribute to a more complete picture of what is occurring in the crop.

In response to LeJeune's query about whether these models can be used to study *A. fumigatus* in waste management to determine strategies for a different portion of the fungi life cycle, Bailey replied that this has not been a priority for his research thus far, but it is important to many applications. His research framework would allow for that type of study, provided that data were available related to residues on plant waste. The local microclimate and dispersion from waste sites could be predicted; therefore, the framework should be able to accommodate this type of study.

Data Challenges in Community Science

LeJeune asked about the strengths and weaknesses of the community science approach to data collection and how these data can be validated to draw stronger conclusions from the results. Meyer responded that community science can involve some discrepancies. For instance, the random distribution of sites results in uneven representation across countries and across the continent. The collection method will also vary, with some people more carefully adhering to the instructions than others. Additionally, the lack of actual agricultural data could conceivably result in challenges, because participants may be willing to collect samples but unwilling to disclose the type of antifungal products being used, potentially out of concern about causing problems for their suppliers. He added that governments can also be hesitant about providing agricultural antifungal usage data. Meyer remarked that the project should provide baseline data that is currently lacking in the literature. Obtaining the isolates to establish genetic characterization across a continent is a challenge. Variance is expected in terms of accurate reporting of sampling position, climate and weather conditions, and description of the setting. LeJeune acknowledged the logistical and economic challenges in conducting a global survey. Meyer noted that he preferred to use the same type of adhesive film as the community science study in the UK, but the expense and supply logistics made this unfeasible. Locally produced film is affordable and available, but ensuring consistent quality over the duration of the project is a challenge. He stated that statistically, having more reproducible points from the same location would have been ideal, but this is difficult with the community science approach.

REFLECTIONS ON DAY TWO

LeJeune reflected that the workshop sessions explored the agricultural use of antifungal agents, the possible effects of that usage on human

medicine, the One Health aspects of that interplay, and the need to balance food security with the risks associated with AMR. That balance may be influenced by climate change and the economics of each country. Historical patterns of AMR related to occupational exposure in agriculture systems and the complexity of fungicide registration requirements were described, including the lengthy timeline involved in registering a new agent. Speakers discussed molecular epidemiology, molecular mechanisms of resistance, and global surveillance methods. Substantial agricultural and environmental data gaps were identified, and nontraditional community science and modeling approaches were detailed. Antifungal resistance is a global, multisectoral problem that will require input from plant and agriculture, human health, and surveillance communities, said LeJeune.

8

One Health Approach to Antimicrobial Resistance and Pathogen Surveillance

The seventh session of the workshop focused on surveillance studies and research investigating the association between the use of agricultural fungicides and the emergence of antifungal-resistant infections, factors contributing to the risk of cross-resistance, and mitigation efforts. Maryn McKenna, senior writer at *WIRED* and Senior Fellow in Health Narrative and Communication at the Emory University Center for the Study of Human Health, moderated the session. Paul Verweij, professor and chair of the Department of Medical Microbiology at Radboud University in the Netherlands, discussed the emergence of antifungal resistance in clinical settings, its association with agricultural use of fungicides, and potential risks for increased development of cross-resistance. Marin Talbot Brewer, associate professor of mycology and plant pathology at the University of Georgia, presented findings from U.S. surveillance studies of *A. fumigatus* in the environment and on consumer food and garden products. Multi-fungicide-resistant isolates have been identified in a variety of agricultural settings and garden and lawn products. Shawn Lockhart, senior clinical laboratory advisor for the Mycotic Disease Branch and senior advisor for antimicrobial resistance at the Centers for Disease Control and Prevention (CDC), described the limited efforts to detect resistant fungal infections in the United States, a lack of available antifungal susceptibility diagnostic products, and low capacity for laboratory processing of these tests. He discussed the value of expanded antifungal resistance surveillance and of greater communication and collaboration between various sectors. Jorge Pinto Ferreira, a veterinarian and food safety officer at the Food and Agricultural Organization (FAO) of the United Nations, described international

guidance and data platforms for AMR surveillance efforts. Bas Zwaan, professor at Wageningen University & Research in the Netherlands, provided an overview of the One Health Aspects of Circularity project, the benefits and risks of a circular system of farming, and research efforts to identify interventions to mitigate the threat of agricultural antifungal resistance to clinical populations.

ONE HEALTH APPROACH TO ANTIMICROBIAL RESISTANCE SURVEILLANCE AND MITIGATION MEASURES

Verweij explored the necessity and benefits of adopting a One Health approach in conducting antimicrobial resistance surveillance and implementing mitigation measures. He described the emergence of azole-resistant *A. fumigatus* (ARAf) and research associating this emergence with the use of azole fungicides. He also discussed barriers to antifungal resistance research and interventions, as well as risk levels for various potential sources of cross-resistance.

Emergence of Azole-Resistant *Aspergillus fumigatus* in Humans

In 2007, several patients developed aspergillosis with azole-resistant isolates found in their clinical samples, said Verweij (Verweij et al., 2007). Four of these patients lacked prior history of azole treatment, indicating that the source of azole-resistance was not medical treatment of the host. Following this discovery, a large collection of *A. fumigatus* isolates was investigated for resistance (Snelders et al., 2008). Prior to 2000, no resistance was present; however, resistant isolates were found in patient samples collected every year thereafter. Furthermore, the majority of these isolates had the same mutation with tandem repeat (TR) insertions that are associated with environmental resistant isolates. This finding indicates the possibility that resistance is selected for in the environment. Verweij and colleagues investigated 35 fungicides for activity against *A. fumigatus* with a wildtype isolate and an isolate with the TR34/L98H resistance mutation (Snelders et al., 2012). Five fungicides from the triazole class were found to have high activity against the wildtype and no activity against these TR34 isolates. Additionally, two fungicides from the diazole class were active against the wildtype and not against TR34.

Modeling studies investigated cross reactivity and found that two of the fungicides—propiconazole and bromuconazole—had very similar core structures to two medical triazoles, itraconazole and posaconazole (Snelders et al., 2012). Moreover, fungicides tebuconazole and epoxiconazole were very similar to another medical triazole, voriconazole. A fifth fungicide, difenoconazole, demonstrated a strong interaction with these three medical

antifungals itraconazole, posaconazole, and voriconazole. Thus, these five fungicides were suspected of driving the clinically observed resistance. The Netherlands first authorized use of these fungicides between 1990 and 1996 (Snelders et al., 2012) and the first resistant isolates detected via susceptibility testing were from clinical samples dating back to 1998. Verweij stated that the time span between the introduction of a fungicide and the appearance of resistance is typically about 10 years, which holds for *A. fumigatus*.

Research and Actions to Address Resistance in *Aspergillus fumigatus*

The emergence of resistant *A. fumigatus* necessitated (1) devising appropriate methods of detection, (2) developing better understanding of its effects on humans, and (3) identifying its source. Verweij noted that several research groups undertook the challenge of detecting resistance, resulting in a commercial polymerase chain reaction (PCR) test to detect TR34 and TR46 (Chong et al., 2015) and a simple agar-based screening assay that enabled straightforward detection of resistant colonies (Buil et al., 2017). A study compared patients with voriconazole-susceptible infections and voriconazole-resistant infections to determine the effects of resistance on human outcomes, finding that the survival rate decreased by 20 percent in individuals with a resistant isolate (Lestrade et al., 2019a). Verweij emphasized that the introduction of voriconazole initially led to a 15% increase in survival rate for patients with invasive aspergillosis (Denning, 1996); the emergence of resistance virtually eliminates this benefit. As more research indicated that resistance to antifungal compounds in the environment was driving resistance observed in clinical isolates, studies have focused on detecting environmental locations with selective pressure for azole resistance in *A. fumigatus* (Schoustra et al., 2019b). Resistance hotspots—which feature both a habitat for *A. fumigatus* and the presence of azole residues—have now been identified. Moreover, new preliminary population genomic analysis have provided more evidence for a link between environmental resistance and clinical resistance (Rhodes et al., 2021).

Verweij presented a timeline of major research and mitigation efforts undertaken to address ARAf in the Netherlands. The first case of a clinical resistant isolate was retrospectively identified and dated back to 1998. Between 2007-2015, research was published on the first case series of azole-resistant invasive aspergillosis (Verweij et al., 2007), the emergence of the TR34 mutation (Snelders et al., 2008), the hypothesis that resistant aspergillosis was associated with environmental fungicide use (Verweij et al., 2009), indication of triazole fungicides inducing cross-resistance to medical triazoles (Snelders et al., 2012), the emergence of the TR46 mutation (van der Linden et al., 2013), and PCR assays to detect resistance (Chong et al., 2015). In 2013, the Netherlands National Institute

for Public Health and the Environment instituted a surveillance network, and five medical centers and five teaching hospitals participate in annual surveyance reporting for this network. In 2015, the Netherlands Ministry of Health awarded Verweij and colleagues a grant to research the implications of resistance, and in 2017 the Netherlands issued a guideline that recommends combination antifungal therapy for all patients suspected of invasive aspergillosis. In 2019, research regarding the mortality of resistant invasive aspergillosis (Lestrade et al., 2019a) and identifying environmental hotspots (Schoustra et al., 2019b) was published. In 2021, a mitigation protocol was implemented in the Netherlands for the first time. Verweij emphasized that it took about 10 years for *A. fumigatus* to develop resistance, yet it took more than twice that time for targeted interventions to be implemented.

Challenges to Implementing Interventions to Address Resistant Aspergillosis

Several challenges contributed to the lengthy timeframe between the identification of azole-resistant invasive aspergillosis and the implementation of interventions, said Verweij. Initially, disbelief in the reports of resistance slowed the research response. He remarked that when he submitted his first research application on this issue, a grant reviewer expressed their incredulity at the possible link between clinical azole-resistance and environmental fungicide use. Additionally, the first time his group's hypothesis was submitted to *The Lancet Infectious Diseases* journal, it was denied publication due to insufficient evidence (it was published two years later after resubmission) (Verweij et al., 2009). Furthermore, Verweij described general feedback that resistant cases were a local problem rather than a national or international issue.

Given that *A. fumigatus* is not a plant pathogen, it was difficult to locate a partner in the agriculture sector. Verweij stated his good fortune in finding collaborators at the Wageningen University who were researching *A. fumigatus* in the environment. Invasive mycosis is not generally perceived as a public health problem, which further complicates research efforts. Although patients with leukemia were initially identified as susceptible to developing mycosis, that susceptibility did not extend to the general public. Now that the risk of resistant infections have broadened to patients with influenza and COVID-19, the public health problem has become more apparent. Moreover, resistance has also developed in other pathogens. Verweij noted that with the exception of the US CDC, most public health institutes do not have a mycotic branch or mycology expertise, and this contributes to the challenge of raising awareness about this public health issue.

Fungal resistance has not been prioritized, and many antimicrobial resistance (AMR) programs exclude it. When fungal resistance is included

in AMR programs, it competes with bacterial resistance, an area with far more researchers. Verweij analyzed infectious disease grants awarded by the Dutch Medical Research Council between 2006–2023, and found that of the 129 projects funded for a total of $66 million, only four of these grants addressed AMR, and a single project addressed fungal resistance. He emphasized that the Netherlands has high fungal resistance rates and low bacterial resistance rates; yet only one project was dedicated to fungal resistance, indicating the challenges in prioritizing this area. Additionally, many stakeholders are involved in this problem, including the Ministries of Health, Agriculture, and Infrastructure; the fungicide authorization board; fungicide producers; fungicide users; medical researchers; and agricultural researchers. With so many stakeholders in this field, leadership can be difficult to establish to move initiatives forward, he remarked.

New Challenges in Research on Resistant Isolates

Verweij described his current research on resistant isolates. Of the nearly 12,000 clinical isolates collected since 1994, approximately 1,800 were azole-resistant, with significant increases since 2008. Moreover, the number of variants is also increasing, with 91 different resistant genotypes identified across the TR34, TR46, and F46Y groups, in addition to isolates with single resistance mutations. This high number of genotypes poses diagnostic challenges. He and colleagues found an isolate in 2021 with TR34/L98H mutations and several single nucleotide polymorphisms (SNP) in the *cyp51A* gene. There are published literature that describe each genetic component: the TR34 genotype is known to confer full resistance to itraconazole, a T289A mutation is known to indicate high resistance to voriconazole and a G448S single point mutation is known to provide resistance to both itraconazole and voriconazole. However, susceptibility testing showed that the isolate was susceptible to itraconazole and resistant to voriconazole and esophogonazole. This example demonstrated how the variety of co-existing SNPs can complicate the process of determining an isolate's resistance phenotype. Available PCR tests detected TR34 and TR46 mutations, but those diagnostics don't detect SNPs and are likely unable to provide full information on the isolate's resistance phenotype (Song et al., 2022).

Managing resistance in *A. fumigatus* involves striking a balance between use and risk assessment, said Verweij. For instance, one might assume that a fungicide with no activity against *A. fumigatus* would be safe for use in the environment, though this fungicide might still have an indirect effect on human health through affecting the microbiome. A fungicide that has activity against *A. fumigatus* but belongs in a class not used in medicine may be problematic if *A. fumigatus* isolates develop resistance to this fungicide, and, as a result, acquire a mutator genotype that increases their probability

of developing more spontaneous mutations (e.g., SNPs that confer azole resistance). The risk for cross-resistance is higher still for a fungicide that features azole compounds but has a different molecular structure. The fungicide could potentially drive SNPs in the *cyp51A* gene, as is the case with imazalil (a diazole fungicide). Verweij remarked that fungicides from the same class as medical antifungal drugs and featuring a similar core structure pose the highest risk of cross-resistance. He added that this question of balance is relevant in regard to dihydro-orotate dehydrogenase (DHODH) inhibitors (see Figure 8-1). Currently, olorofim is being developed for clinical use and ipflufenoquin simultaneously has been authorized for use in the environment. Given that both of these products are DHODH inhibitors, potential for the development of cross-resistance is present.

GENOMIC SURVEILLANCE AND EPIDEMIOLOGY

Brewer presented findings from U.S. surveillance studies of *A. fumigatus* in the environment. Conducted in settings where azoles are used, these studies aimed to identify hotspots—i.e., locations where resistance is likely to be developing or is already abundant—in order to target mitigation strategies. Multi-fungicide-resistant isolates were identified in a variety of agricultural settings. Genomics analysis was applied to isolates collected from different settings and uncovered genotypes that were present in both

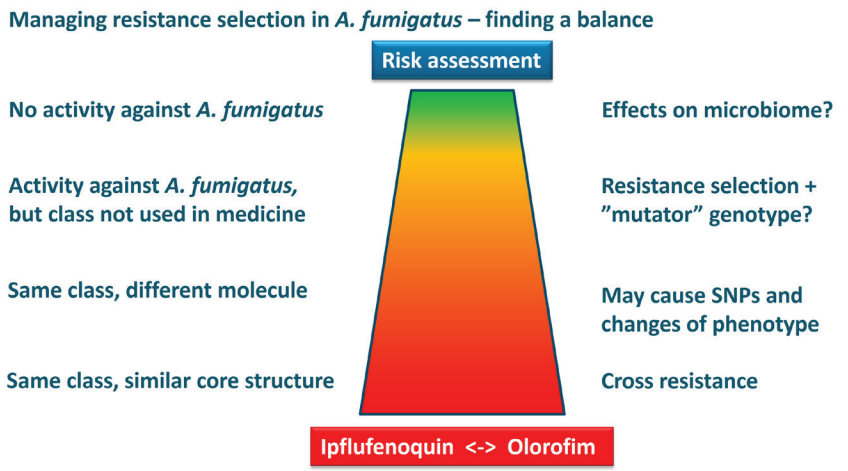

FIGURE 8-1 Managing resistance selection in *A. fumigatus*.
NOTE: SNP = single nucleotide polymorphisms.
SOURCE: Verweij presentation, June 27, 2022.

the environment and in the clinic. Genomic surveillance is a tool for gaining a better understanding of ARAf transmission from their origin site to the patient, she explained.

Evidence Supporting the Agricultural Origin of Clinical Pan-Azole Resistance

Cases of azole-naïve patients acquiring pan-azole resistant infections indicate that ARAf infections in humans come from the environment, said Brewer. Isolates from the environment and isolates from the clinic share resistance mechanisms—the most common of which are TR34 and TR46 mutations—that have been detected around the world. Additionally, human and environmental ARAf isolates share multilocus genotypes and nearly identical whole-genome sequencing genotypes (Burks et al., 2021). Thus, evidence that these infections come from the environment is strong, but the patterns of ARAf movement are not yet known, Brewer remarked. In addition, questions remain about whether azoles used in wood preservation contribute to resistance. Likewise, the role of topical azole residues in resistance development remains unknown. After such residues enter waterways, they could become part of biosludge that is applied in the environment.

In an effort to determine the movement of ARAf isolates from agricultural environments into patients, Brewer and colleagues conducted surveillance in the United States. Prior to their work, the only identified azole-resistant isolates were found in peanut crop waste debris in Georgia. They collected over 700 isolates from 50 agricultural sites in Georgia and Florida where azoles are heavily used. These locations included soil and plant debris from fields and orchards growing peanut, grape, pecan, apple, strawberry, tomato, and orange crops, in addition to compost and pecan-processing debris. After screening, they conducted broth microdilution assays to determine the MIC of 172 of the 700 isolates, testing them against tebuconazole, itraconazole, voriconazole, and posaconazole. They found pan-azole resistance in 12 of the isolates. By sequencing the *cyp51* gene, the TR46 allele was found in all 12 pan-azole resistant isolates. She noted that the samples came from compost or pecan debris, but the peanut production debris they anticipated would be a hotspot did not yield any pan-azole resistant samples.

Brewer sought to determine whether the resistant isolates found in patients featured a signature that could be found in isolates from agricultural sites. She posited that the presence of resistance to multiple fungicide classes would indicate that *A. fumigatus* are adapting in agricultural environments. To ascertain whether azole-resistant clinical and agricultural isolates are resistant to other classes of fungicides, these isolates were tested against quinone outside inhibitor (QoI) fungicides, also known as strobilurins. This class of fungicides is not used to treat humans and the

G143A mutation in cytochrome B is known to be associated with QoI resistance, making it easily identifiable in both plant pathogenic fungi and *A. fumigatus*. Genome sequencing was also performed on environmental isolates from the research team's collected samples and on publicly available environmental and human isolates. Researchers scanned for QoI resistance and for resistance to additional fungicide classes, the benzimidazoles and the succinate dehydrogenase inhibitors (SDHI). She emphasized that a single gene can feature multiple signatures of resistance.

Multi-Fungicide Resistance

Using a representative wildtype isolate—which is sensitive to QoI, benzimidazole, and SDHI—for comparison, approximately 120 isolates containing either the TR34 or TR46 allele were sequenced. Derived from humans or from the environment in the United States, the Netherlands, and India, many of these isolates were found to have the G143A allele associated with QoI resistance. All of the isolates tested had the F219Y allele, indicating resistance to benzimidazoles. A minority of the isolates contained H270Y, the genomic signature of SDHI resistance. Furthermore, the phenotype of these isolates matched the genotype for resistance to these various fungicide classes. Thus, pan-azole resistant isolates were found to be multi-fungicide resistant, Brewer stated.

