Evaluation of Agricultural and Clinical Antifungal Agents Against Members of the *Fusarium solani* Species Complex

Amelia Flesner[1,] and Jeffrey Coleman[2]*

[1] Undergraduate Student, Department of Entomology and Plant Pathology, Auburn University
[2] Associate Professor, Department of Entomology and Plant Pathology, Auburn University

Members of the *Fusarium solani* species complex (FSSC) are important pathogens in both agricultural and clinical settings. The Coleman lab has successfully demonstrated that isolates from a clinical setting are capable of infecting and causing disease on produce as well as proving that some agricultural isolates can cause human infection [1]. This pathogenicity has caused the need for further antifungal testing in both clinical and agricultural realms. Treating fungal infections is difficult because fungal cells and human cells share many basic structures as well as possessing the ability to easily develop resistance to drugs. The objective of this research is to investigate how the FSSC isolates from varying environments react to different antifungal agents, determining potential treatment options and exposing possible resistance. The minimum inhibitory concentration of various antifungal agents will be analyzed and compared to determine effectiveness. Ten-fold antifungal dilutions are added to a set number of spores and incubated for three days at thirty-five degrees Celsius. Fungal growth within each dilution is examined macroscopically to determine the minimum amount of antifungal needed to inhibit the growth of that particular infection (Fig. 2). Agricultural fungicides such as prochloraz and clinical antifungals such as amphotericin B show inhibition of both clinical and antifungal isolates. The concentration needed of each can vary by isolates, indicating possible resistance. The FSSC has been denoted as a fungal species complex of particular concern by the WHO in 2022, and this research will contribute significant information surrounding treatment in both clinical and agricultural environments.

This research aims to investigate the response of FSSC isolates from different environments to various antifungal agents, providing valuable insight for treating infections in both clinical and agricultural settings, especially considering the WHO's designation of FSSC as a fungal pathogen of particular concern.

The *Fusarium solani* species complex comprises filamentous fungi that are globally distributed and capable of causing disease in both crops and humans. This species complex is notably recognized for its pathogenicity in economically significant crops such as cotton and soybean. The spores of the FSSC can also lead to human mycoses either through direct inoculation or inhalation, posing a significant risk, particularly to immunocompromised individuals. The risk for immunocompromised farmers is even higher as engaging in agricultural activities involving *Fusarium*-infected crops elevates the risk of spore exposure. Consequently, the identification of optimal treatment methods for these diseases has become crucial over recent years.

This study involved the evaluation of both clinical and agricultural antifungal agents due to the ability of these fungal isolates to infect both humans and crops. The clinical antifungal agents investigated are: amphotericin B, fluconazole, voriconazole, triamcinolone, caspofungin, ketoconazole, miconazole, clotrimazole, and posaconazole. The agricultural antifungals examined are: tebuconazole and prochloraz.

In clinical practice, amphotericin B and voriconazole are the preferred drugs for combating *Fusarium solani* species complex infections. This study revealed that both antifungals demonstrated inhibitory effects at lower concentrations compared to other options such as fluconazole, which is widely used for other medical-

* Corresponding author: ajf0058@auburn.edu

ly important fungi. Amphotericin B exhibited a lower inhibitory concentration than voriconazole against the same isolates but is often associated with more severe side effects. Because of this, voriconazole has become the more frequently prescribed treatment due to its relatively lower adverse effects. However, this research indicates potential alternative options like clotrimazole, which exhibited the same minimum inhibitory concentration (MIC) as voriconazole (Fig. 1).

In the agricultural domain, prochloraz is extensively used to manage FSSC infection of crops in other countries. This study has shown that even a minute concentration of this fungicide, approximately 0.06 µg/mL, can impede the growth of *Fusarium* isolates. However, prochloraz is considered a hazardous substance with significant long-term environmental repercussions and is banned within the United States. Tebuconazole also inhibits isolate growth but at a higher concentration. It is deemed less hazardous and has additional benefits such as controlling bacterial and viral pathogens affecting plants. Assessing the efficacy of higher verses lower concentrations requires a comprehensive consideration of the associated benefits and consequences, as well as the specific species complex causing the infection.

The minimum inhibitory concentrations were determined according to a protocol for filamentous fungi from the Clinical and Laboratory Standards Institute [2].

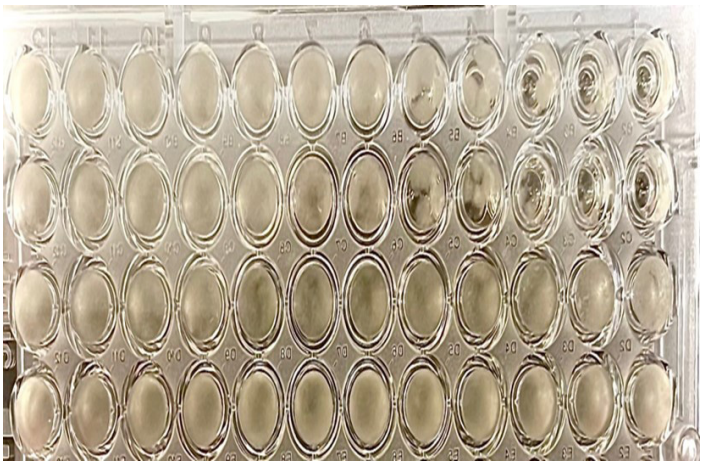

Fig. 2 involves a study involving the isolate Cherokee 2-1 and four distinct antifungal agents. The first row denotes a minimum inhibitory concentration of 4 µg/mL with voriconazole. The second row signifies an MIC of 2 µg/mL with tebuconazole. Conversely, rows three and four indicate MIC values exceeding 32 µg/mL with caspofungin and triamcinolone respectively. Demonstrating the inability of these antifungals to impede the growth of the isolate at the highest concentration tested.