To determine how the *A. fumigatus* isolates were evolving, whole genome sequences of isolates were used to construct a phylogenetic tree. The isolates did not necessarily cluster according to their collection source (e.g., environmental or clinical) (Kang et al., 2022) (see Figure 8-2). Instead, the pan-azole resistant isolates with TR34 or TR46 alleles clustered together into a single clade. Moreover, all of the multi-fungicide-resistant isolates clustered within this same clade. Brewer described another pattern in the pan-azole resistant isolate phenotypes: most of the pan-azole resistant isolates were resistant to benzimidazoles, many of them were resistant to QoIs, and a few were resistant to SDHI, a progression that follows the chronology of the introduction of these fungicide classes. The longer a class had been in use in the environment, the higher the proportion of resistant fungal isolates were detected. Thus, evidence supporting an agricultural origin of pan-azole resistance includes (1) the presence of multi-fungicide-resistant *A. fumigatus* in both the environment and the clinic, (2) the discovery of geographically widespread multi-fungicide-resistant isolates across the world, (3) the phylogenetic clustering of clinical and agricultural isolates to a single clade, and (4) the presence of isolates with fungicide resistance markers found in patients, said Brewer. She added that resistance to multiple classes of fungicides may complicate any efforts to preserve the azoles for clinical use. Minimizing the use of agricultural azoles by more exclusively using

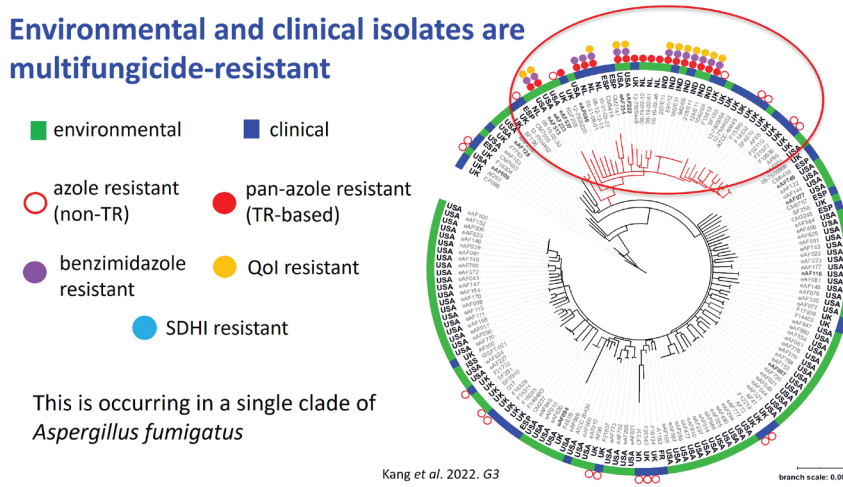

FIGURE 8-2 Multifungicide-resistant environmental and clinical isolates in a single clade of *A. fumigatus*.
NOTE: QoI = quinone outside inhibitor; SDHI = succinate dehydrogenase inhibitor; TR = tandem repeat.
SOURCE: Brewer presentation, June 27, 2022; adapted from Kang et al., 2022.

other fungicide classes may not successfully preserve azole therapies, as other classes may still indirectly select for azole-resistant isolates.

Broadened Surveillance Efforts

Brewer and colleagues extended surveillance efforts to additional regions and crops to determine whether hotspots can be found across the United States. In 2018 and 2019, soil and plant debris were sampled from 52 sites from 8 states along the East and West Coasts. Sampled crops included tulip, hemp, wheat, apple, grape, herbs, flowers, brassica, cucurbit, peanut, peach, corn, and soybean. Compost was also tested, with samples taken from an organic farm to compare with samples from farms using fungicides. The resulting 727 isolates were screened and MICs were determined using broth microdilution assays. A greater range of phenotypes was found compared with the previous surveillance study performed in Georgia and Florida. Additionally, they identified isolates with mid-range MICs that did not carry *cyp51* mutations associated with resistance. Approximately 20 isolates were pan-azole resistant and contained the TR34 and TR46 alleles. These came from grape, wheat, herbs, peach, tulip, and compost, with the majority coming from the latter two categories. As of June 2022, the phylogeny analyses

are still in progress. Brewer noted that one of the tulip fields sampled was organic, but all bulbs were imported from the Netherlands, indicating a potential method of global movement of resistant isolates.

Azole Resistance in Food Supply and Plant-Based Retail Products

Another research effort investigated the possibility of human exposure to azoles used on crops via food and garden products by testing food and plant-based retail products for ARAf, said Brewer. Samples from grocery store produce included grapes, almond, peanuts, pecan, and apple. Compost, soil, and flower bulbs were selected for home and garden product sampling due to the high amounts of azoles typically used on these elements. Most of the products sampled originated in the United States, with some of the gardening supplies coming from other countries. Over 500 *A. fumigatus* isolates were identified, with the majority found in peanuts, compost, flower bulbs, and soil. All *A. fumigatus* isolates were screened on selective media, and 130 isolates were tested for MIC levels. The majority of isolates from compost and flower bulbs were found to have pan-azole resistance. Approximately 47 percent of the 130 isolates were azole-sensitive, 18 percent were resistant only to tebuconazole (an azole fungicide), 6 percent were resistant to one type of medical azole, and 29 percent were resistant to tebuconazle and more than one medical azole.

Sequencing the *cyp51A* gene identified TR34 or TR46 alleles in nearly a quarter of these isolates, Brewer explained. However, the remainder of the 130 isolates featured a variety of *cyp51A* genotypes. Some isolates carried the wildtype version of the gene, while others had alleles not shared by any other isolates in the samples. The most common genotype—found in 40 percent of the isolates—contained Y46F/V172M/T248N/E2555D/K427E alleles. Brewer referred to this genotype as "type 3." In investigating the association of resistant phenotypes and *cyp51A* genotypes, the majority of pan-azole-resistant isolates had the TR34 or TR46 allele. The "type 3" genotype was featured in just 5 of the pan-azole resistant isolates, but also in the majority of azole-sensitive isolates. Therefore, the "type 3" genotype on its own does not underlie resistance, and there must be another mechanism involved in conferring the azole resistance, Brewer remarked. She added that most of the pan-azole-resistant isolates with TR alleles were from compost or tulips, with a single such isolate obtained from raw peanut. Very low levels of *A. fumigatus* were found on peanuts that had been roasted or salted.

Genotyping was used to compare isolates from retail products, field samples, and clinical samples. Brewer noted that none of the clinical isolates were azole-resistant. Multivariate analyses and k-mer analysis revealed six clusters of isolates, four of which were fairly similar to one another and the other two being outliers. Azole-resistance was lowest in the four similar

clusters, with 87 percent of these isolates indicating azole susceptibility. The clinical isolates were spread across the four similar clusters. One of the outlier clusters was comprised of isolates from retail products, 61 percent of which were azole-susceptible. The other outlier cluster was comprised of isolates from compost, flower bulbs, peanuts, and pecans. All of these isolates were azole-resistant or pan-azole-resistant, and they all featured the TR34 or TR46 allele.

Brewer reiterated that pan-azole resistance was found in commercial peanuts, soil, compost, and flower bulbs, primarily in compost and bulbs. Although most of the pan-resistant isolates had TR34 or TR46 alleles, other alleles were also identified that are not associated with azole resistance. Non-*cyp51A*-based mechanisms of resistance warrant further investigation. *A. fumigatus* populations in the United States appear to cluster genetically based on their pan-azole-resistant phenotype, not by whether they are derived from the clinic or environment. Of the food and garden supplies sampled, lawn and garden products contain the most pan-azole resistant isolates. Brewer remarked that these products thereby pose the greatest risk for people who are at risk of aspergillosis. Her current research involves azole residue profiling on substrates with high levels of ARAf to determine associations between azole residues and hotspots. Genotyping will be performed on fungal isolates from agricultural samples and on publicly available clinical samples (Etienne et al., 2021) to identify common genotypes present in both the environment and clinic in the United States. This research will aid in identifying hotspots for targeting mitigation efforts.

DIAGNOSTICS, RESISTANCE TESTING, AND SURVEILLANCE CAPABILITIES IN HEALTH CARE AND AGRICULTURAL SYSTEMS

Lockhart described the limited efforts to detect antifungal resistant infections in the United States and associated the country's low rate of resistant *Aspergillus* infections with a lack of detection, rather than absence of ARAf in clinical settings. He outlined the lack of antifungal susceptibility diagnostic products and the low number of laboratory facilities that process these tests. He also discussed the need for more antifungal resistance surveillance and greater communication and collaboration between various sectors.

Fungal Infection Diagnostics

Lockhart remarked that aspergillosis is a serious problem that often goes unrecognized. Before addressing antifungal resistance, fungal infections must be detected in patients. Autopsy studies indicate that aspergillosis is among the most common missed diagnoses among patients in intensive care units

(Winters et al., 2012), underscoring the need for better diagnostics to detect fungal infections in general and *Aspergillus* infections specifically. Delays in diagnosing an *Aspergillus* infection can reduce the likelihood that antifungal treatment will be effective. Prompt diagnosis requires the development of point-of-care tests and affordable tests for use in resource-limited settings. He noted that some countries do not have a single laboratory with the capacity to diagnose fungal infections. Furthermore, the problem of resistant antifungal infections is expected to grow.

Since azoles drugs came into use in the 1990s, resistance has been periodically observed in patients on long-term antifungal therapy, with rare cases observed in patients with severe acute illness (CDC, 2021b). Many different mutations have been identified in these infections, which are rarely transmissible. He added that repeated spraying of azole fungicides in agricultural fields leads to repeated selection for *Aspergillus* resistance, raising the likelihood that resistance rate will increase over time. Thus, the absence of resistance at this current point in time does not signify that resistance will not be detected 5-10 years later.

In contrast to Europe, where up to 20 percent of *Aspergillus* infections in hospitals are resistant (Fuhren et al., 2015), only a small number of cases have been detected in the United States. Lockhart associated this low case rate with the limited efforts to detect cases. In 2010, the first resistant *A. fumigatus* isolates with TR34 and TR46 mutations were identified in the United States (Wiederhold et al., 2016). In 2018, CDC published a report of TR34-carrying *A. fumigatus* isolates found in seven U.S. patients (Beer et al., 2018), leading the agency to include ARAf on the antibiotic resistance watch list (CDC, 2019). Lockhart highlighted a persisting general misconception that a low number of identified U.S. cases signifies that resistant *A. fumigatus* is not a pressing issue in this country. In reality, the 16 human cases of ARAf with TR34 or TR46 alleles identified in the United States to date likely reflects a small percentage of the true scope of the pathogen, he said.

Antifungal Susceptibility Testing

Lack of laboratory participation and susceptibility testing products are contributing to low levels of antifungal susceptibility testing (AFST) conducted in the United States, Lockhart remarked. In order for a laboratory to perform AFST, it must meet regulations that include demonstration of proficiency demonstration. The College of American Pathologists is the only U.S. group that evaluates commercial proficiency for AFST. Only a tenth of the approximately 4,000 laboratories in the United States participate in the proficiency demonstration. Moreover, the majority of AFST conducted by these 400 laboratories is on yeast, not on mold species such as *Aspergillus*.

Currently, less than a dozen U.S. laboratories perform AFST on molds, with the majority of isolates being sent to three commercial laboratories located in Minnesota, Utah, and Texas. As a result of the low number and proximity of laboratories, the turnaround time for AFST results is typically two weeks. Moreover, only one product is available in the United States for general mold susceptibility testing. This gradient diffusion product is sold commercially, but it is not licensed for mold susceptibility testing. Lockhart remarked that no licensed mold susceptibility test products approved by the U.S. Food and Drug Administration are available in the United States.

Lockhart stated that many medical professionals do not view this lack of diagnostics as a problem. Two mold-active antifungal classes—azoles and polyenes—are available for patient treatment; thus, a patient who does not respond to one of these drugs can be prescribed the other without waiting for AFST results. He remarked that the available therapies may soon widen to five different classes of mold-active antifungals. Both a DHODH inhibitor and a Gwt1 inhibitor have been developed, and tetrazoles have demonstrated some efficacy against ARAf. Particularly in the case of molds, AFST is required to determine the best antifungal therapy available to guide medical professionals in providing the best treatment options to their patients. Thus, expanded AFST capacity would better inform treatment options in a timely fashion.

A commercially available, agar-based screening assay enables detection of azole resistance in *A. fumigatus*. Lockhart described this product as an effective screening tool, but not a replacement for AFST. He noted that the European Commission for Antifungal Susceptibility Testing recommends the product for screening only, not for resistance testing (Guinea et al., 2019). The assay is being utilized in the United States and has been incorporated into CDC's Antimicrobial Resistance Laboratory Network (ARLN), a group of seven participating laboratories across the country that screen for bacterial and fungal resistance. *A. fumigatus* samples can be sent to two of these laboratories, located in Tennessee and Maryland, where they are prescreened for antifungal resistance using agar plates, and broth microdilution can be performed.

Antifungal Resistance Surveillance

The screening conducted by ARLN is the only screening for molds currently taking place in the United States. Lockhart remarked that a problem cannot be solved without understanding it, so learning the extent of antifungal resistance will require more surveillance. ARAf has been identified in the United States (Hurst et al., 2017). While Brewer's research demonstrated that ARAf is present in multiple types of crops in various parts of the country (Kang et al., 2022), environmental surveillance efforts to screen

for resistance related to specific pathogens have thus far been limited, said Lockhart. He added that close collaboration between agricultural and clinical groups could enable progress toward shared environmental surveillance objectives by combining resources and sharing specimens. Highlighting Brewer's research, he emphasized that azole-resistant strains indicating cross-resistance to other fungicide classes have been identified in humans (Kang et al., 2020). Similarly, in his own research, Lockhart found that azole-resistant yeast isolates taken from humans in 2010 were resistant to other fungicide classes (Pfaller et al., 2012). He pointed out earlier research that revealed an azole-resistant yeast isolate from the bloodstream is more likely to also be resistant to echinocandins and posited that a "mutator phenotype" could predispose an isolate to be resistant to both echinocandins and azoles.

Communication in the Mycology Research Community

The similarities in antifungal resistance in plants and in humans warrant communication between mycologists who study plants and those who study human fungal infections, said Lockhart. Findings from each of these communities are often published in journals typically only read by that group. Furthermore, most meetings invite members from the plant or human side of mycology, but not from both. However, these scientific communities are focusing on some of the same questions, and communication could foster deeper understanding. He highlighted the *Journal of Fungi,* which was launched in 2015 to help to address this need for communication by publishing articles on any fungal problem, be it related to human pathogenic fungi, plant pathogenic fungi, or environmental fungi. This journal draws an audience of various mycology specialties, helping readers access new findings they might otherwise be unaware of. However, further efforts are needed to connect the different branches of mycologists. CDC is working to enhance collaboration by engaging in bimonthly meetings with the U.S. Environmental Protection Agency and the U.S. Department of Agriculture. These meetings enable the agencies to identify shared problems and collectively work toward solutions. For example, ipflufenoquin—a DHODH inhibitor—has been approved for use as a fungicide on plants. Meanwhile, another DHODH inhibitor, olorofim, is in phase III clinical trials. No crosstalk has been taking place about the potential for development of resistance, Lockhart remarked. He added that the agricultural and human health communities should be discussing this potential problem now, before resistance emerges in the field. Likewise, communication and collaboration between plant and human health professionals can help generate solutions to the threat of antifungal resistance.

INTERNATIONAL ONE HEALTH APPROACH TO ANTIMICROBIAL RESISTANCE AND PATHOGEN SURVEILLANCE

Pinto Ferreira described international guidance and data platforms for AMR surveillance efforts. He outlined how the platforms address privacy considerations and the expected timeline for implementation.

International Surveillance Guidance

Pinto Ferreira highlighted World Health Organization (WHO) AMR surveillance guidelines for resistance in foodborne bacteria, noting that international guidance avoid being overly prescriptive to allow flexibility for different countries to meet their varying needs (WHO, 2017). He added that this guidance is focused on antibiotics and antibacterial products, with no mention of antifungals as part of the antimicrobial entities to be included in integrated surveillance efforts. Since 2021, FAO and WHO have issued additional guidance documents for the monitoring and surveillance of foodborne AMR (FAO and WHO, 2021, 2022). Given the recent nature of their release, implementation of this guidance is still taking shape. The Republic of Korea has funded an FAO initiative—the AMR Codex Texts (ACT) project—to support the implementation of these international integrated surveillance guidelines at the field level. The ACT project, which is currently being conducted in Bolivia, Cambodia, Columbia, Mongolia, Nepal, and Pakistan, will assess the use and impact of Codex standards related to AMR.[1]

Antimicrobial Use and Resistance Data Platforms

At the international level, the Tripartite Integrated Surveillance System for Antimicrobial Resistance and Antimicrobial Use (TISSA) platform is currently being developed for displaying AMR-related data. Pinto Ferreira noted that given that the former Tripartite Joint Secretariat on AMR officially became a quadripartite in March 2022, TISSA could potentially face a future name change. The TISSA platform will integrate data from WHO, FAO, and the World Organisation for Animal Health (WOAH). WHO will collect data on humans related to both AMR and antimicrobial use (AMU), as well as some environmental and food data. WOAH (formerly known as OIE until May 2022) will collect antimicrobial use data in animals. Pinto Ferreira remarked that with WOAH providing AMU in animals data and WHO providing data on human AMU and AMR, a data gap remains for AMR in animals and food and AMU in plant production and protection.

[1] More information about the ACT project is available at https://www.fao.org/antimicrobial-resistance/projects/ongoing/project-10/en/ (accessed August 23, 2022).

To fill this data gap, FAO is simultaneously developing the International FAO Antimicrobial Resistance Monitoring (InFARM) platform, which will link with TISSA. Although concurrently developing two platforms provides challenges, Pinto Ferreira noted that the process has advantages and progress is well underway, with invitations to countries to participate in the platform's pilot program forthcoming. Obtaining AMR and AMU data is part of FAO's strategic framework (FAO, 2021b). The Monitoring and Evaluation of the Global Action Plan on Antimicrobial Resistance includes an indicator that countries use the FAO platform to report "levels and trends in sales or use of pesticides for the purpose of controlling bacterial or fungal disease in plant production" (WHO et al., 2019).

Privacy Considerations

The InFARM platform will collect AMR and AMU data from countries and report it via the annual FAO report and the TISSA platform. Pinto Ferreira stated that resistance reporting is highly sensitive, given that it can impact a nation's trade activity. To help mitigate reservations governments may have about sharing resistance data, InFARM will include three reporting levels. The first level is private, and this data will only be viewable by the country it pertains to. Another level contains public data that are geographically aggregated and reported by region or subregion, rather than by country, to protect each country's identity. A third level is fully transparent and public, with data identifiable by country. Governments may select from these three data reporting privacy levels. In addition, InFARM will automatically generate reports and graphics to aid countries in visualizing the data being collected.

Data Platform Timeline

Development of the InFARM platform will continue through 2022, with simultaneous collection of AMR data from animals and food. In 2023, a global roll out of InFARM will take place through annual open calls for data. Additionally, FAO will contribute data to TISSA. By 2024, data from additional AMR and AMU surveillance programs on plant production and protection will be included in InFARM. Pinto Ferreira highlighted that FAO is already collecting information regarding agricultural fungicide use reported by countries on a voluntary basis.[2] He remarked that data contributions are inconsistent and can vary depending on a country's economic, human, and technical resources. Furthermore, these data do not include the

[2] Fungicide use data collected by FAO is available at https://www.fao.org/faostat/en/#search/fungicides (accessed August 23, 2022).

purpose for fungicide use. Although this database does not offer granularity, its data are public and can be downloaded and printed. He described the current website as a starting point for the development of the new platforms that will offer greater functionality.

ONE HEALTH ASPECTS OF CIRCULARITY

Zwaan provided an overview of the One Health Aspects of Circularity project. The use of chemicals to combat bacteria, insects, or fungi ultimately faces the development of resistance in the targeted population of organisms. He remarked that good stewardship and prudent use of these compounds are first and foremost in addressing potential emerging health risks from different organisms. In the Netherlands and in other countries in Europe and around the world, current agricultural practices involve high input of chemicals, wasted resources, and a fairly linear process. Creating a more circular system in which plant waste is composted and used in future crop cycles has benefits, but current chemical usage within this system poses risks.

Zwaan described this type of circular system within the context of the flower bulb industry. Azoles are used to combat fungal plant pathogens; the fungicide residue then accumulates in plant waste. *A. fumigatus* plays a role in the plant waste composting process, but the presence of azole residue selects for the fungus to develop resistance in order to survive. *Aspergillus* produces plentiful spores that can cause disease when inhaled by humans. The similarities in the azoles used in agriculture and in medicine lead to treatment challenges because *A. fumigatus* with acquired resistance in the environment may not respond to medical therapy. Zwaan stated that combining the use of chemicals and microbes within the process of circular agriculture poses risk for the emergence of One Health problems.

Agricultural Antifungal Resistance

Multiple factors relevant to the development of AMR should be considered in research, including resistance mechanisms, ecological settings, biology of the species, and the nature of the selection pressure, Zwaan explained. The One Health Aspects of Circularity project is currently at its midway point. The project initially focused on the bulb industry in the Netherlands due to the established knowledge base about the growth of ARAf in bulb plant waste. Using the bulb industry as a case study, the project aims to identify (1) other sectors in which plant waste contributes to the development of resistance and (2) interventions to prevent the growth and spread of azole-resistant *A. fumigatus*. Zwaan highlighted recent research funded by the Netherlands Ministry of Health to

investigate potential resistance hotspots. *A. fumigatus* spores resistant to both itraconazole, a medical therapy, and tebuconazole, an agricultural product, were found in wood chips, strawberry, onion, potato, and other crops. A subset of samples was tested for resistance mechanism identification, and TR34 and TR46 mutations in combination with point mutations were detected similar to those reported in clinical azole-resistant isolates. These findings suggest that the issue of resistance extends beyond the bulb industry and the Netherlands, warranting collaborative efforts from multiple nations.

In an effort to better understand how resistant spores spread, Zwaan conducted air sampling around plant waste heaps. A machine that actively samples the air was used to collect samples before and after the turning of waste heaps. No spores were captured in samples taken before the heaps were turned. During the process of being turned, the heaps released profusive amounts of spores. After the turning, the spore count returned to very low levels. Zwaan stated that this finding indicates that avoiding disturbance to waste heaps could reduce the risk of ARAf spores being released into the air. He added that the concept of circularity offers benefits in reusing waste material to return nutrients to the soil, and different management techniques could decrease associated risks.

Noting inspiration from the community science research conducted by Jennifer Shelton and Matthew Fisher at Imperial College London, Zwaan described air sampling surveillance efforts his team is developing. They created a sampling method in which a PCR seal (i.e., a thin film with an adhesive side) is exposed to the air for a determined length of time at a sampling site, then collected, trimmed to fit inside a petri dish, covered with agar, and incubated to recover fungal isolates that grow from spores collected on the film. Zwaan noted they are in the process of determining the best approach to conducting systematic community science sampling throughout the Netherlands. Data on the presence and location of resistant spores will contribute to understanding their origin.

Linking the Environment to the Clinic

Zwaan emphasized that learning how long it takes for an ARAf spore to travel from its origin to a potential human host could contribute to understanding how to disrupt the spore's route. He and colleagues have investigated samples collected from flower bulb waste heaps in three different locations from 2016–2019 that include both susceptible and resistant fungal isolates. They compared these with clinical samples collected from three medical facilities in Amsterdam during roughly the same time period. Zwaan noted the similarities in the genotypes identified from the clinical and environmental samples, suggesting a link between the fungal isolates

recovered in these two settings. Efforts to identify specific links between patients and environmental isolates are ongoing.

Research Considerations

Zwaan summarized that resistance hotspots exist outside of the flower bulb sector, and organic waste treatment affects the growth of *A. fumigatus*. He added that hotspots will continue to be a focus in understanding how resistance develops and pointed out that little is known about the ecological niches of *A. fumigatus* in settings where it has no contact with azoles and other chemicals. The community science approach could potentially contribute data to help answer this question. Whole genome sequencing analysis can then be used to explore whether there is a causal link between resistance in the environment and in clinical settings. Zwaan posited that the ability to prevent resistance from developing seems unlikely in light of the discovery that resistant *A. fumigatus* spores appear to be ubiquitously present. Therefore, his current research target is the prevention of transmission. Moreover, he believed that the necessary development of new drug treatments for fungal diseases in humans should be coupled with efforts to ensure that new chemicals used in medicine are not also used in agriculture.

DISCUSSION

Potential Interventions and Policy Changes

McKenna asked the participants to provide interventions or policy considerations they view as priorities in addressing antifungal resistance. Verweij replied that, like Zwaan, he too has come to the realization that preventing resistance from emerging is unlikely. Numerous experts have indicated that the same modes of action should not be used in the environment and in patients, and yet this practice continues. Therefore, efforts to identify hotspots and apply interventions to turn them into coldspots are needed to prevent transmission from occurring. Pinto Ferreira stated that the ideal scenario would be different sectors avoiding use of the same molecules; however, given that the ideal does not always transpire, collaborative surveillance data collection should be a focus. Varying recommendations from the human, animal, and plant perspectives can be difficult for governmental stakeholders to navigate. He remarked that collaboratively generating integrated One Health policy recommendations for managing molecules would facilitate policymaking at the country level.

Brewer remarked that an integrated disease management approach can minimize the overall amount of fungicide in the environment. This approach includes multiple strategies, such as using fungicides for disease

control but not for plant growth promotion. Additionally, crops can be bred for resistance to decrease reliance on chemical inputs. Lockhart commented that integrated crop management has not been a focus for addressing azole usage, and communication is needed between the clinical and agricultural sectors to encourage the prioritization of these approaches. Food security and disease management are equally important to public health, and threats to these areas are global problems requiring collaborative problem-solving, said Lockhart. Zwaan replied that the Dutch government is considering control measure protocols to address resistance, because having mechanisms in place to enforce protocols can be more impactful than mere recommendations. Stewardship practices to utilize alternative approaches and limit chemical use to specific circumstances can also be beneficial. Zwaan described how the Dutch government formerly ran a knowledge center that advised farmers on issues such as chemical use, providing them with an information source other than the companies selling the products and systems. Such consultation services, stewardship practices, and approaches to plant waste could be helpful in decreasing the spread of resistant fungi.

Resistance and Fitness Cost

Kent Kester, vice chair of the Forum on Microbial Threats, asked about the fitness of resistant environmental isolates. Often, antibiotic-resistant bacteria are not as fit as typical strains, and when antibiotics are withdrawn and selective pressure is reduced, the wildtype or susceptible strains repopulate. He questioned whether a similar phenomenon occurs in antifungal resistant organisms. Brewer replied that much is known about the fitness of plant pathogenic fungi resistant to antifungals. For instance, resistance to benzimidazoles or QoIs generally does not carry a fitness cost. Additionally, benzimidazole-resistant isolates from pecan pathogens have been found in locations where benzimidazoles are no longer used. A fitness cost has been seen in relation to triazole resistance. She remarked that she is currently investigating whether resistance to triazoles carries a fitness cost for *A. fumigatus* under different environmental conditions. Her team has considered whether a strategy of reducing azole use could have results similar to those Kester described in bacteria. However, multi-fungicide resistance may enable continued selection for azole-resistant isolates in the presence of other single site fungicides. Brewer added that this is yet to be determined, and that although current studies do not indicate fitness costs in isolates from humans or in mouse models, additional research is needed to determine fitness costs in various environmental settings. Zwaan remarked that he has found little evidence of fitness cost related to resistance, which may be related to compensatory mutations in the strains. He noted that

the fungi being investigated are in a food-rich environment that could prevent fitness cost. Researching fungi in stressful conditions is needed to determine whether this factors into fitness cost. His group is also examining whether *A. fumigatus* is more susceptible to pressure from competing fungi or bacteria.

Farm Worker Safety

McKenna asked about farm worker safety issues related to resistant fungi. Verweij replied that it is not yet known whether a worker in a field is at a higher risk of developing a resistant infection than the general population. Researching the occupations or residence locations of patients who develop resistant infections is a potential method of assessing associated risk level, but privacy issues complicate this type of research.

Food Security Considerations

Given that agricultural triazoles provide high yield stabilization and food security, McKenna asked about the balance in preserving food security while monitoring resistance. Brewer replied that fungi can cause 20 percent yield losses in crop production, and therefore managing fungal diseases is important to food security. Furthermore, azoles can contribute to food safety by reducing mycotoxin production in wheat with Fusarium head blight, as noted by Pierce Paul in Chapter 5. Reducing the input of fungicides and utilizing integrated pest management are approaches to limiting use of these chemicals to situations where they are necessary to save crops, thus achieving balance between clinical issues and plant pathogen control. Brewer added that overuse of azoles can also lead to the development of resistance in plant pathogens. Awareness efforts are needed to inform growers that minimal, effective triazole practices can avoid resistance in plant pathogenic fungi as well as helping to reduce human pathogen risk. Pinto Ferreira noted that in the past, discussion of ending the use of growth promotion in animal production featured disputes that growth promotion was required to generate adequate animal protein. He remarked that economic data indicate that producing adequate animal protein is possible in the absence of growth promotion. He questioned whether economic data are available to indicate what effect ending azole usage in plant production could have on food security.

Addressing Potential Overlapping Use in Agriculture and Medicine

McKenna asked how the potential use of DHODH inhibitors in medicine and in agriculture should be approached to avoid repeating more resistant fungal pathogens. Verweij replied that risk assessment is the first step.

This involves determining whether the agricultural and clinical targets are the same, the binding affinity of the chemical compound to each target, and the spectrum of activity against *A. fumigatus*. Similar molecular structure of the targets will pose a high risk for resistance selection, thus the level of similarity needs to be determined. Additionally, the entrance of a new class into the fungicide arena increases the likelihood that other new compounds in this class will emerge, each of these may have a closer molecular structure to that of the human medication olorofim. The risk of such developments needs to be investigated, said Verweij. Zwaan added that microbes tend to eventually overcome the chemical. This dynamic drives the development of new chemicals for applications in agriculture and other fields. Lockhart added that this situation provides an opportunity for the fields of agriculture and medicine to collaboratively create a surveillance system and share samples to detect any resistance before it takes hold. Brewer remarked that ipflufenoquin will soon be used on crops in spite of the possibility that it shares the same target with olorofim. She suggested that, moving forward, a system should be put into place to avoid approval of chemicals that will be used in both agriculture and medicine. A system could be implemented for investigating the potential for cross-resistance in new compounds. When cross-resistance is likely, compounds could be regulated for use in food production, but not for ornamentals and landscaping.

9

Antifungal Drug Development and Stewardship in Health Care

The eighth session of the workshop focused on the role of resistance in driving a continued need for new antifungal drugs featuring novel modes of action and challenges inherent in antifungal discovery and development. Maryn McKenna, senior writer at *WIRED* and Senior Fellow in Health Narrative and Communication at the Emory University Center for the Study of Human Health, moderated the session. David Andes, William A. Craig Professor and chief of the Division of Infectious Diseases and Medical Microbiology and Immunology at the University of Wisconsin, provided an overview of the current antifungal armamentarium, its gaps, and the status of development efforts underway for new antifungal treatments. John Rex, chief medical officer of F2G Ltd, discussed the challenges in developing new antifungal drugs—particularly in terms of the time and expense involved—and incentive funding structures to encourage antimicrobial development activity.

NEW ANTIFUNGAL DRUGS IN DEVELOPMENT

Andes discussed currently approved antifungals, gaps in the antifungal armamentarium, progress toward discovery and development of new antifungals, and the status of research on new antifungal treatments moving toward clinical use. Over 1 billion fungal infections occur annually around the world, resulting in 1.6 million deaths (Pianalto and Alspaugh, 2016). The fungal diseases with the greatest number of estimated cases per year worldwide are cryptococcosis, candidiasis, pneumocystis, and aspergillosis (see Figure 9-1).

Fungal disease	Estimated cases per year	Estimated mortality
Cryptococcosis	>1,000,000	20%–70%
Candidiasis	>400,000	10%–75%
Aspergillosis	>200,000	30%–95%
Mucormycosis	>11,000	30%–90%
Blastomycosis	~3000	<2%–68%
Coccidioidomycosis	~20,000	<1%–70%
Histoplasmosis	~25,000	28%–50%
Paracoccidioidomycosis	~4000	5%–27%
Penicilliosis	>8000	2%–75%
Pneumocystis	>400,000	20%–80%

FIGURE 9-1 Estimated cases per year and estimated mortality of fungal diseases worldwide.
SOURCE: Andes presentation, June 27, 2022; adapted from (Pianalto and Alspaugh, 2016).

Current Antifungal Therapies

The current armamentarium of antifungals to treat invasive fungal infections is limited to three classes: polyenes, triazoles, and echinocandins. Andes explained that these three classes primarily target two sites in the fungus. Polyenes and triazoles both target ergosterol: polyenes bind and sequester ergosterol, while triazoles inhibit a step in its synthesis. Echinocandins comprise the most recent mechanistic class to enter clinical use, and these drugs inhibit the production of a fungal-specific component of the cell wall, 1,3-ß-D-glucane.

Andes emphasized the difficulty in identifying compounds that kill fungi without hurting mammalian hosts. Polyenes target ergosterol, the primary sterol in fungi, which is structurally similar to cholesterol, the primary sterol in humans. This similarity can cause off-target toxicities including renal insufficiency that can become life-threatening. Likewise, triazoles bind to CYP51A, an enzyme that is structurally similar to mammalian metabolic enzymes. Triazoles can cause off-target toxicities, which can result in limited treatment options and drug interactions in hospital patients.

Antifungal Therapy Gaps

The emergence of resistance among *Candida* species is concerning, said Andes, given the high mortality rates associated with triazole-resistant invasive candidiasis and aspergillosis (Alexander et al., 2013; Wiederhold and Verweij, 2020). Moreover, mold pathogens are emerging that feature intrinsic resistance to the current treatments, including species of *Fusarium*, *Scedosporium,* and Mucorales. The current antifungal armamentarium has pharmacokinetic liabilities. Many of the fungi that affect human health can be disseminated to different parts of the body. Andes remarked that fungal infections commonly disseminate to the central nervous system (CNS) or inside the eye, and very few antifungals can penetrate those infection sites at therapeutic levels. Fluconazole can accumulate in the CNS or eye at 80 percent penetration, flucytosine can achieve 75 percent, and voriconazole can reach 60 percent (Nett and Andes, 2016). All other currently available antifungals have CNS or eye accumulation rates below 1 percent.

Antifungal Discovery and Development

All antifungals have shortcomings that the field of antifungal discovery is working to overcome, said Andes. For instance, the azoles have toxicity and drug interaction considerations. Echinocandins are only available in intravenous delivery formulations. Antifungals can have limited pharmacokinetic distribution and resistant pathogens are emerging. He remarked that the holy grail in antifungal discovery and development is a novel mechanism of action that avoids cross-resistance to existing agents. Short of developing novel targets, repurposing and medicinal chemistry approaches can be used (Pianalto and Alspaugh, 2016). Repurposing involves exploring drugs that are already approved by the U.S. Food and Drug Administration (FDA) or European Medicines Agency for antifungal activity. Medicinal chemistry involves making improvements to existing drug classes.

Antifungals Developed Via Medicinal Chemistry

In the medicinal chemistry approach, a new drug's target is similar to previous drugs, but it has improved features. Oteseconazole, an azole antifungal approved by the FDA in April 2022, is one of several recently developed antifungals. This drug is the first of the tetrazole class, so named for the four nitrogen atoms in the compound, in comparison to the three nitrogen atoms in triazoles. This new chemistry affects the compounds' metal binding group that interacts with the CYP51 enzyme (Warrilow et al., 2014). This alteration improves drug potency against fungal pathogens and improves the stability of the molecules, making them less likely to

be metabolized by human CYP enzymes. This lengthens half-life of the drug and results in fewer off-target effects, both in terms of direct toxicities and drug interactions. In comparison to older azoles, the change of chemistry in oteseconazole causes a significant effect on the ratio of the binding constant to *Candida* drug targets relative to the human drug target ortholog (Warrilow et al., 2014). Clotrimazole and itraconazole demonstrated binding rates of less than 5, indicating a relatively low affinity to bind to *Candida* over the human CYP protein. The ratios were higher for voriconazole and fluconazole—two existing azoles used in systemic therapy—at 229 and 543, respectively. This signifies that these drugs are far more likely to bind to *Candida* than to human CYP. Oteseconazole had a ratio greater than 2,000, indicating a far greater affinity for the fungal target relative to the mammalian target. Andes explained that this results in oteseconazole achieving a much greater effect on *Candida* than triazoles used at similar concentrations.

Based upon the results of two phase III treatment studies (NIH, 2021a), oteseconazole was recently approved by the FDA for the treatment of vulvovaginal candidiasis. The drug has been tested in phase II trials against onychomycosis (NIH, 2020), a fungal infection of the toenails, and tinea pedis (NIH, 2018), commonly referred to as "athlete's foot." The results of these treatment studies demonstrate a superiority over the standard of care—i.e., fluconazole—with fewer drug interactions and toxicity. Andes remarked that these findings hold promise for future generations of drugs developed using this technology.

Novel Modes of Action

Andes emphasized that a number of compounds featuring novel modes of action are in clinical development. Fosmanogepix is a compound that inhibits Gwt1, a glycosyl-phosphatidylinositol (GPI) acyl-transfer protein. Inhibition of this enzyme results in changes in the cell wall integrity and cell death. Fosmanogepix has little to no activity against a human ortholog. In the context of current antifungal gaps, this compound has an extremely broad spectrum of activity against yeast and mold, including triazole-resistant *Aspergillus*. Andes added that its pharmacologic properties are favorable. Both oral and intravenous formulations of fosmanogepix have been developed, with high oral absorption and bioavailability greater than 90 percent. He emphasized that fungal infections often require months of therapy, which poses challenges for intravenous delivery. Pharmacokinetically, fosmanogepix achieves penetration into the CNS and eye and holds promise of effective concentrations for these sites of dissemination. A completed phase II trial for fosmanogepix against invasive candidiasis demonstrated efficacy and did not identify a concerning safety signal, suggesting

further development is warranted (NIH, 2021b). Two phase II trials for fosmanogepix are ongoing, one for *C. auris* (NIH, 2022a) and another against aspergillosis and rare molds (NIH, 2022b). An expanded access study for the treatment of *Fusarium* is also underway. Andes emphasized that fosmanogepix addresses multiple antifungal gaps, including (1) efficacy against multidrug-resistant organisms, (2) a novel mechanism of action, (3) penetration into the CNS, (4) formulations allowing both intravenous and oral administration, and (5) no significant toxicity signal indicated thus far.

Olorofim also has a novel target, being the first compound from the orotamide class, Andes explained. This class inhibits dihydro-orotate dehydrogenase (DHODH), causing depletion of pyrimidine and subsequent inhibition of DNA and RNA synthesis. DHODH has a human ortholog for which this compound has minimal binding (Oliver et al., 2016). Olorofim has a broad spectrum of activity, particularly against molds and endemic fungi. He noted the drug has not shown appreciable activity against *Candida, Cryptococcus,* or Zygomycetes. Based on its favorable pharmacologic features, olorofim has both intravenous and oral formulations, with oral bioavailability of 45-82 percent. Furthermore, the compound achieves CNS penetration. Olorofim does appear to be a substrate for CYP3A4 metabolism, which poses a risk for drug-drug interactions. A phase II trial is underway for patients with refractory fungal infections for which no other treatment options exist (NIH, 2022c). Andes stated that presentation from the results of this trial thus far have been promising. A phase III trial is also being conducted against *Aspergillus* and rare molds (NIH, 2022d). He remarked that, like fosmanogepix, olorofim appears to address several antifungal gaps, including a novel mode of action, spectrum of activity, safety, and pharmacokinetics.

Several additional drugs featuring unique mechanisms of action are in early clinical or preclinical studies. Andes highlighted the challenges in antifungal development in noting that three of the five drug studies in early clinical trials have been stalled. The reasons for the stalled status range from the science of the mechanism of action to treatment failure to finances. Thus, despite the appearance of safety and efficacy of these compounds during preclinical studies, they will not be moving forward within the near future. Currently, seven additional antifungals with novel modes of action are in the preclinical phases. These drugs target multi-drug-resistant pathogens and are demonstrating safety and efficacy in preclinical models. Andes noted that at this early research phase, it remains unclear whether any of these compounds will eventually be used to treat patients. However, the number of novel targets being developed holds promise. He added that One Health stewardship is important for the management of new antifungal options.

ANTIFUNGAL RESEARCH AND DEVELOPMENT: CHALLENGES AND NEED FOR INCENTIVES

Rex discussed the challenges in developing new antifungal drugs, the time and expense involved in this process, and the value of having effective treatments available in the context of emerging resistance to current therapies. He highlighted financial incentives that could stimulate increased antimicrobial development activity.

Challenges in Antifungal Development

Inventing and delivering a new drug is difficult, slow, and costly, said Rex. The challenging nature of discovering and developing antifungals is reflected in the low number of novel mechanisms on the market and the limited antifungals available in oral formulation. Furthermore, antifungal resistance is increasing and spreading worldwide, affecting both plants and humans (Fisher et al., 2018). Rex explained that finding fungal targets is not difficult and numerous fungal genomes are fully sequenced. Although methods of killing fungi are readily available, identifying drugs that kill fungi and that are well tolerated in the human body is a formidable process. Prospective drugs can fail due to physical properties, pharmacology, and tolerability. The process for discovering a new molecule is lengthy, and bringing a molecule from discovery through development requires years of research. Rex noted that the compound for olorofim—a drug he is involved in developing—was discovered in 2010 and is still being developed 12 years later.

The time and effort required to develop new antifungal and antibiotic drugs drive up the expense of this process. The average research and develop cost to bring a human medicine to approval is $1.3 billion (Wouters, McKee, and Luyten, 2020). Rex explained that this figure encompasses a process in which numerous compounds do not proceed to approval but contribute to the insights needed to generate an effective drug that is ultimately approved. Drug development can last a decade, with costs incurred throughout that timeframe. Once a drug is approved, funding is required to finance post-approval commitments, operational costs for manufacturing the drug, and surveillance and pharmacovigilance activities. During the first 10 years after approval, these costs average $350 million. Thus, costs to develop a drug and fund the first decade post-market can total $1.7 billion per molecule, but usage-based income will not recover these costs (Drakeman, 2014; Rex and Krause, 2021). Rex remarked that these costs cannot be substantially decreased, given the absence of discounts and regulatory shortcuts regardless of company size, for-profit or non-profit status, or the degree of novelty involved. Thus, each antifungal is incredibly valuable, the release of new

antifungal drugs is rare, and the importance of maintaining the efficacy of existing drugs is considerable, he added.

Evolving Payer Paradigms

Rex described the current economic model for antimicrobials as "broken." When a new antibiotic is approved, its use is delayed and deferred in an effort to preserve it. He emphasized that such measures constitute necessary stewardship to avoid decreasing a new drug's efficacy while existing drugs can still be used. However, the innovator faces tremendous financial loss—and in some cases bankruptcy and business closure—from the current pay-per-use model that only reimburses a portion of the value (Carr and Stringer, 2019). Rex drew an analogy between antimicrobials and fire extinguishers. A person might not regard a fire extinguisher stored in their home or office building as being used. Rex argued that these fire extinguishers are in fact being used in that they are immediately available in the event a fire breaks out. Similarly, an infection can emerge quickly and, if untreated, spread to other susceptible individuals. Therefore, the availability of an effective antimicrobial treatment holds value regardless of whether the drug is currently being administered to humans. This value is captured in the acronym STEDI: **s**pectrum, **t**ransmission, **e**nablement, **d**iversity, and **i**nsurance. Rex exemplified the value of preventing transmission with a scenario in which a person contracts a serious infection from a highly resistant microbe. The availability of an effective treatment enables the microbe to be killed before it is passed to other people. Those prospective microbe hosts spared from being infected have benefitted from the treatment, despite that they may never become aware that the microbe posed a threat to them.

Incentive models can encourage the development of new antifungals for inventory against future microbial threats.[1] Incentives can be characterized as "push" and "pull" models. "Push" incentives include grants and grant coordination. Rex noted that a variety of groups—such as Combating Antibiotic-Resistant Bacteria (i.e., CARB-X), Replenishing and Enabling the Pipeline for Anti-Infective Resistance Impact Fund (i.e., REPAIR), BARDA, and the National Institute of Allergy and Infectious Diseases—have coalesced to fund academic groups and small companies to develop new antibacterial and antifungal products.[2] "Pull" incentives disrupt the current pay-per-use model. Rather than paying innovators for drug usage,

[1] More information about the rationale for economic incentives for the development of antimicrobials is available at http://drive-ab.eu/, https://amr-review.org/, and https://amr.solutions/incentives/ (accessed August 27, 2022).

[2] More information about CARB-X is available at https://carb-x.org/. More information about the REPAIR Impact Fund is available at https://www.repair-impact-fund.com/ (accessed August 27, 2022).

and defining usage as the administration of a drug into a human, the pull model offers market entry reward (MER). The MER is a defined sum of money that is paid over time to the creator of a new antimicrobial independent of the volume of use. Rex noted that the United Kingdom recently announced plans to buy two antibacterial agents for £10 million per year for 10 years, for a total of £100 million paid to the innovator (NHS, 2022; NICE, 2022; Outterson and Rex, 2022). Irrespective of the amount of drug used, the innovator will be compensated for developing needed drugs. Stewardship and access requirements can be added to MER agreements to stipulate that the company should not actively market the compound. Thus, pull incentives can create alignment of all parties on stewardship, said Rex. He reflected that new antifungals for human use (1) will be limited due to the difficulty in creating them, (2) will be expensive to discover and develop, (3) need to be preserved via careful stewardship, and (4) can be encouraged via a different payer model.

DISCUSSION

Strategies to Avoid Antifungal Dual Use

McKenna asked about action steps or policy changes that can be taken to address problems associated with resistant fungal infections. Rex commented that a shift has occurred in the animal agricultural industry in response to concerns about the use of antibacterial compounds. Approaches that avoid overlap with human clinical products are being taken, including changing animal husbandry practices. Rex said that substantial use of an antifungal agent in plant or animal agriculture that shares the same class as a clinical product would be unfortunate. Andes noted the inevitability that some antifungals will fail because of toxicity or pharmacologic features. He remarked that failed clinical antifungal products could potentially be used in nonhuman health circumstances in order to avoid overlapping usage. Tim Widmer, national program leader for plant health at the U.S. Department of Agriculture Agricultural Research Service, asked whether developers of agricultural and plant products have access to antimicrobial products that have failed clinical trials in humans—i.e., whether a pipeline is established for this purpose or whether intellectual property rights and the exorbitant cost of drug development pose barriers to data sharing between companies. Andes replied that he is not aware of any systematic approach to sharing these data, and that methods to increase communication between the agricultural and human health are needed. Rex remarked that topical agents will not become systemic human medicinal products; this pathway could offer advantages in agricultural applications.

Communication Structures to Reduce Resistance Potential

Given that the FDA has a framework for assessing the potential of plant and animal agricultural antibiotic use to affect resistance in humans, McKenna asked whether a similar framework could be created for fungicides. Rex remarked that this issue is structural in nature, so it should be feasible to overcome the structural issue and set up such a framework. He added that the pragmatic approaches used to eliminate antibiotics used in animal agriculture are an example of success in changing processes. Recalling a visit to a facility applying these animal practices, Rex said that the workers preferred the new methods to the former status quo and that full implementation took place over a decade. McKenna asked how communication systems targeted to either health care or the environmental and agricultural sectors could be addressed to facilitate communication between these sectors. Andes replied that this workshop is a method of shifting those communications patterns. Rex stated that he sat on the U.S. Presidential Advisory Council for Antimicrobial Resistance, which was comprised of experts from both agriculture and medicine. Councils such as that and workshops such as this shift siloed communication patterns.

Lessons from Antibacterial Research

Jeff LeJeune, food safety officer in the Food Systems and Food Safety Division of the Food and Agriculture Organization of the United Nations, asked about whether phages are a potential avenue for treatment against fungal infections. Rex replied that he cannot speak to phages for fungal pathogens, but there are phages that clearly do kill bacteria. However, they tend to be highly specific in their target bacteria and require substantial effort to develop as therapeutics, he added.

McKenna asked whether the funding models in place for antibacterial development apply to antifungal development. Andes remarked that until recently, antifungal research was not eligible for many funding programs. Rex stated that antifungal drugs need to be developed for problems anticipated to occur 10–15 years into the future. These compounds lose efficacy over time, thus new products need to be available to take their place once they are no longer effective. He emphasized that in situations where an effective tool against the most common varieties of fungal pathogens is already available, it is challenging to obtain approval for a new product that treats common varieties as well as less frequent types of infections. Moreover, successfully demonstrating superiority in efficacy trials is rare, especially given that serious infections require aggressive dosing. In the United States, a pull incentive to develop antimicrobial innovations established by the PASTEUR

Act is currently being considered by Congress.[3] Although the bill's first iteration did not include antifungals, advocacy efforts achieved inclusion of these products in the current version, which is expected to be voted on by the end of 2022. Rex stated that 2–4 new drugs are needed each decade to stay ahead of the resistance that continues to emerge against products after they are in use for several years.

[3] *PASTEUR Act of 2021*, HR 3932, 117th Congress, 1st session (June 16, 2021).

10

Integrated Plant Disease Management

The ninth session of the workshop focused on agricultural technologies and strategies to mitigate the threat of antifungal resistance by reducing the use of fungicides and addressing conditions that foster development of resistant fungi. Tim Widmer, national program leader for plant health at the U.S. Department of Agriculture Agricultural Research Service (USDA/ARS), moderated the session. Melanie Lewis Ivey, associate professor of plant pathology at The Ohio State University (OSU), discussed technology that reduces fungicide output, decreases water usage, and yields financial savings while also being as effective as traditional fungicide application methods in controlling disease. Walt Mahaffee, research plant pathologist at the USDA/ARS, detailed challenges in detecting grape powdery mildew, new detection methods, and efforts to detect azole resistance in this fungus. Jianhua Zhang, evolutionary microbiologist at the Netherlands National Institute for Health and Environment, discussed dynamics of *A. fumigatus* hotspots and strategies to decrease the total number of *A. fumigatus* or remove the fungicide pressure to select for resistance within these settings.

Widmer noted concerns expressed by multiple workshop participants regarding the possible connection between agricultural application of azoles and resistant pathogens in humans, as well as the challenges in verifying and communicating that connection. Regardless of a relationship between agricultural fungicide use and human pathology, more judicial and reduced use of fungicides is advantageous for plant health and thereby for growers, by decreasing the likelihood that the target pathogen will develop resistance. Therefore, an integrated disease approach could reduce resistance-induced

pressure on the plant pathogen, which, in turn, could potentially lead to a reduction of resistance in human pathogens.

PRECISION APPLICATION TECHNOLOGIES AND STAKEHOLDER COMMUNICATION

Ivey discussed technology designed to reduce chemical use, resulting in decreased volume of fungicide application within the environment and savings in cost and water usage. She detailed validation measures used to demonstrate that this technology offers spray coverage and disease control equivalent to that of traditional fungicide application methods. Ivey is a fruit pathologist who works to identify economical and sustainable strategies to control diseases of fruit, hop, and nut crops, with a particularly focus on reduced reliance on pesticides, pesticide resistance mitigation, and pesticide stewardship.

Laser Guided Intelligent Sprayer Technology

Ivey described how fruit crops are sprayed using airblast technology, which creates a huge plume of pesticide; some of the pesticide settles on the crop, but the majority goes into the air or drops to the ground. Heping Zhu, a professor in the department of Food, Agricultural and Biological Engineering at OSU and agricultural engineer at USDA/ARS, approached Ivey in 2016 to determine whether a laser-guided intelligent sprayer he had developed for the ornamental industry could be used in the fruit crop industry. Using lidar sensor technology, the sprayer can detect the canopy architecture and density, as well as measure the speed at which a tractor is traveling. The sprayer collects these measurements as it moves along a row of crops, using these data to determine the quantity and location of nozzles to engage and the variable spray pressure setting appropriate for the density of the canopy. Whereas conventional airblast technology sprays continuously as it travels each row of crops, the intelligent sprayer utilizes a pulsating spray pattern; it issues spray as it detects plants, and it ceases to spray as it detects the spaces and posts in between plants. Similarly, the sprayer only engages the nozzles required to treat the specific height of each plant. This technology results in reduced drift during application.

Technology Efficacy Validation

During initial tests of the intelligent sprayer, growers expressed doubt that the chemicals were coming into contact with the plants due to the low levels of product being used and the lack of visible droplets, said Ivey. Traditional airblast technology utilized since the 1950s produces a spray

that is visible on plants, on farmers themselves, and in the drift in the air. Thus, validation efforts became a component of stakeholder communication. Validation criteria included (1) provision of adequate coverage for pest control, (2) provision of disease control equivalent to airblast technology, and (3) economic and environmental sustainability. Ivey remarked that in the fruit crop industry, environmental and economic sustainability are associated, because growers can increase market share by labeling products as "reduced pesticide" or "pesticide free" without meeting organic status.

Adequate Coverage and Disease Control

Optimal spray coverage for pest control purposes is 25–30 percent, with coverage below 25 percent deemed inadequate and greater than 50 percent considered excessive, Ivey explained. Excessive spray coverage indicates that greater quantities of pesticides are being applied than what is required to control pests. To test the spray coverage of the intelligent sprayer, water sensitive cards were attached to apple and grape plants. Samples were taken from various locations of the plant canopy and trunk or vine for both the intelligent mode and the airblast mode. The pesticide coverage of the intelligent sprayer measured between 42–56 percent, with the center of the plant indicating higher percentage coverage than the samples taken from the edge of the canopy. In comparison, the pesticide coverage with the airblast sprayer was 61–79 percent, with samples from all parts of the plant surpassing the 50 percent threshold for excessive coverage. The mean percentage for all intelligent sprayer samples was 50 percent, compared to a mean of 70 percent for the airblast method. To validate adequate disease control, grape plants were investigated for foliar fungal diseases including powdery mildew, downy mildew, and black rot. The same fungicide program was utilized for the crops treated by the intelligent sprayer and the crops receiving airblast application. The two types of sprayers demonstrated similar percentages of disease severity and progression, which were significantly better than the non-treated control group. This finding indicates equivalent disease control for the intelligent and airblast sprayers, said Ivey.

Environmental and Ecological Sustainability

Pesticide volume and water usage are equivalent, and pesticide volume output was measured to determine water usage for the two application methods, Ivey explained. Measurements were taken for grape plant application at various phenological stages. When grape plants are small, they feature a larger ratio of trunk to green growth. As they become larger, the canopy expands. The intelligent sprayer moves between rows of plants and applies pesticide when it senses canopy or trunk. Therefore, as plants have

wider canopies, pesticide is applied a greater proportion of time, and the pesticide volume output increases. The intelligent sprayer pesticide output for plants at 10–12 inches of growth on the grape vine was less that 0.2 liters per vine. This rate increased with plant size until reaching a rate of approximately 0.7 liters per vine for grape at pre-harvest stage. In contrast, the airblast pesticide output is fairly consistent across all phenological stages, measuring between 0.7 to just over 1 liter per vine for all stages. Depending on the phenological stage, the intelligent sprayer demonstrated a 29–91 percent reduction in pesticide and water usage over the airblast technology. Thus, the intelligent sprayer achieves equivalent disease control as the airblast, while using far less water and releasing smaller quantities of pesticide into the environment.

Ivey emphasized that California is experiencing drought to the extent that farmers with minimal access to water must drive miles to refill water tanks. This reduction in water usage would necessitate fewer trips for water for farmers using the intelligent sprayer. She added that the results were validated in an apple orchard. The farmer conducting the pesticide application for the demonstration trial decided to use the new technology on the remainder of his 100-acre orchard due to the reduction in chemical and water usage. The reduced pesticide output translates to financial savings, with the pesticide cost dropping $469 per hectare when using the intelligent sprayer. Ivey noted that this figure is a 3-year average of the same chemical program used each year based on 2019 prices. The price of fungicides has since skyrocketed, and this price increase could alter the reduction in cost.

To validate the economic sustainability of the intelligent sprayer, Guilherme Signorini, assistant professor of value chain management in the Department of Horticulture and Crop Science at OSU, conducted a cost-benefit analysis of utilization of the new technology in a 20-hectare vineyard. The analysis assumed that investment is made in year 0, the plants require three years to produce grapes, and the vines will have 23 years of productivity in a viniferous planting. Fixed and variable costs were based on an enterprise budget Signorini developed, and extreme weather conditions and grape cost were excluded from the analysis. Ivey noted that the cost of the intelligent sprayer is considerable, and her figures are based on the price after Heping sold the technology to a sprayer company. Demand for the intelligent sprayer has been high, and thus the current cost may have increased since the analysis was last updated before being submitted for publication. At the time of this analysis, a new intelligent sprayer costs $70,000. Retrofitting an existing sprayer with the intelligent technology is approximately $36,000. Whereas an intelligent sprayer purchased in new condition decreases in value over time, with a net present value of under $50,000, the present value of a retrofitted sprayer is over $51,000, indicating an increase in value. The internal rate of return is comparable for both

the new and retrofitted sprayers, at 14.3 and 14.8 percent, respectively. Similarly, the payback terms are nearly equivalent at 11.7 years for the new sprayer and 11.4 years for the retrofitted. The return on investment is 3.53 for a new machine and 4.45 for a retrofitted one. Ivey noted that an economic analysis is currently being conducted for an apple orchard, and the payback term will likely be about half the length of the grape vineyard setting.

Ivey remarked that the intelligent sprayer provides environmental benefit by achieving reduced pesticide and water usage, which is particularly valuable as extended droughts become more frequent across the United States. Targeted pesticide applications decrease the pesticide in the ground and air, resulting in less contact with non-target organisms. She added that the growers involved in the demonstration trials have become promoters of the intelligent sprayer, suggesting that farmers would be open to adopting this new technology.

FINDING NEEDLES IN HAYSTACKS: DISEASE MONITORING AND RISK ASSESSMENT

Mahaffee gave an overview of fungal disease management, challenges in detecting grape powdery mildew, recently developed detection methods involving samples from the air and from field workers' gloves, and efforts to detect azole-resistant powdery mildew. Grape production within the United States has a farm gate value of $6 billion, and it has an economic impact of almost $220 billion in grape-producing regions. Mahaffee described that 95 percent of grape yield is attributed to fungicide-based disease management of grape powdery mildew (Gianessi and Reigner, 2006). Thus, 78 percent of pesticide used in grape production targets this pathogen (Fuller et al., 2014).

Fungal Disease Management

Because disease will inevitably develop in an agricultural crop, the goal of disease control is to delay that development to limit economic impact, Mahaffee remarked. Disease control works toward the aim of pushing the logistic curve of a disease further into the future. The threat from any given plant pathogen varies annually, posing high risk during some years and low risk during others. No single tool addresses all threats. Rather, successful disease management involves combining tools in the locations, times, and sequences that are most effective. These tools include pesticide application, disease forecasting, disease scouting, and cultural practices related to fertility, canopy management, and planting times. Mahaffee noted that he has seen the frequency of fungicide applications for grape

powdery mildew range from 3–17 applications per year. The majority of fungicide compounds only affect the infection process—rather than curing established disease—and therefore most fungicide applications are primarily prophylactic. Pesticide product labels specify regulatory stipulations regarding the frequency of use, rotation, and other products with which a compound can be mixed within a single application.

Crop disease risk is not uniform nor random across the field. Identifying a localized approach to replace broadcast fungicide application is challenging due to the difficulty in detecting and locating powdery mildew. Detection tools for the fungus are limited to spectral data and visual observation, and powdery mildew must be identified within a small portion of the pathogen lifecycle in order to be controlled (Aylor, 2017). Visual observation often results in false positives, and visual scouting data Mahaffee collected indicate that the percentage of false positives sometimes exceeds the percentage of incidence during an observation period. To improve pathogen monitoring, Mahaffee and his team have investigated mechanisms for detecting spores during the dispersion segment of the pathogen lifecycle, while they are still in the air and have not yet attached to hosts (Mahaffee et al., 2022) (see Figure 10-1). Various types of mobile spore trap detection devices have been deployed for this purpose.

Inoculum Detection

Mahaffee has engaged in research on using inoculum detection to inform fungicide intervals (Thiessen et al., 2016; Thiessen et al., 2017, 2018). In the initial phase of this research, fungicide application was based on pathogen detection in the air using spore traps. The application intervals

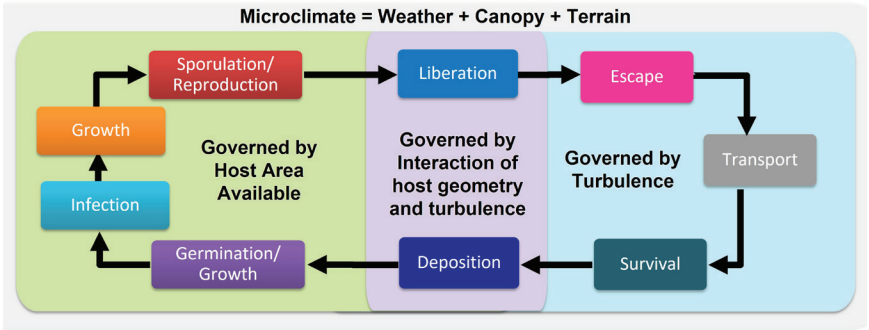

FIGURE 10-1 Pathogen life cycle.
SOURCE: Mahaffee presentation, June 27, 2022; Mahaffee et al., 2022; The American Phytopathological Society, 2023.

were then adjusted based on pathogen concentration. Over an interval of eight field seasons, fungicide frequency was reduced by an average of 3.8 applications per season, resulting in a decrease of over 13 pounds of fungicide per acre. This reduction equated to a cost savings of $150–250 per acre in fungicide costs, depending on the chemicals being used. He and his colleagues are working with several companies to commercially implement this method. In collaboration with Rob Stoll at University of Utah and Brian Bailey at University of California, Davis, his team is using modeling to develop a rational strategy for deployment of inoculum samplers.

Powdery mildew does not become visually detectable until the leaf is at least 5 percent infected, Mahaffee noted, highlighting the difficulty of detecting pathogens on plants. At that point, even the most intense scouting efforts have less than a 50 percent probability of detecting disease. Sarah Lowder, a doctoral student of plant pathology at Oregon State University, conducted research using swabs of vineyard workers' gloves as a method for monitoring disease and fungicide resistance risk. Given that workers interact with the plants, she investigated whether pathogens could be identified by sampling workers' gloves. A receiver operating characteristic (i.e., ROC) analysis found that visual detection methods resulted in a high number of false negatives. The glove sampling detected pathogens at lower levels before they became visible. The study found that disease can be detected almost a month earlier using the glove sampling technique over visual observation.

Resistance Detection

In addition to pathogen detection, the spore trap and glove swab techniques have been used to test for quinone outside inhibitor (QoI) resistance (Miles et al., 2021). The G143A allele associated with QoI resistance began appearing in field populations in 2013 and prevalence steadily progressed until 2016, when major disease control failure events took place across much of the Western United States. In 2017, Mahaffee and colleagues worked with growers to cease use of QoI fungicides, and the frequency of the resistant allele decreased as QoI usage was reduced. From 2018–2020, nearly 5,000 samples from 107 vineyards in 17 West Coast counties were tested for QoI resistance. A higher frequency of resistance was found in California than in Oregon or Washington, but California also has a higher density of grape production. Therefore, it has not been determined whether the resistance is related to the density of production, given that spores can travel from one vineyard to another neighboring one. A public database enables growers to view the frequency of QoI resistance detection in near-real time.[1]

[1] The Fungicide Resistance Assessment, Mitigation and Extension (i.e., FRAME) Network database is available at https://framenetworks.wsu.edu/ (accessed August 29, 2022).

Research is underway to detect azole resistance in grape powdery mildew, which is associated with the Y136F mutation (Golan and Pringle, 2017). Approximately 4,000 historical air samples collected from conventional and organic vineyards throughout the season were investigated, confirming the Y136F mutation in 273 of the samples. These samples with Y136F mutation were then tested for the TR34 and TR46 alleles associated with azole resistance in *Aspergillus fumigatus (A. fumigatus)*, but *A. fumigatus* was not identified within the samples. However, *Botrytis cinerea*—a fungus similar in spore morphology and size to *A. fumigatus*—was detected in all samples. Mahaffee expressed that the detection of *Botrytis cinerea* indicates that the DNA collection technique was suitable for isolating *A. fumigatus* DNA. Mahaffee is researching whether sentinel data can be used to determine the probable source of an inoculum plume and predict the area at risk for deposition and disease development.

MITIGATION MEASURES IN COMPOST HOTSPOTS

Zhang discussed features of *A. fumigatus* hotspots—settings in which resistant fungi proliferate—and strategies to transform these into coldspots by reducing the total number of *A. fumigatus* or removing the fungicide pressure that selects for resistance. In 2015, she discovered that samples taken from flower bulb plant waste heaps contained high numbers of azole-resistant genotypes (Zhang et al., 2017a). Research revealed that these heaps also contained substantial amounts of fungicide residues. At the time, she worked at the Wageningen University and Research, and she found that a plant waste heap located near the institution had low levels of azole residues and azole-resistant fungal isolates. This sparked her investigation into why some plant waste heaps become resistance hotspots.

Dynamics of Azole-Resistance in Hotspots

Zhang tested numerous potential types of natural waste for *A. fumigatus*. Flower bulb compost, wood chippings, and green waste were all determined to be hotspots for the fungus (Schoustra et al., 2019a; Schoustra et al., 2019b; Zhang et al., 2021c). She noted that fruit waste, silage cow feed, wood waste, grain storage, and manure and straw from chickens and cows were not found to have substantial levels of *A. fumigatus*. The process of composting bulb waste was explored in order to determine the portion(s) of the chain during which *A. fumigatus* developed resistance. Composting begins during summer harvest, with a small pile of bulb plant leaves that becomes larger as more waste is discarded. Multiple processes are at play as pre-compost degrades into mature compost—such as explosive growth of bacteria and fungi and increases in temperature from less than 40 degrees

Celsius to 65 degrees—which enable the compost to kill plant pathogens (van der Wurff et al., 2016).[2] Approximately 3-4 months into the composting process, machinery is used to turn the piles on a bi-weekly basis until the compost reaches maturity, at which point it is used in planting new crops of flowers. Zhang found that the practice of turning the compost piles releases clouds of spores into the air.

Samples from the flower waste heaps were taken over the course of a year to determine whether emergence of resistant *A. fumigatus* is dependent on the season or stage of the operation (Zhang et al., 2021c). The heaps were tested for total amount of *A. fumigatus*, concentration of azole fungicides, and fraction of resistant isolates compared with the total population. *A. fumigatus* was found throughout the year, independent of the season, whereas azole fungicide concentration varied over time. The samples indicated a high fraction of resistant isolates in the population with initial readings of approximately 50 percent. Zhang described challenges in isolating *A. fumigatus* in the samples arising from the presence of other fungi that were difficult to remove from the culture plates. She and her team developed a new "flamingo" medium—so named for its pink color—which resulted in better *A. fumigatus* isolation over five other media (Zhang et al., 2021a). The flamingo medium resulted in significant reductions in surface area covered by non-*A. fumigatus* in plant waste samples, as well as in samples from ditchwater, grass, soil, and wood.

Once the *A. fumigatus* was isolated, seven types of resistant genotypes were identified, all of which contained TR34 and TR46 mutations (Zhang et al., 2021c). Zhang noted that the dominant genotype varied over time. During the course of a year, resistant genotypes appeared and remained present for variable durations, with some genotypes no longer detectable one month after appearing, while other genotypes remained present for multiple months. She described that the *cyp51A* gene involved in azole resistance has evolved over time. The first identified clinical azole-resistant isolate—TR34/L98H—appeared in 1998 (Snelders et al., 2012). In the years since, TR34/L98H has been found in bulb waste heaps, in addition to additional resistant *cyp51A* genotypes that have emerged. With these genotypes, both the length of the promoter region and the variation and single nucleotide polymorphism mutation have increased over time.

Flower bulb waste material has been found to be a natural niche for the sexual cycle in *A. fumigatus* (Zhang et al., 2021d). In researching how bulb waste heaps promote sexual reproduction of *A. fumigatus*, Zhang found that this substrate provides favorable conditions for reproduction, including adequate nutrition, a dark environment, and 30-degree Celsius temperature.

[2] More information about compost research conducted at Wageningen University and Research is available at www.biogreenhouse.org (accessed August 30, 2022).

Furthermore, sexual reproduction promotes the survival of *A. fumigatus* in the flower bulb waste, because ascospores produced during this process withstand extreme conditions (Zhang et al., 2015, 2021b). Sexual reproduction also can generate diverse genotypes able to survive varying conditions. When the spore begins to grow and undergoes mitotic division, de novo mutations can occur. In the prese\nce of azole fungicide residue, a resistant genotype can be selected over time. Thus, the sexual cycle and sporulation of *A. fumigatus* are important functions for adaptation within the waste heaps.

Zhang explained that 1 gram of bulb waste compost can contain 100,000 *A. fumigatus* spores. Moreover, a single stockpile measuring 50 meters wide by 50 meters long and 10 meters high may contain 2,500,000,000,000,000 *A. fumigatus* spores, half of which could be azole-resistant, given a 50 percent abundance approximation. Furthermore, these resistant spores can involve various genotypes resulting from asexual and sexual reproduction and azole selection.

Strategies to Mitigate Resistant *A. fumigatus*

The development of bulb plant waste composting offers ecological benefits. In the 1970s, plant waste in Europe was burned, releasing carbon dioxide into the air that contributed to climate change. Zhang noted that many developing countries continue to burn plant waste on large scales. Although the circular economy of reusing plant waste to fertilize future crops offers ecological sustainability, it generates enormous quantities of resistant *A. fumigatus* spores, which can pose health risks to susceptible patients. Thus, approaches that balance both ecological and health needs should be considered. Strategies to alter the plant waste stream to transform hotspots into coldspots are being investigated in an effort to capitalize on the ecological benefits of composting without increasing health threats. Wageningen University and Research is conducting ongoing studies on self-composting and compost fermentation processes to determine whether these methods can reduce the total number of *A. fumigatus* spores or remove the selective pressure of azoles. They are experimenting under controlled conditions toward understanding the factors that can reduce *A. fumigatus* and the pressure of azoles; they plan to apply these learnings in validating strategies within the agricultural setting.

A strategy to reduce the total number of *A. fumigatus* spores involves interrupting its propagation and germination processes. Waste plant material is put into boxes that vary in substance and conditions. Given the different conditions can influence the growth of *A. fumigatus*, Zhang stated that it may be possible to identify conditions that reduce *A. fumigatus* growth. Freestanding plant waste piles were compared with plant waste placed in wooden boxes, plastic boxes, and plastic boxes with water added.

Preliminary data found prolific growth of *A. fumigatus* in wooden boxes and significant reduction of *A. fumigatus* in non-watered plastic boxes. Although the plant waste in watered plastic boxes had more *A. fumigatus* than the freestanding piles, the percentage of resistant isolates was lower in the watered boxes. These findings demonstrate that oxygen and water content could play a role in reducing *A. fumigatus*, said Zhang. Studies are also exploring whether adjusting the ratio of bacteria, yeast, and mold in compost can reduce the amount of *A. fumigatus* in compost. Bacteria are being added to plant waste to determine whether (1) the compost ecosystem will still function with an adjusted ratio and (2) *A. fumigatus* will remain at a lower proportion.

Zhang described another approach to transforming hotspots involving removal of fungicide pressure and selection. Fungicide residues constitute a source of carbon and nitrogen, and microorganisms could potentially degrade these residues. A laboratory study found that *Pseudomonas aeruginosa* can biodegrade propiconazole, a fungicide often found in agricultural plant waste (Satapute and Kaliwal, 2016). If *Pseudomonas* or a similar organism can degrade triazoles in agricultural settings, this strategy could reduce the fungicide pressure that causes *A. fumigatus* to select for resistance.

Plant waste is an excellent niche for *A. fumigatus* to complete its life cycle, evolve, and cope with extreme conditions. Zhang noted that much has been learned about hotspots, but the factor(s) driving the emergence of the TR genotype has not yet been identified. Collaboration is needed to determine the most effective strategies to mitigate azole resistance in *A. fumigatus*.

DISCUSSION

Jeff LeJeune, food safety officer in the Food Systems and Food Safety Division of the Food and Agriculture Organization of the United Nations, asked whether integrated pest management (IPM) or other approaches can prevent the use of fungicides by reducing the need for it, particularly in low-income settings. Mahaffee replied that pathogens can evolve quickly—slowing this evolution to overcome resistance requires the incorporation of fungicides into the disease management program. Thus, IPM minimizes the use of fungicides to levels that are necessary to achieve desired outcomes, but this approach does not eliminate the need for fungicides. He added that a pesticide can be targeted to the stage of the pathosystem where it will have the most effect. For instance, in grape production, this often involves using sulfur fungicides early and limiting synthetic fungicides to the susceptible period during bloom, when maintaining crop quality is most critical. Ivey added that education and outreach efforts in low-income settings could

contribute to ensuring that pesticides are used correctly. The misuse of pesticides in terms of the number of applications used is common, thus education in this area is important in reducing use and mitigating resistance. Widmer remarked that social scientists can play a role in plant pathology by facilitating buy-in among growers to incorporate practices that limit fungicide use. Ivey noted that adopting new technologies poses challenges for mid-size growers. However, sustainability grants for new equipment could be established for farmers working to reduce the input of pesticides and increase environmental sustainability.

REFLECTIONS ON DAY THREE

Paige Waterman, interim chair of medicine and vice chair for clinical research at the F. Edward Herbert School of Medicine at the Uniformed Services University of the Health Sciences, Bethesda, offered reflections on the third day of the workshop. The sessions highlighted the value of stepping outside of professional comfort zones, whether these be surveillance, diagnostics, therapeutics, mitigation, product development, laboratory research, agricultural environments, clinical settings, or policy arenas. Addressing antifungal resistance is an ongoing endeavor, and this workshop emphasized priority areas in bridging the gap between plant agriculture and human health. She noted her appreciation of the integrated approach the workshop utilized in examining the complexities involved in antifungal resistance.

References

Ahn, I. E., T. Jerussi, M. Farooqui, X. Tian, A. Wiestner, and J. Gea-Banacloche. 2016. Atypical *Pneumocystis jirovecii* pneumonia in previously untreated patients with CLL on single-agent ibrutinib. *Blood* 128(15):1940-1943.

Al-Yasiri, M. H., A. C. Normand, C. L'Ollivier, L. Lachaud, N. Bourgeois, S. Rebaudet, R. Piarroux, J. F. Mauffrey, and S. Ranque. 2016. Opportunistic fungal pathogen *Candida glabrata* circulates between humans and yellow-legged gulls. *Sci Rep* 6(1):36157.

Alexander, B. D., M. D. Johnson, C. D. Pfeiffer, C. Jimenez-Ortigosa, J. Catania, R. Booker, M. Castanheira, S. A. Messer, D. S. Perlin, and M. A. Pfaller. 2013. Increasing echinocandin resistance in *Candida glabrata*: Clinical failure correlates with presence of *FKS* mutations and elevated minimum inhibitory concentrations. *Clin Infect Dis* 56(12):1724-1732.

Ali, M. E., M. Gunn, T. Stackhouse, S. Waliullah, B. Guo, A. Culbreath, and T. Brenneman. 2021. Sensitivity of *Aspergillus flavus* isolates from peanut seeds in Georgia to azoxystrobin, a quinone outside inhibitor (QOI) fungicide. *J Fungi (Basel)* 7(4):284.

Aylor, D. 2017. *Aerial dispersal of pollen and spores*, *Epidemiology*. St. Paul, MN: The American Phytopathological Society.

Barber, A. E., J. Riedel, T. Sae-Ong, K. Kang, W. Brabetz, G. Panagiotou, H. B. Deising, and O. Kurzai. 2020. Effects of agricultural fungicide use on *Aspergillus fumigatus* abundance, antifungal susceptibility, and population structure. *mBio* 11(6):e02213-02220.

Beardsley, J., C. L. Halliday, S. C. Chen, and T. C. Sorrell. 2018. Responding to the emergence of antifungal drug resistance: Perspectives from the bench and the bedside. *Future Microbiol* 13(10):1175-1191.

Beer, K. D., E. C. Farnon, S. Jain, C. Jamerson, S. Lineberger, J. Miller, E. L. Berkow, S. R. Lockhart, T. Chiller, and B. R. Jackson. 2018. Multidrug-resistant *Aspergillus fumigatus* carrying mutations linked to environmental fungicide exposure—three states, 2010-2017. *MMWR Morb Mortal Wkly Rep* 67(38):1064-1067.

Benedict, K., B. R. Jackson, T. Chiller, and K. D. Beer. 2019. Estimation of direct healthcare costs of fungal diseases in the United States. *Clin Infect Dis* 68(11):1791-1797.

Benedict, K., S. V. Tsay, M. G. Bartoces, S. Vallabhaneni, B. R. Jackson, and L. A. Hicks. 2022a. Outpatient antifungal prescribing patterns in the United States, 2018. *Antimicrob Steward Healthc Epidemiol* 1(1):e68.

Benedict, K., H. K. Whitham, and B. R. Jackson. 2022b. Economic burden of fungal diseases in the United States. *Open Forum Infectious Diseases* 9(4):ofac097.

Bensasson, D., J. Dicks, J. M. Ludwig, C. J. Bond, A. Elliston, I. N. Roberts, and S. A. James. 2019. Diverse lineages of *Candida albicans* live on old oaks. *Genetics* 211(1):277-288.

Berman, J., and D. J. Krysan. 2020. Drug resistance and tolerance in fungi. *Nat Rev Microbiol* 18(6):319-331.

Bhattacharya, S., B. D. Esquivel, and T. C. White. 2018. Overexpression or deletion of ergosterol biosynthesis genes alters doubling time, response to stress agents, and drug susceptibility in *Saccharomyces cerevisiae*. *mBio* 9(4):e01291-01218.

Bitar, D., O. Lortholary, Y. Le Strat, J. Nicolau, B. Coignard, P. Tattevin, D. Che, and F. Dromer. 2014. Population-based analysis of invasive fungal infections, France, 2001–2010. *Emerging Infectious Diseases* 20(7):1163-1169.

Bongomin, F., S. Gago, R. O. Oladele, and D. W. Denning. 2017. Global and multi-national prevalence of fungal diseases—estimate precision. Journal of Fungi 3(4):57.

Bongomin, F., C. R. Batac, M. D. Richardson, and D. W. Denning. 2018. A review of onychomycosis due to *Aspergillus* species. *Mycopathologia* 183(3):485-493.

Brackin, A. P., J. M. G. Shelton, A. Abdolrasouli, M. C. Fisher, and T. R. Sewell. 2020. A low-cost tebuconazole-based screening test for azole-resistant *Aspergillus fumigatus*. *Curr Protoc Microbiol* 58(1):e112.

Break, T. J., V. Oikonomou, N. Dutzan, J. V. Desai, M. Swidergall, T. Freiwald, D. Chauss, O. J. Harrison, J. Alejo, D. W. Williams, S. Pittaluga, C. R. Lee, N. Bouladoux, M. Swamydas, K. W. Hoffman, T. Greenwell-Wild, V. M. Bruno, L. B. Rosen, W. Lwin, A. Renteria, S. M. Pontejo, J. P. Shannon, I. A. Myles, P. Olbrich, E. M. N. Ferre, M. Schmitt, D. Martin, Genomics, C. Computational Biology, D. L. Barber, N. V. Solis, L. D. Notarangelo, D. V. Serreze, M. Matsumoto, H. D. Hickman, P. M. Murphy, M. S. Anderson, J. K. Lim, S. M. Holland, S. G. Filler, B. Afzali, Y. Belkaid, N. M. Moutsopoulos, and M. S. Lionakis. 2021. Aberrant type 1 immunity drives susceptibility to mucosal fungal infections. *Science* 371(6526).

Brilhante, R. S. N., L. P. Alencar, S. P. Bandeira, J. A. Sales, A. J. J. Evangelista, R. Serpa, R. A. Cordeiro, W. A. Pereira-Neto, J. J. C. Sidrim, D. Castelo-Branco, and M. F. G. Rocha. 2019. Exposure of *candida parapsilosis* complex to agricultural azoles: An overview of the role of environmental determinants for the development of resistance. *Sci Total Environ* 650(Pt 1):1231-1238.

Brown, L., A. K. Leck, M. Gichangi, M. J. Burton, and D. W. Denning. 2021. The global incidence and diagnosis of fungal keratitis. *Lancet Infect Dis* 21(3):e49-e57.

Bueid, A., S. J. Howard, C. B. Moore, M. D. Richardson, E. Harrison, P. Bowyer, and D. W. Denning. 2010. Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009. *J Antimicrob Chemother* 65(10):2116-2118.

Buil, J. B., E. Snelders, L. B. Denardi, W. J. G. Melchers, and P. E. Verweij. 2019. Trends in azole resistance in *Aspergillus fumigatus*, the Netherlands, 1994-2016. *Emerg Infect Dis* 25(1):176-178.

Buil, J. B., H. A. L. van der Lee, A. Rijs, J. Zoll, J. Hovestadt, W. J. G. Melchers, and P. E. Verweij. 2017. Single-center evaluation of an agar-based screening for azole resistance in *Aspergillus fumigatus* by using VIPcheck. *Antimicrob Agents Chemother* 61(12):e01250-01217.

Burks, C., A. Darby, L. Gomez Londono, M. Momany, and M. T. Brewer. 2021. Azole-resistant *Aspergillus fumigatus* in the environment: Identifying key reservoirs and hotspots of antifungal resistance. *PLoS Pathog* 17(7):e1009711.

Bustamante, B., L. R. Illescas, A. Posadas, and P. E. Campos. 2020. Azole resistance among clinical isolates of *Aspergillus fumigatus* in Lima, Peru. *Med Mycol* 58(1):54-60.

Carr, A., and J. Stringer. 2019. Achaogen, Inc. (AKAO). Needham & Company, LLC. https://needham.bluematrix.com/docs/html/e821e131-b928-4b4d-86dd-63ec995e6e21.html (accessed February 24, 2023).

Casadevall, A. 2020. Climate change brings the specter of new infectious diseases. *J Clin Invest* 130(2):553-555.

Casadevall, A., D. P. Kontoyiannis, and V. Robert. 2019. On the emergence of *Candida auris*: Climate change, azoles, swamps, and birds. *mBio* 10(4).

CDC (Centers for Disease Control and Prevention). 2019. *Antibiotic resistance threats in the United States: 2019*. Atlanta, GA: U.S. Department of Health and Human Services, CDC.

CDC. 2020. *Antifungal susceptibility testing and interpretation*. https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html (accessed October 5, 2022).

CDC. 2021a. *Antifungal-resistant* Aspergillus. https://www.cdc.gov/fungal/diseases/aspergillosis/antifungal-resistant.html (accessed August 31, 2022).

CDC. 2021b. *Antifungal resistance: People and environment*. https://www.cdc.gov/fungal/diseases/aspergillosis/azole-resistant-aspergillus.html (accessed August 22, 2022).

CDC. 2021c. *Healthcare-associated infections—community interface*. https://www.cdc.gov/hai/eip/haicviz.html (accessed July 29, 2022).

Chen, Y., F. Dong, J. Zhao, H. Fan, C. Qin, R. Li, P. E. Verweij, Y. Zheng, and L. Han. 2020. High azole resistance in *Aspergillus fumigatus* isolates from strawberry fields, China, 2018. *Emerg Infect Dis* 26(1):81-89.

Chong, G. L., W. W. van de Sande, G. J. Dingemans, G. R. Gaajetaan, A. G. Vonk, M. P. Hayette, D. W. van Tegelen, G. F. Simons, and B. J. Rijnders. 2015. Validation of a new *Aspergillus* real-time PCR assay for direct detection of *Aspergillus* and azole resistance of *Aspergillus fumigatus* on bronchoalveolar lavage fluid. *J Clin Microbiol* 53(3):868-874.

Chow, N. A., L. Gade, S. V. Tsay, K. Forsberg, J. A. Greenko, K. L. Southwick, P. M. Barrett, J. L. Kerins, S. R. Lockhart, T. M. Chiller, A. P. Litvintseva, and U.S. *Candida auris* Investigation Team. 2018. Multiple introductions and subsequent transmission of multidrug-resistant *Candida auris* in the USA: A molecular epidemiological survey. *Lancet Infect Dis* 18(12):1377-1384.

Collar, A. L., M. Swamydas, M. O'Hayre, M. S. Sajib, K. W. Hoffman, S. P. Singh, A. Mourad, M. D. Johnson, E. M. Ferre, J. M. Farber, J. K. Lim, C. M. Mikelis, J. S. Gutkind, and M. S. Lionakis. 2018. The homozygous CX3CR1-M280 mutation impairs human monocyte survival. *JCI Insight* 3(3).

Culbreath, A. K., T. B. Brenneman, R. C. Kemerait, K. L. Stevenson, and D. J. Anco. 2019. Combinations of elemental sulfur with demethylation inhibitor fungicides for management of late leaf spot (*Nothopassalora personata*) of peanut. *Crop Protection* 125:104911.

Cunningham, A. 2021. A deadly fungus behind hospital outbreaks was found in nature for the first time. *ScienceNews*, April 10, 2021.

Denning, D. W. 1996. Therapeutic outcome in invasive aspergillosis. *Clinical Infectious Diseases* 23(3):608-615.

Doughty, K. J., H. Sierotzki, M. Semar, and A. Goertz. 2021. Selection and amplification of fungicide resistance in *Aspergillus fumigatus* in relation to DMI fungicide use in agronomic settings: Hotspots versus coldspots. *Microorganisms* 9(12).

Drakeman, D. L. 2014. Benchmarking biotech and pharmaceutical product development. *Nat Biotechnol* 32(7):621-625.

Drummond, R. A., J. V. Desai, E. E. Ricotta, M. Swamydas, C. Deming, S. Conlan, M. Quinones, V. Matei-Rascu, L. Sherif, D. Lecky, C. R. Lee, N. M. Green, N. Collins, A. M. Zelazny, D. R. Prevots, D. Bending, D. Withers, Y. Belkaid, J. A. Segre, and M. S. Lionakis. 2022. Long-term antibiotic exposure promotes mortality after systemic fungal infection by driving lymphocyte dysfunction and systemic escape of commensal bacteria. *Cell Host Microbe* 30(7):1020-1033 e1026.

Drummond, R. A., M. Swamydas, V. Oikonomou, B. Zhai, I. M. Dambuza, B. C. Schaefer, A. C. Bohrer, K. D. Mayer-Barber, S. A. Lira, Y. Iwakura, S. G. Filler, G. D. Brown, B. Hube, J. R. Naglik, T. M. Hohl, and M. S. Lionakis. 2019. CARD9(+) microglia promote antifungal immunity via IL-1beta- and CXCL1-mediated neutrophil recruitment. *Nat Immunol* 20(5):559-570.

Duong, T. M. N., P. T. Nguyen, T. V. Le, H. L. P. Nguyen, B. N. T. Nguyen, B. P. T. Nguyen, T. A. Nguyen, S. C. Chen, V. R. Barrs, C. L. Halliday, T. C. Sorrell, J. N. Day, and J. Beardsley. 2020. Drug-resistant *Aspergillus flavus* is highly prevalent in the environment of Vietnam: A new challenge for the management of aspergillosis? *J Fungi (Basel)* 6(4).

Duong, T. N., T. V. Le, K. H. Tran, P. T. Nguyen, B. T. Nguyen, T. A. Nguyen, H. P. Nguyen, B. T. Nguyen, M. C. Fisher, J. Rhodes, G. Marks, G. J. Fox, S. C. Chen, M. G. Walsh, V. R. Barrs, J. Talbot, C. L. Halliday, T. C. Sorrell, J. N. Day, and J. Beardsley. 2021. Azole-resistant *Aspergillus fumigatus* is highly prevalent in the environment of Vietnam, with marked variability by land use type. *Environ Microbiol* 23(12):7632-7642.

Edwards, S. G., and N. P. Godley. 2010. Reduction of Fusarium head blight and deoxynivalenol in wheat with early fungicide applications of prothioconazole. *Food Additives & Contaminants: Part A* 27(5):629-635.

EPA (Environmental Protection Agency). 2022. *EPA proposes registration of the new fungicide ipflufenoquin*. https://www.epa.gov/pesticides/epa-proposes-registration-new-fungicide-ipflufenoquin (accessed August 1, 2022).

Etienne, K. A., E. L. Berkow, L. Gade, N. Nunnally, S. R. Lockhart, K. Beer, I. K. Jordan, L. Rishishwar, and A. P. Litvintseva. 2021. Genomic diversity of azole-resistant *Aspergillus fumigatus* in the United States. *mBio* 12(4):e0180321.

Fan, X., M. Xiao, K. Liao, T. Kudinha, H. Wang, L. Zhang, X. Hou, F. Kong, and Y. C. Xu. 2017. Notable increasing trend in azole non-susceptible *Candida tropicalis* causing invasive candidiasis in China (August 2009 to July 2014): Molecular epidemiology and clinical azole consumption. *Front Microbiol* 8:464.

FAO (Food and Agriculture Organization). 2016. *The FAO action plan on antimicrobial resistance, 2016–2020*. https://www.fao.org/3/i5996e/i5996e.pdf (accessed August 31, 2022).

FAO. 2021a. *Climate change fans spread of pests and threatens plants and crops, new fao study*. https://www.fao.org/news/story/en/item/1402920/icode/ (accessed August 31, 2022).

FAO. 2021b. *Strategic framework: 2022-31*. Rome: Food and Agriculture Organization of the United Nations.

FAO, and WHO (World Health Organization). 2021. Guidelines on integrated monitoring and surveillance of foodborne antimicrobial resistance. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B94-2021%252FCXG_94e.pdf (accessed August 23, 2022).

FAO, and WHO. 2022. *Foodborne antimicrobial resistance: Compendium of codex standards*. Rome: FAO; WHO.

Faria-Ramos, I., S. Farinha, J. Neves-Maia, P. R. Tavares, I. M. Miranda, L. M. Estevinho, C. Pina-Vaz, and A. G. Rodrigues. 2014. Development of cross-resistance by *Aspergillus fumigatus* to clinical azoles following exposure to prochloraz, an agricultural azole. *BMC Microbiol* 14:155.

FDA. 2018. FDA approves fostamatinib tablets for ITP. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-fostamatinib-tablets-itp

Fei, Z., A. G. Olenskyj, B. N. Bailey, and M. Earles. 2021. Enlisting 3D crop models and GANs for more data efficient and generalizable fruit detection. Paper read at Proceedings of the IEEE/CVF International Conference on Computer Vision.

Fernández-Ortuño, D., J. A. Torés, A. De Vicente, and A. Pérez-García. 2010. *The QOI fungicides, the rise and fall of a successful class of agricultural fungicides*: IntechOpen Croatia.

Fisher, M. C., A. Alastruey-Izquierdo, J. Berman, T. Bicanic, E. M. Bignell, P. Bowyer, M. Bromley, R. Bruggemann, G. Garber, O. A. Cornely, S. J. Gurr, T. S. Harrison, E. Kuijper, J. Rhodes, D. C. Sheppard, A. Warris, P. L. White, J. Xu, B. Zwaan, and P. E. Verweij. 2022. Tackling the emerging threat of antifungal resistance to human health. *Nat Rev Microbiol* 20(9):557-571.

Fisher, M. C., N. J. Hawkins, D. Sanglard, and S. J. Gurr. 2018. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science* 360(6390):739-742.

Fraaije, B., S. Atkins, S. Hanley, A. Macdonald, and J. Lucas. 2020. The multi-fungicide resistance status of *Aspergillus fumigatus* populations in arable soils and the wider european environment. *Front Microbiol* 11:599233.

Fuhren, J., W. S. Voskuil, C. H. Boel, P. J. Haas, F. Hagen, J. F. Meis, and J. G. Kusters. 2015. High prevalence of azole resistance in *Aspergillus fumigatus* isolates from high-risk patients. Journal of Antimicrobial Chemotherapy 70(10):2894-2898.

Fuller, K. B., J. M. Alston, and O. S. Sambucci. 2014. The value of powdery mildew resistance in grapes: Evidence from California. *Wine Economics and Policy* 3(2):90-107.

Fungicide Resistance Action Committee. 2022. *Frac code list 2022: Fungal control agents sorted by cross-resistance pattern and mode of action*. https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2022--final.pdf?sfvrsn=b6024e9a_2 (accessed August 1, 2022).

Gabaldón, T., and L. Carreté. 2016. The birth of a deadly yeast: Tracing the evolutionary emergence of virulence traits in *Candida glabrata*. *FEMS Yeast Research* 16(2):fov110.

Garcia-Rubio, R., M. Cuenca-Estrella, and E. Mellado. 2017. Triazole resistance in *Aspergillus* species: An emerging problem. *Drugs* 77(6):599-613.

Garrett, K. A. 2021. Impact network analysis and the INA R package: Decision support for regional management interventions. *Methods in Ecology and Evolution* 12(9):1634-1647.

Gianessi, L., and N. Reigner. 2006. The importance of fungicides in US crop production. *Outlooks on Pest Management* 17(5):209.

Global Action for Fungal Infections (Gaffi). 2022. *Policy brief: Chronic pulmonary aspergillosis: Global misdiagnosis of TB-like fungal lung disease.* Gaffi.

Golan, J. J., and A. Pringle. 2017. Long-distance dispersal of fungi. *Microbiol Spectr* 5(4):5.4.03.

Gold, J. A. W., E. E. Seagle, J. Nadle, D. M. Barter, C. A. Czaja, H. Johnston, M. M. Farley, S. Thomas, L. H. Harrison, J. Fischer, B. Pattee, R. K. Mody, E. C. Phipps, S. S. Davis, B. L. Tesini, A. Y. Zhang, T. M. Markus, W. Schaffner, S. R. Lockhart, S. Vallabhaneni, B. R. Jackson, and M. Lyman. 2021. Treatment practices for adults with candidemia at 9 active surveillance sites—United States, 2017-2018. *Clin Infect Dis* 73(9):1609-1616.

Goncalves, P., A. Melo, M. Dias, B. Almeida, L. A. Caetano, C. Verissimo, C. Viegas, and R. Sabino. 2020. Azole-resistant *Aspergillus fumigatus* harboring the TR34/L98H mutation: First report in portugal in environmental samples. *Microorganisms* 9(1).

Govender, N. P., J. Patel, R. E. Magobo, S. Naicker, J. Wadula, A. Whitelaw, Y. Coovadia, R. Kularatne, C. Govind, S. R. Lockhart, I. L. Zietsman, and T. R.-S. A. group. 2016. Emergence of azole-resistant *Candida parapsilosis* causing bloodstream infection: Results from laboratory-based sentinel surveillance in South Africa. *J Antimicrob Chemother* 71(7):1994-2004.

Gsaller, F., P. Hortschansky, T. Furukawa, P. D. Carr, B. Rash, J. Capilla, C. Muller, F. Bracher, P. Bowyer, H. Haas, A. A. Brakhage, and M. J. Bromley. 2016. Sterol biosynthesis and azole tolerance is governed by the opposing actions of SrbA and the CCAAT binding complex. *PLoS Pathog* 12(7):e1005775.

Guinea, J., P. E. Verweij, J. Meletiadis, J. W. Mouton, F. Barchiesi, M. C. Arendrup, and Subcommittee on Antifungal Susceptibility Testing of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2019. How to: EUCAST recommendations on the screening procedure e.Def 10.1 for the detection of azole resistance in *Aspergillus fumigatus* isolates using four-well azole-containing agar plates. *Clin Microbiol Infect* 25(6):681-687.

Hagan, A. 2018. *Invasive fungal infections: A creeping public health threat*. https://asm.org/Articles/2018/September/Invasive-Fungal-Infections-A-Creeping-Public-Healt (accessed July 26, 2022).

Hammond, E. E., C. S. McDonald, J. Vestbo, and D. W. Denning. 2020. The global impact of *Aspergillus* infection on COPD. *BMC Pulm Med* 20(1):241.

Herbrecht, R., P. Bories, J. C. Moulin, M. P. Ledoux, and V. Letscher-Bru. 2012. Risk stratification for invasive aspergillosis in immunocompromised patients. *Ann N Y Acad Sci* 1272:23-30.

Hernandez Nopsa, J. F., G. J. Daglish, D. W. Hagstrum, J. F. Leslie, T. W. Phillips, C. Scoglio, S. Thomas-Sharma, G. H. Walter, and K. A. Garrett. 2015. Ecological networks in stored grain: Key postharvest nodes for emerging pests, pathogens, and mycotoxins. *Bioscience* 65(10):985-1002.

Hevey, M. A., I. A. George, K. Raval, W. G. Powderly, and A. Spec. 2019. Presentation and mortality of cryptococcal infection varies by predisposing illness: A retrospective cohort study. *Am J Med* 132(8):977-983 e971.

HHS. 2021. *Presidential advisory council on combatting antibiotic-resistant bacteria*. https://www.hhs.gov/ash/advisory-committees/paccarb/meetings/past-meetings/index.html (accessed August 31, 2022).

Howard, S. J., D. Cerar, M. J. Anderson, A. Albarrag, M. C. Fisher, A. C. Pasqualotto, M. Laverdiere, M. C. Arendrup, D. S. Perlin, and D. W. Denning. 2009. Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* 15(7):1068-1076.

Hurst, S. F., E. L. Berkow, K. L. Stevenson, A. P. Litvintseva, and S. R. Lockhart. 2017. Isolation of azole-resistant *Aspergillus fumigatus* from the environment in the south-eastern USA. *J Antimicrob Chemother* 72(9):2443-2446.

International Chronic Granulomatous Disease Cooperative Study, G. 1991. A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. *N Engl J Med* 324(8):509-516.

Jackson, B. R., N. Chow, K. Forsberg, A. P. Litvintseva, S. R. Lockhart, R. Welsh, S. Vallabhaneni, and T. Chiller. 2019. On the origins of a species: What might explain the rise of *Candida auris*? *J Fungi (Basel)* 5(3).

Kang, S. E., L. G. Sumabat, T. Melie, B. Mangum, M. Momany, and M. T. Brewer. 2020. Evidence for the agricultural origin of antimicrobial resistance in a fungal pathogen of humans. *bioRxiv*:2020.2005.2024.113787.

Kang, S. E., L. G. Sumabat, T. Melie, B. Mangum, M. Momany, and M. T. Brewer. 2022. Evidence for the agricultural origin of resistance to multiple antimicrobials in *Aspergillus fumigatus*, a fungal pathogen of humans. *G3 (Bethesda)* 12(2).

Kelly, S. L., and D. E. Kelly. 2013. Microbial cytochromes P450: Biodiversity and biotechnology. Where do cytochromes P450 come from, what do they do and what can they do for us? *Philos Trans R Soc Lond B Biol Sci* 368(1612):20120476.

Kumar, V., S. C. Cheng, M. D. Johnson, S. P. Smeekens, A. Wojtowicz, E. Giamarellos-Bourboulis, J. Karjalainen, L. Franke, S. Withoff, T. S. Plantinga, F. L. van de Veerdonk, J. W. M. van der Meer, L. A. B. Joosten, P. Y. Bochud, O. Marchetti, J. R. Perfect, R. Xavier, B. J. Kullberg, C. Wijmenga, and M. G. Netea. 2014. Immunochip SNP array identifies novel genetic variants conferring susceptibility to candidaemia. *Nature Communications* 5:4675.

Lass-Florl, C., G. M. Salzer, T. Schmid, W. Rabl, H. Ulmer, and M. P. Dierichi. 1999. Pulmonary *Aspergillus* colonization in humans and its impact on management of critically ill patients. *Br J Haematol* 104(4):745-747.

Latgé, J. P. 1999. *Aspergillus fumigatus* and aspergillosis. *Clinical Microbiology Reviews* 12(2):310-350.

Lestrade, P. P., R. G. Bentvelsen, A. Schauwvlieghe, S. Schalekamp, W. van der Velden, E. J. Kuiper, J. van Paassen, B. van der Hoven, H. A. van der Lee, W. J. G. Melchers, A. F. de Haan, H. L. van der Hoeven, B. J. A. Rijnders, M. T. van der Beek, and P. E. Verweij. 2019a. Voriconazole resistance and mortality in invasive aspergillosis: A multicenter retrospective cohort study. *Clin Infect Dis* 68(9):1463-1471.

Lestrade, P. P. A., J. F. Meis, W. J. G. Melchers, and P. E. Verweij. 2019b. Triazole resistance in *Aspergillus fumigatus*: Recent insights and challenges for patient management. *Clin Microbiol Infect* 25(7):799-806.

Lionakis, M. S., N. D. Albert, M. Swamydas, C. R. Lee, P. Loetscher, and D. P. Kontoyiannis. 2017a. Pharmacological blockade of the chemokine receptor CCR1 protects mice from systemic candidiasis of hematogenous origin. *Antimicrob Agents Chemother* 61(3).

Lionakis, M. S., K. Dunleavy, M. Roschewski, B. C. Widemann, J. A. Butman, R. Schmitz, Y. Yang, D. E. Cole, C. Melani, C. S. Higham, J. V. Desai, M. Ceribelli, L. Chen, C. J. Thomas, R. F. Little, J. Gea-Banacloche, S. Bhaumik, M. Stetler-Stevenson, S. Pittaluga, E. S. Jaffe, J. Heiss, N. Lucas, S. M. Steinberg, L. M. Staudt, and W. H. Wilson. 2017b. Inhibition of B cell receptor signaling by ibrutinib in primary CNS lymphoma. *Cancer Cell* 31(6):833-843 e835.

Lionakis, M. S., B. G. Fischer, J. K. Lim, M. Swamydas, W. Wan, C. C. Richard Lee, J. I. Cohen, P. Scheinberg, J. L. Gao, and P. M. Murphy. 2012. Chemokine receptor CCR1 drives neutrophil-mediated kidney immunopathology and mortality in invasive candidiasis. *PLoS Pathog* 8(8):e1002865.

Lyman, M., K. Forsberg, J. Reuben, T. Dang, R. Free, E. E. Seagle, D. J. Sexton, E. Soda, H. Jones, D. Hawkins, A. Anderson, J. Bassett, S. R. Lockhart, E. Merengwa, P. Iyengar, B. R. Jackson, and T. Chiller. 2021. Notes from the field: Transmission of pan-resistant and echinocandin-resistant *Candida auris* in health care facilities—Texas and the District of Columbia, January-April 2021. *MMWR Morb Mortal Wkly Rep* 70(29):1022-1023.

Magill, S. S., E. O'Leary, S. J. Janelle, D. L. Thompson, G. Dumyati, J. Nadle, L. E. Wilson, M. A. Kainer, R. Lynfield, S. Greissman, S. M. Ray, Z. Beldavs, C. Gross, W. Bamberg, M. Sievers, C. Concannon, N. Buhr, L. Warnke, M. Maloney, V. Ocampo, J. Brooks, T. Oyewumi, S. Sharmin, K. Richards, J. Rainbow, M. Samper, E. B. Hancock, D. Leaptrot, E. Scalise, F. Badrun, R. Phelps, J. R. Edwards, and T. Emerging Infections Program Hospital Prevalence Survey. 2018. Changes in prevalence of health care-associated infections in U.S. hospitals. *N Engl J Med* 379(18):1732-1744.

Mahaffee, W. F., F. Margairaz, L. D. Ulmer, B. N. Bailey, and R. Stoll. 2023. Catching spores: Linking epidemiology, pathogen biology, and physics to ground-based airborne inoculum monitoring. *Plant Disease* 107(1):13-33.

Mazi, P. B., M. A. Olsen, D. Stwalley, A. M. Rauseo, C. Ayres, W. G. Powderly, and A. Spec. 2022. Attributable mortality of *Candida* bloodstream infections in the modern era: A propensity score analysis. *Clin Infect Dis* 75(6):1031-1036.

Messina, J. A., E. K. Maziarz, A. Spec, D. P. Kontoyiannis, and J. R. Perfect. 2017. Disseminated cryptococcosis with brain involvement in patients with chronic lymphoid malignancies on ibrutinib. *Open Forum Infect Dis* 4(1):ofw261.

Miles, T. D., T. M. Neill, M. Colle, B. Warneke, G. Robinson, I. Stergiopoulos, and W. F. Mahaffee. 2021. Allele-specific detection methods for QoI fungicide-resistant *Erysiphe necator* in vineyards. *Plant Dis* 105(1):175-182.

Mullick, A., M. Elias, S. Picard, L. Bourget, O. Jovcevski, S. Gauthier, A. Tuite, P. Harakidas, C. Bihun, B. Massie, and P. Gros. 2004. Dysregulated inflammatory response to *Candida albicans* in a C5-deficient mouse strain. *Infection and Immunity,* 72(10):5868-5876.

Nett, J. E., and D. R. Andes. 2016. Antifungal agents: Spectrum of activity, pharmacology, and clinical indications. *Infect Dis Clin North Am* 30(1):51-83.

Nguyen, N. T. B., H. Le Ngoc, N. V. Nguyen, L. V. Dinh, H. V. Nguyen, H. T. Nguyen, and D. W. Denning. 2021. Chronic pulmonary aspergillosis situation among post tuberculosis patients in Vietnam: An observational study. *J Fungi (Basel)* 7(7).

NHS (National Health Service) England. *NHS lands breakthrough in global battle against superbugs*. June 15, 2022. https://www.england.nhs.uk/2022/06/nhs-lands-breakthrough-in-global-battle-against-superbugs/

NICE (National Institute for Health Care Excellence). *NICE reaches important milestone in the UK's efforts to tackle antimicrobial resistance*. April 12, 2022. https://www.nice.org.uk/news/article/nice-reaches-important-milestone-in-the-uk-s-efforts-to-tackle-antimicrobial-resistance

NIH (National Institutes of Health). 2018. A study to evaluate the efficacy and safety of oral VT-1161 in patients with moderate - severe interdigital tinea pedis. https://clinicaltrials.gov/ct2/show/NCT01891305?term=NCT01891305&draw=2&rank=1 (accessed August 27, 2022).

NIH. 2020. A study to evaluate the efficacy and safety of oral VT-1161 in patients with onychomycosis of the toenail. https://clinicaltrials.gov/ct2/show/NCT02267356?term=NCT02267356&draw=2&rank=1 (accessed August 27, 2022).

NIH. 2021a. A study of oral oteseconazole (VT-1161) for the treatment of patients with recurrent vaginal candidiasis (yeast infection). https://clinicaltrials.gov/ct2/show/NCT03561701?term=NCT03561701&draw=2&rank=1 (accessed August 27, 2022).

NIH. 2021b. An efficacy and safety study of APX001 in non-neutropenic patients with candidemia. https://clinicaltrials.gov/ct2/show/NCT03604705?term=NCT03604705&draw=2&rank=1 (accessed August 27, 2022).

NIH. 2022a. An open-label study of APX001 for treatment of patients with candidemia/invasive candidiasis caused by *Candida auris*. https://clinicaltrials.gov/ct2/show/NCT04148287?term=NCT04148287&draw=2&rank=1 (accessed August 27, 2022).

NIH. 2022b. Open-label study of APX001 for treatment of patients with invasive mold infections caused by *Aspergillus* or rare molds. https://clinicaltrials.gov/ct2/show/NCT04240886?term=NCT04240886&draw=2&rank=1 (accessed August 27, 2022).

NIH. 2022c. Evaluate F901318 treatment of invasive fungal infections in patients lacking treatment options. https://clinicaltrials.gov/ct2/show/NCT03583164?term=NCT+03583164&draw=2&rank=1 (accessed August 27, 2022).

NIH. 2022d. Olorofim *Aspergillus* infection study. https://clinicaltrials.gov/ct2/show/NCT05101187?term=NCT05101187&draw=2&rank=1 (accessed August 27, 2022).

OECD (Organisation for Economic Co-operation and Development). 2018a. *Guidance document for the assessment of the equivalence of technical grade active ingredients for identical microbial strains and isolates*, *Series on pesticides and biocides*. Paris: OECD Publishing. https://www.oecd.org/chemicalsafety/guidance-document-for-the-assessment-of-the-equivalence-of-technical-grade-active-ingredients-for-identical-microbial-strains-489def79-en.htm

OECD. 2018b. *Working document on the risk assessment of secondary metabolites of microbial biocontrol agents*, *OECD environment, health and safety publications series on pesticides, no. 98*. Paris: OECD Publishing. https://www.oecd.org/chemicalsafety/working-document-on-the-risk-assessment-of-secondary-metabolites-of-microbial-biocontrol-agents-6ca93ea9-en.htm

OECD. 2019. *Customisation opportunities of iuclid for the management of chemical data*, *OECD series on testing and assessment, no. 297*. Paris: OECD Publishing.

Oerke, E.-C., A.-K. Mahlein, and U. Steiner. 2014. Proximal sensing of plant diseases. In *Detection and diagnostics of plant pathogens*, edited by M. L. Gullino and P. J. M. Bonants. Dordrecht: Springer Netherlands. Pp. 55-68.

Oliver, J. D., G. E. M. Sibley, N. Beckmann, K. S. Dobb, M. J. Slater, L. McEntee, S. du Pre, J. Livermore, M. J. Bromley, N. P. Wiederhold, W. W. Hope, A. J. Kennedy, D. Law, and M. Birch. 2016. F901318 represents a novel class of antifungal drug that inhibits dihydroorotate dehydrogenase. *Proceedings of the National Acad Sci U S A* 113(45):12809-12814.

Outterson, K. and J. Rex. *UK Pull (Netflix) Pilot: STEDI-Based QALY Value Of New Antibiotics Implies Societal Value > 10m GBP/Year!* April 11, 2022. AMR Solutions. https://amr.solutions/2022/04/11/uk-pull-netflix-pilot-qaly-value-of-new-antibiotics-implies-societal-value-10m-gbp-year/

Pappas, P. G., C. A. Kauffman, D. R. Andes, C. J. Clancy, K. A. Marr, L. Ostrosky-Zeichner, A. C. Reboli, M. G. Schuster, J. A. Vazquez, T. J. Walsh, T. E. Zaoutis, and J. D. Sobel. 2016. Clinical practice guideline for the management of candidiasis: 2016 update by the infectious diseases society of america. *Clin Infect Dis* 62(4):e1-50.

Pfaller, M. A., M. Castanheira, S. R. Lockhart, A. M. Ahlquist, S. A. Messer, and R. N. Jones. 2012. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of *Candida glabrata*. *J Clin Microbiol* 50(4):1199-1203.

Pianalto, K. M., and J. A. Alspaugh. 2016. New horizons in antifungal therapy. *J Fungi (Basel)* 2(4).

Pontes, L., C. A. G. Beraquet, T. Arai, G. L. Pigolli, L. Lyra, A. Watanabe, M. L. Moretti, and A. Z. Schreiber. 2020. *Aspergillus fumigatus* clinical isolates carrying CYP51A with TR34/L98H/S297T/F495I substitutions detected after four-year retrospective azole resistance screening in Brazil. *Antimicrob Agents Chemother* 64(3).

Price, C. L., J. E. Parker, A. G. Warrilow, D. E. Kelly, and S. L. Kelly. 2015. Azole fungicides - understanding resistance mechanisms in agricultural fungal pathogens. *Pest Manag Sci* 71(8):1054-1058.

Pristov, K. E., and M. A. Ghannoum. 2019. Resistance of *Candida* to azoles and echinocandins worldwide. *Clin Microbiol Infect* 25(7):792-798.

Protsiv, M., C. Ley, J. Lankester, T. Hastie, and J. Parsonnet. 2020. Decreasing human body temperature in the United States since the industrial revolution. *Elife* 9:e49555.

Rao, V. U. S., G. Arakeri, G. Madikeri, A. Shah, R. S. Oeppen, and P. A. Brennan. 2021. COVID-19 associated mucormycosis (CAM) in India: A formidable challenge. *Br J Oral Maxillofac Surg* 59(9):1095-1098.

Rauseo, A. M., A. Coler-Reilly, L. Larson, and A. Spec. 2020. Hope on the horizon: Novel fungal treatments in development. *Open Forum Infect Dis* 7(2):ofaa016.

Ren, J., X. Jin, Q. Zhang, Y. Zheng, D. Lin, and Y. Yu. 2017. Fungicides induced triazole-resistance in *Aspergillus fumigatus* associated with mutations of TR46/Y121F/T289A and its appearance in agricultural fields. *J Hazard Mater* 326:54-60.

Resendiz-Sharpe, A., K. Dewaele, R. Merckx, B. Bustamante, M. C. Vega-Gomez, M. Rolon, J. Jacobs, P. E. Verweij, J. Maertens, and K. Lagrou. 2021. Triazole-resistance in environmental *Aspergillus fumigatus* in Latin American and african countries. *J Fungi (Basel)* 7(4).

Rex, J., and Krause, K. *All-In Cost Of A New Antibiotic From Discovery To 10 Years On Market*. January 9, 2021. AMR Solutions. https://amr.solutions/2021/01/09/all-in-cost-of-a-new-antibiotic-from-discovery-to-10-years-on-market/

Rhodes, J., A. Abdolrasouli, K. Dunne, T. R. Sewell, Y. Zhang, E. Ballard, A. P. Brackin, N. van Rhijn, H. Chown, A. Tsitsopoulou, R. B. Posso, S. H. Chotirmall, N. G. McElvaney, P. G. Murphy, A. F. Talento, J. Renwick, P. S. Dyer, A. Szekely, P. Bowyer, M. J. Bromley, E. M. Johnson, P. Lewis White, A. Warris, R. C. Barton, S. Schelenz, T. R. Rogers, D. Armstrong-James, and M. C. Fisher. 2022. Population genomics confirms acquisition of drug-resistant *Aspergillus fumigatus* infection by humans from the environment. *Nat Microbiol* 7(5):663-674.

Rhodes, J., A. Abdolrasouli, K. Dunne, T. R. Sewell, Y. Zhang, E. Ballard, A. P. Brackin, N. van Rhijn, A. Tsitsopoulou, R. B. Posso, S. H. Chotirmall, N. G. McElvaney, P. G. Murphy, A. F. Talento, J. Renwick, P. S. Dyer, A. Szekely, M. J. Bromley, E. M. Johnson, P. L. White, A. Warris, R. C. Barton, S. Schelenz, T. R. Rogers, D. Armstrong-James, and M. C. Fisher. 2021. Tracing patterns of evolution and acquisition of drug resistant *Aspergillus fumigatus* infection from the environment using population genomics. *bioRxiv*:2021.2004.2007.438821.

Rhodes, J., and M. C. Fisher. 2019. Global epidemiology of emerging *Candida auris*. *Curr Opin Microbiol* 52:84-89.

Richtel, M., and A. Jacobs. 2019. Fungus immune to drugs quietly sweeps the globe. *The New York Times*, April 7, 2019.

Robert, V. A., and A. Casadevall. 2009. Vertebrate endothermy restricts most fungi as potential pathogens. *J Infect Dis* 200(10):1623-1626.

Rocchi, S., E. Daguindau, F. Grenouillet, E. Deconinck, A.-P. Bellanger, D. Garcia-Hermoso, S. Bretagne, G. Reboux, and L. Millon. 2014. Azole-resistant *Aspergillus fumigatus* isolate with the TR34/L98H mutation in both a fungicide-sprayed field and the lung of a hematopoietic stem cell transplant recipient with invasive aspergillosis. *Journal of Clinical Microbiology* 52(5):1724-1726.

Roschewski, M., M. S. Lionakis, C. Melani, J. A. Butman, S. Pittaluga, A. N. Lucas, S. M. Steinberg, E. S. Jaffe, R. F. Little, L. M. Staudt, and W. H. Wilson. 2018. Dose-adjusted TEDDI-R induces durable complete remissions in relapsed and refractory primary CNS lymphoma. *Blood* 132(Supplement 1):4195-4195.

Rutjanawech, S., C. Mejia-Chew, C. Ayres, and A. Spec. 2021. A canker barking at the wrong knee: *Thyronectria austroamericana* septic arthritis. *Open Forum Infect Dis* 8(8):ofab381.

Satapute, P., and B. Kaliwal. 2016. Biodegradation of the fungicide propiconazole by *Pseudomonas aeruginosa* PS-4 strain isolated from a paddy soil. *Annals of Microbiology* 66(4):1355-1365.

Savary, S., L. Willocquet, S. J. Pethybridge, P. Esker, N. McRoberts, and A. Nelson. 2019. The global burden of pathogens and pests on major food crops. *Nat Ecol Evol* 3(3):430-439.

Schnabel, G., and D. R. Layne. 2004. Comparison of reduced-application and sulfur-based fungicide programs on scab intensity, fruit quality, and cost of disease control on peach. *Plant Dis* 88(2):162-166.

Schoustra, S., J. Zhang, B. Zwaan, A. Debets, P. Verweij, D. Buijtenhuijs, and A. Rietveld. 2019a. *New insights in the development of azole-resistance in Aspergillus fumigatus*. National Institute for Public Health and the Environment.

Schoustra, S. E., A. J. M. Debets, A. Rijs, J. Zhang, E. Snelders, P. C. Leendertse, W. J. G. Melchers, A. G. Rietveld, B. J. Zwaan, and P. E. Verweij. 2019b. Environmental hotspots for azole resistance selection of *Aspergillus fumigatus*, the Netherlands. *Emerg Infect Dis* 25(7):1347-1353.

Shelton, J. M. G. 2021. *Environmental drivers of azole-resistance in Aspergillus fumigatus and its relevance to human health (dissertation)*. London, UK: Imperial College London.

Shelton, J. M. G., R. Collins, C. B. Uzzell, A. Alghamdi, P. S. Dyer, A. C. Singer, and M. C. Fisher. 2022. Citizen science surveillance of triazole-resistant *Aspergillus fumigatus* in United Kingdom residential garden soils. *Appl Environ Microbiol* 88(4):e0206121.

Shelton, J. M. G., M. C. Fisher, and A. C. Singer. 2020. Campaign-based citizen science for environmental mycology: The science solstice and summer soil-stice projects to assess drug resistance in air- and soil-borne *Aspergillus fumigatus*. *Citizen Science: Theory and Practice* 5(1):20.

Singla, R., R. Singhal, R. Rathore, A. Gupta, P. Sethi, V. P. Myneedu, A. Chakraborty, and V. Kumar. 2021. Risk factors for chronic pulmonary aspergillosis in post-TB patients. *Int J Tuberc Lung Dis* 25(4):324-326.

Smeekens, S. P., A. Ng, V. Kumar, M. D. Johnson, T. S. Plantinga, C. van Diemen, P. Arts, E. T. Verwiel, M. S. Gresnigt, K. Fransen, S. van Sommeren, M. Oosting, S. C. Cheng, L. A. Joosten, A. Hoischen, B. J. Kullberg, W. K. Scott, J. R. Perfect, J. W. van der Meer, C. Wijmenga, M. G. Netea, and R. J. Xavier. 2013. Functional genomics identifies type I interferon pathway as central for host defense against *Candida albicans*. *Nature Communications* 4:1342.

Smothers, H. 2016. A deadly, drug-resistant yeast infection is spreading around the world, it's fine guys. *Cosmopolitan*.

Snelders, E., S. M. Camps, A. Karawajczyk, G. Schaftenaar, G. H. Kema, H. A. van der Lee, C. H. Klaassen, W. J. Melchers, and P. E. Verweij. 2012. Triazole fungicides can induce cross-resistance to medical triazoles in *Aspergillus fumigatus*. *PLoS One* 7(3):e31801.

Snelders, E., W. J. Melchers, and P. E. Verweij. 2011. Azole resistance in *Aspergillus fumigatus*: A new challenge in the management of invasive aspergillosis? *Future Microbiol* 6(3):335-347.

Snelders, E., H. A. van der Lee, J. Kuijpers, A. J. Rijs, J. Varga, R. A. Samson, E. Mellado, A. R. Donders, W. J. Melchers, and P. E. Verweij. 2008. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* 5(11):e219.

Song, Y., J. B. Buil, W. J. G. Melchers, J. Zoll, and P. E. Verweij. 2022. P001 characteristics and dynamics of azole-resistant *Aspergillus fumigatus* variants emerging over a 28-year period in the Netherlands. *Medical Mycology* 60(Supplement_1).

Southwick, K., E. H. Adams, J. Greenko, B. Ostrowsky, R. Fernandez, R. Patel, M. Quinn, S. Vallabhaneni, R. J. Denis, R. Erazo, S. Chaturvedi, V. B. Haley, L. Leach, Y. C. Zhu, R. Giardina, E. C. Lutterloh, and D. S. Blog. 2018. 2039. New york state 2016–2018: Progression from *Candida auris* colonization to bloodstream infection. *Open Forum Infectious Diseases* 5(suppl_1):S594-S595.

Souza, A. C., B. B. Fuchs, H. M. Pinhati, R. A. Siqueira, F. Hagen, J. F. Meis, E. Mylonakis, and A. L. Colombo. 2015. *Candida parapsilosis* resistance to fluconazole: Molecular mechanisms and in vivo impact in infected *Galleria mellonella* larvae. *Antimicrob Agents Chemother* 59(10):6581-6587.

Spec, A., G. Escota, C. Chrisler, and B. Davies. 2019. *Comprehensive review of infectious diseases*. Cambridge, MA: Elsevier.

Steffen, W., W. Broadgate, L. Deutsch, O. Gaffney, and C. Ludwig. 2015. The trajectory of the anthropocene: The great acceleration. *The Anthropocene Review* 2(1):81-98.

Stevens, D. 2011. Systemic antifungal agents. In *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, 7 ed.*, edited by G. Mandell, J. Bennett and R. Dolin. Philadelphia, PA: Churchill Livingstone. Pp. 549-563.

Sucher, A. J., E. B. Chahine, and H. E. Balcer. 2009. Echinocandins: The newest class of antifungals. *Ann Pharmacother* 43(10):1647-1657.

Swamydas, M., J. L. Gao, T. J. Break, M. D. Johnson, M. Jaeger, C. A. Rodriguez, J. K. Lim, N. M. Green, A. L. Collar, B. G. Fischer, C. C. Lee, J. R. Perfect, B. D. Alexander, B. J. Kullberg, M. G. Netea, P. M. Murphy, and M. S. Lionakis. 2016. CXCR1-mediated neutrophil degranulation and fungal killing promote *Candida* clearance and host survival. *Sci Transl Med* 8(322):322ra310.

Tenforde, M. W., A. M. Gertz, D. S. Lawrence, N. K. Wills, B. L. Guthrie, C. Farquhar, and J. N. Jarvis. 2020. Mortality from hiv-associated meningitis in sub-Saharan Africa: A systematic review and meta-analysis. *Journal of the International AIDS Society* 23(1):e25416-e25416.

Thiessen, L. D., J. A. Keune, T. M. Neill, W. W. Turechek, G. G. Grove, and W. F. Mahaffee. 2016. Development of a grower-conducted inoculum detection assay for management of grape powdery mildew. *Plant Pathology* 65(2):238-249.

Thiessen, L. D., T. M. Neill, and W. F. Mahaffee. 2017. Timing fungicide application intervals based on airborne *Erysiphe necator* concentrations. *Plant Dis* 101(7):1246-1252.

Thiessen, L. D., T. M. Neill, and W. F. Mahaffee. 2018. Development of a quantitative loop-mediated isothermal amplification assay for the field detection of *Erysiphe necator*. *PeerJ* 6:e4639.

Toda, M., K. D. Beer, K. M. Kuivila, T. M. Chiller, and B. R. Jackson. 2021. Trends in agricultural triazole fungicide use in the united states, 1992-2016 and possible implications for antifungal-resistant fungi in human disease. *Environ Health Perspect* 129(5):55001.

Toda M., Williams S.R., Berkow E.L., et al. Population-Based Active Surveillance for Culture-Confirmed Candidemia — Four Sites, United States, 2012–2016. MMWR Surveillance Summmary 2019;68(No. SS-8):1–15. DOI: http://dx.doi.org/10.15585/mmwr.ss6808a1

Trevino-Rangel, R. J., H. Villanueva-Lozano, A. Bonifaz, L. R. Castanon-Olivares, A. Andrade, M. A. Becerril-Garcia, M. F. Martinez-Resendez, J. Ayala-Gaytan, A. M. Montoya, and G. M. Gonzalez. 2021. Species distribution and antifungal susceptibility patterns of *Aspergillus* isolates from clinical specimens and soil samples in Mexico. *Med Mycol* 59(10):1006-1014.

Ullmann, A. J., J. M. Aguado, S. Arikan-Akdagli, D. W. Denning, A. H. Groll, K. Lagrou, C. Lass-Flörl, R. E. Lewis, P. Munoz, P. E. Verweij, A. Warris, F. Ader, M. Akova, M. C. Arendrup, R. A. Barnes, C. Beigelman-Aubry, S. Blot, E. Bouza, R. J. M. Brüggemann, D. Buchheidt, J. Cadranel, E. Castagnola, A. Chakrabarti, M. Cuenca-Estrella, G. Dimopoulos, J. Fortun, J. P. Gangneux, J. Garbino, W. J. Heinz, R. Herbrecht, C. P. Heussel, C. C. Kibbler, N. Klimko, B. J. Kullberg, C. Lange, T. Lehrnbecher, J. Löffler, O. Lortholary, J. Maertens, O. Marchetti, J. F. Meis, L. Pagano, P. Ribaud, M. Richardson, E. Roilides, M. Ruhnke, M. Sanguinetti, D. C. Sheppard, J. Sinkó, A. Skiada, M. J. G. T. Vehreschild, C. Viscoli, and O. A. Cornely. 2018. Diagnosis and management of *Aspergillus* diseases: Executive summary of the 2017 ESCMID-ECMM-ERS guideline. *Clinical Microbiology and Infection* 24:e1-e38.

van den Bosch, F., F. Lopez-Ruiz, R. Oliver, N. Paveley, J. Helps, and F. van den Berg. 2018. Identifying when it is financially beneficial to increase or decrease fungicide dose as resistance develops. *Plant Pathology* 67(3):549-560.

van der Linden, J. W., S. M. Camps, G. A. Kampinga, J. P. Arends, Y. J. Debets-Ossenkopp, P. J. Haas, B. J. Rijnders, E. J. Kuijper, F. H. van Tiel, J. Varga, A. Karawajczyk, J. Zoll, W. J. Melchers, and P. E. Verweij. 2013. Aspergillosis due to voriconazole highly resistant *Aspergillus fumigatus* and recovery of genetically related resistant isolates from domiciles. *Clin Infect Dis* 57(4):513-520.

Van Der Linden, J. W., A. Warris, and P. E. Verweij. 2011. *Aspergillus* species intrinsically resistant to antifungal agents. *Med Mycol* 49 Suppl 1(Supplement_1):S82-89.

van der Wurff, A. W., J. Fuchs, M. Raviv, and A. Termorshuizen. 2016. *Handbook for composting and compost use in organic horticulture*: BioGreenhouse.

Vergidis, P., C. B. Moore, L. Novak-Frazer, R. Rautemaa-Richardson, A. Walker, D. W. Denning, and M. D. Richardson. 2020. High-volume culture and quantitative real-time PCR for the detection of *Aspergillus* in sputum. *Clin Microbiol Infect* 26(7):935-940.

Verweij, P. E., M. Ananda-Rajah, D. Andes, M. C. Arendrup, R. J. Bruggemann, A. Chowdhary, O. A. Cornely, D. W. Denning, A. H. Groll, K. Izumikawa, B. J. Kullberg, K. Lagrou, J. Maertens, J. F. Meis, P. Newton, I. Page, S. Seyedmousavi, D. C. Sheppard, C. Viscoli, A. Warris, and J. P. Donnelly. 2015. International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*. *Drug Resist Updat* 21-22:30-40.

Verweij, P. E., A. Chowdhary, W. J. Melchers, and J. F. Meis. 2016. Azole resistance in *Aspergillus fumigatus*: Can we retain the clinical use of mold-active antifungal azoles? *Clin Infect Dis* 62(3):362-368.

Verweij, P. E., E. Mellado, and W. J. Melchers. 2007. Multiple-triazole-resistant aspergillosis. *N Engl J Med* 356(14):1481-1483.

Verweij, P. E., E. Snelders, G. H. Kema, E. Mellado, and W. J. Melchers. 2009. Azole resistance in *Aspergillus fumigatus*: A side-effect of environmental fungicide use? *Lancet Infect Dis* 9(12):789-795.

Viegas, C., B. Almeida, L. Aranha Caetano, A. Afanou, A. Straumfors, C. Verissimo, P. Goncalves, and R. Sabino. 2022. Algorithm to assess the presence of *Aspergillus fumigatus* resistant strains: The case of Norwegian sawmills. *Int J Environ Health Res* 32(5):963-971.

Viegas, C., T. Faria, L. A. Caetano, E. Carolino, A. Q. Gomes, and S. Viegas. 2017. *Aspergillus* spp. Prevalence in different portuguese occupational environments: What is the real scenario in high load settings? *J Occup Environ Hyg* 14(10):771-785.

Warrilow, A. G., C. M. Hull, J. E. Parker, E. P. Garvey, W. J. Hoekstra, W. R. Moore, R. J. Schotzinger, D. E. Kelly, and S. L. Kelly. 2014. The clinical candidate VT-1161 is a highly potent inhibitor of *Candida albicans* CYP51 but fails to bind the human enzyme. *Antimicrob Agents Chemother* 58(12):7121-7127.

Weiner-Lastinger, L. M., K. Haass, C. Gross, D. Leaptrot, E. Wong, H. Wu, and M. A. Dudeck. 2022. Pathogens attributed to central line-associated bloodstream infections in US acute care hospitals during the first year of the COVID-19 pandemic. *Infect Control Hosp Epidemiol*:1-13.

WHO (World Health Organization). 2017. *Integrated surveillance of antimicrobial resistance in foodborne bacteria: Application of a One Health approach*. Geneva: World Health Organization.

WHO. 2018. *Mycotoxins*. https://www.who.int/news-room/fact-sheets/detail/mycotoxins (accessed August 31, 2022).

WHO. 2019. *No time to Wait: Securing the future from drug-resistant infections*. https://www.who.int/publications/i/item/no-time-to-wait-securing-the-future-from-drug-resistant-infections (accessed February 17, 2023).

WHO. 2020. *First meeting of the who antifungal expert group on identifying priority fungal pathogens: Meeting report*. Geneva: World Health Organization.

WHO. 2021. *Antimicrobial resistance*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance (accessed August 31, 2022).

WHO, FAO, and OIE (World Organisation for Animal Health). 2019. *Monitoring and evaluation of the global action plan on antimicrobial resistance: Framework and recommended indicators*. Geneva: World Health Organization.

WHO, FAO, OIE, UNEP (United Nations Environment Programme). 2022a. *Antimicrobial resistance multi-partner trust fund annual report 2021*. Geneva: WHO, FAO, OIE, UNEP.

WHO, FAO, OIE, UNEP. 2022b. *Strategic framework for collaboration on antimicrobial resistance—together for One Health*. Geneva: World Health Organization, Food and Agriculture Organization of the United Nations and World Organization for Animal Health.

Wiederhold, N. P., V. G. Gil, F. Gutierrez, J. R. Lindner, M. T. Albataineh, D. I. McCarthy, C. Sanders, H. Fan, A. W. Fothergill, and D. A. Sutton. 2016. First detection of TR34 L98H and TR46 Y121F T289A *cyp51* mutations in *Aspergillus fumigatus* isolates in the United States. *J Clin Microbiol* 54(1):168-171.

Wiederhold, N. P., and P. E. Verweij. 2020. *Aspergillus fumigatus* and pan-azole resistance: Who should be concerned? *Curr Opin Infect Dis* 33(4):290-297.

Wiegand, S., R. Berner, A. Schneider, E. Lundershausen, and A. Dietz. 2019. Otitis externa: Investigation and evidence-based treatment. *Deutsches Ärzteblatt International* 116: 224–34.

Winters, B., J. Custer, S. M. Galvagno, Jr., E. Colantuoni, S. G. Kapoor, H. Lee, V. Goode, K. Robinson, A. Nakhasi, P. Pronovost, and D. Newman-Toker. 2012. Diagnostic errors in the intensive care unit: A systematic review of autopsy studies. *BMJ Qual Saf* 21(11):894-902.

Wouters, O. J., M. McKee, and J. Luyten. 2020. Estimated research and development investment needed to bring a new medicine to market, 2009-2018. *JAMA* 323(9):844-853.

Wu, P., T. E. Nielsen, and M. H. Clausen. 2016. Small-molecule kinase inhibitors: An analysis of FDA-approved drugs. *Drug Discov Today* 21(1):5-10.

Xing, Y., J. F. Hernandez Nopsa, K. F. Andersen, J. L. Andrade-Piedra, F. D. Beed, G. Blomme, M. Carvajal-Yepes, D. L. Coyne, W. J. Cuellar, G. A. Forbes, J. F. Kreuze, J. Kroschel, P. L. Kumar, J. P. Legg, M. Parker, E. Schulte-Geldermann, K. Sharma, and K. A. Garrett. 2020. Global cropland connectivity: A risk factor for invasion and saturation by emerging pathogens and pests. *Bioscience* 70(9):744-758.

Xu, H., L. Li, W. J. Huang, L. X. Wang, W. F. Li, and W. F. Yuan. 2012. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: A case control study from China. *Clin Microbiol Infect* 18(4):403-408.

Zarakas, M. A., J. V. Desai, G. Chamilos, and M. S. Lionakis. 2019. Fungal infections with ibrutinib and other small-molecule kinase inhibitors. Curr Fungal Infect Rep 13(3):86-98.

Zhang, J., A. J. Debets, P. E. Verweij, W. J. Melchers, B. J. Zwaan, and S. E. Schoustra. 2015. Asexual sporulation facilitates adaptation: The emergence of azole resistance in *Aspergillus fumigatus*. *Evolution* 69(10):2573-2586.

Zhang, J., A. J. M. Debets, P. E. Verweij, and S. E. Schoustra. 2021a. Selective flamingo medium for the isolation of *Aspergillus fumigatus*. *Microorganisms* 9(6).

Zhang, J., A. J. M. Debets, P. E. Verweij, and E. Snelders. 2021b. Azole-resistance development; how the *Aspergillus fumigatus* lifecycle defines the potential for adaptation. *J Fungi (Basel)* 7(8).

Zhang, J., L. Lopez Jimenez, E. Snelders, A. J. M. Debets, A. G. Rietveld, B. J. Zwaan, P. E. Verweij, and S. E. Schoustra. 2021c. Dynamics of *Aspergillus fumigatus* in azole fungicide-containing plant waste in the Netherlands (2016-2017). *Appl Environ Microbiol* 87(2):e02295-02220.

Zhang, J., E. Snelders, B. J. Zwaan, S. E. Schoustra, J. F. Meis, K. van Dijk, F. Hagen, M. T. van der Beek, G. A. Kampinga, J. Zoll, W. J. G. Melchers, P. E. Verweij, and A. J. M. Debets. 2017a. A novel environmental azole resistance mutation in *Aspergillus fumigatus* and a possible role of sexual reproduction in its emergence. *mBio* 8(3).

Zhang, J., J. van den Heuvel, A. J. M. Debets, P. E. Verweij, W. J. G. Melchers, B. J. Zwaan, and S. E. Schoustra. 2017b. Evolution of cross-resistance to medical triazoles in *Aspergillus fumigatus* through selection pressure of environmental fungicides. *Proc Biol Sci* 284(1863).

Zhang, J., P. E. Verweij, A. Rijs, A. J. M. Debets, and E. Snelders. 2021d. Flower bulb waste material is a natural niche for the sexual cycle in *Aspergillus fumigatus*. *Front Cell Infect Microbiol* 11:785157.

Zhou, D., G. A. Korfanty, M. Mo, R. Wang, X. Li, H. Li, S. Li, J. Y. Wu, K. Q. Zhang, Y. Zhang, and J. Xu. 2021. Extensive genetic diversity and widespread azole resistance in greenhouse populations of *Aspergillus fumigatus* in Yunnan, China. *mSphere* 6(1).

Appendix A

Statement of Task

THE ROLE OF PLANT AGRICULTURAL PRACTICES ON DEVELOPMENT OF ANTIMICROBIAL RESISTANT FUNGI AFFECTING HUMAN HEALTH: A WORKSHOP

A planning committee of the National Academies of Sciences, Engineering, and Medicine will organize and conduct a public workshop series to shed light on: 1) the magnitude of environmentally induced/selected antimicrobial resistance (AMR) in agricultural practices worldwide, with a focus on plant crop production; 2) the practices that contribute to AMR in human pathogens, 3) surveillance strategies, and 4) mitigation strategies.

The public workshop series will feature invited presentations and discussions to explore the following questions:

- What is the magnitude of antifungal use in crop production in high-, middle- and low-income countries? How are such uses regulated?
- What are the mechanisms of AMR in plant pathogens and non-target environmental microbiota? How might this influence AMR in human pathogens?
- Which practices promote, prevent, or reduce the development of AMR in plant production environments, specifically in fungal pathogens? How does this affect risk of produce contamination with AMR pathogens?
- Are sampling and testing technologies for AMR surveillance in plant production systems adequate? What further evidence is

needed to inform the use of antimicrobials worldwide? What further evidence is needed to understand the presence and effects of environmental AMR on human health?

The planning committee will organize the workshop, develop the agenda, select and invite speakers and discussants, and moderate or identify moderators for the discussions. One proceedings publication that reports on the presentations and discussions held during this workshop will be prepared by a designated rapporteur in accordance with institutional guidelines.

Appendix B

Workshop Agenda

THE ROLE OF PLANT AGRICULTURAL PRACTICES ON DEVELOPMENT OF ANTIMICROBIAL RESISTANT FUNGI AFFECTING HUMAN HEALTH: A WORKSHOP

TUESDAY, JUNE 21, 2022 (all times in EDT)

Bridging the Two Worlds: Fungal Pathogens in Plant and Human Health

10:00 AM–10:10 AM	**Welcome Remarks, Workshop Overview, and Goals** **Paige Waterman,** Uniformed Services University of Health Sciences *Workshop co-chair*
10:10 AM–11:05 AM	Opening Panel **Tom Chiller,** U.S. Centers for Disease Control and Prevention *Moderator*
	Antifungal drug uses in human medicine **Arturo Casadevall,** Johns Hopkins University Bloomberg School of Public Health

	Fungicide uses in plant agriculture **Tony Dorn**, U.S. Department of Agriculture
	TBD
11:05 AM-11:20 AM	Break
11:20 AM-12:35 PM	Fungal Diseases, Antifungal Resistance, and Human Health **Tom Chiller**, U.S. Centers for Disease Control and Prevention *Moderator*
	Impact of invasive fungal diseases and antifungal drug resistance on human health **Andrej Spec**, Washington University at St. Louis
	Overview of aspergillosis and mechanisms of antifungal drug resistance **David Denning**, Global Action For Fungal Infections
	Overview of drug-resistant, invasive *Candida* infections **Brendan Jackson**, U.S. Centers for Disease Control and Prevention
	Current research in understanding pathogenesis and host immunity toward developing treatment options **Michail Lionakis**, National Institute of Allergy and Infectious Diseases
12:35 PM–1:15 PM	Fungicide Resistance in Plant Protection Use **Marin Talbot Brewer**, University of Georgia *Moderator*
	Genetics and mechanisms of fungicide resistance **Matthew Fisher**, Imperial College London
	Azole-resistant *Aspergillus fumigatus* in agronomic settings: hotspots and coldspots **Kevin Doughty**, CropLife International

1:15 PM–1:55 PM Discussion

1:55 PM–2:00 PM Summary and Adjourn

END OF DAY 1

WEDNESDAY, JUNE 22, 2022 (all times in EDT)

Effects of Fungicide Use in Plants on Humans and the Environment

10:00 AM–10:05 AM Welcome Remarks, Review of Day 1
Jeff LeJeune, Food and Agriculture Organization of the United Nations

10:05 AM–10:45AM **Role of Fungicide Use in Food Safety and Security**
Phil Taylor, Centre for Agriculture and Bioscience International (CABI)
Moderator

Case examples to illustrate (1) the role of azole fungicides in safeguarding food safety and security, and (2) potential drivers of fungicide use in the US

Tim Brenneman, University of Georgia
Pierce Paul, The Ohio State University

10:45 AM-12:15PM Dimensions of Fungicide Regulations, Use Management, and Risk Assessment
Lynn Goldman, George Washington University Milken Institute School of Public Health
Moderator

Documentation of azole fungicide use in plants in low- and middle-income countries
Phil Taylor, Centre for Agriculture and Bioscience International (CABI)

	Registration and health threats assessment of fungicides **Nathan Mellor,** US Environmental Protection Agency **Magdalini Sachana,** Organisation for Economic Co-operation and Development
	Occupational exposure to environmental resistant fungi and possible implications in human health **Raquel Sabino,** Portuguese National Institute of Health Dr. Ricardo Jorge
12:15 PM-12:30 PM	**Break**
12:30 PM–1:15 PM	**Innovations in Antimicrobial Resistance Surveillance Tools and Technologies** **Jeff LeJeune,** Food and Agriculture Organization of the United Nations *Moderator*
	Aspergillus fumigatus azole resistance survey by air sampling through a citizen science approach **Wieland Meyer,** University of Sydney Westmead Institute for Medical Research
	Innovation in data collection tools for surveillance and mitigation: crop and environment simulation tools **Brian Bailey,** University of California, Davis
	Sampling strategies, interpretation of sampling outcomes, and evaluation of surveillance and management technologies **Karen Garrett,** University of Florida
1:15 PM–1:55 PM	**Discussion**
1:55 PM–2:00 PM	Summary and Adjourn

END OF DAY 2

MONDAY, JUNE 27, 2022

Mitigation Strategies for Fungicide/Antifungal Resistance

8:00 AM–10:00 AM	In-Person Networking and Breakfast
10:00 AM–10:15 AM	**Welcome virtual attendees; Review of workshop thus far** **Paige Waterman**, Uniformed Services University of Health Sciences *workshop co-chair*
10:15 AM–11:45 AM	One Health Approach to Antimicrobial Resistance (AMR) and Pathogen Surveillance Maryn McKenna *Moderator* The necessity and benefits of taking a One Health approach in AMR surveillance and mitigation measures **Paul Verweij**, Radboud University **Bas Zwaan**, Wageningen University Genomic surveillance and epidemiology **Marin Talbot Brewer**, University of Georgia Diagnostics, resistance testing, and surveillance capabilities in local and national levels: health care systems and agricultural systems **Shawn Lockhart**, U.S. Centers for Disease Control and Prevention One Health approach to AMR and pathogen surveillance at the international level **Jorge Pinto Ferreira**, Food and Agriculture Organization of the United Nations
11:45 AM–12:15 PM	Break
12:15 PM–1:00 PM	**Antifungal Drug Development and Stewardship in Health Care** **Maryn McKenna** *Moderator*

	New antifungal drugs in development with different modes of action **David Andes,** University of Wisconsin
	Incentives and market for drug development and global access **John Rex,** F2G
1:00 PM–1:50 PM	**Integrated Disease Management** **Tim Widmer,** U.S. Department of Agriculture *Moderator*
	Precision application technologies and communication with growers and other stakeholders **Melanie Ivey,** The Ohio State University
	Current research toward targeted approaches and considering localized risk factors **Walt Mahaffee,** U.S. Department of Agriculture
	Mitigation measures in compost "hot spots" **Jianhua Zhang,** Wageningen University
1:50 PM–2:00 PM	Summary of Workshop
2:30 PM–4:30 PM	Poster Session

END OF WORKSHOP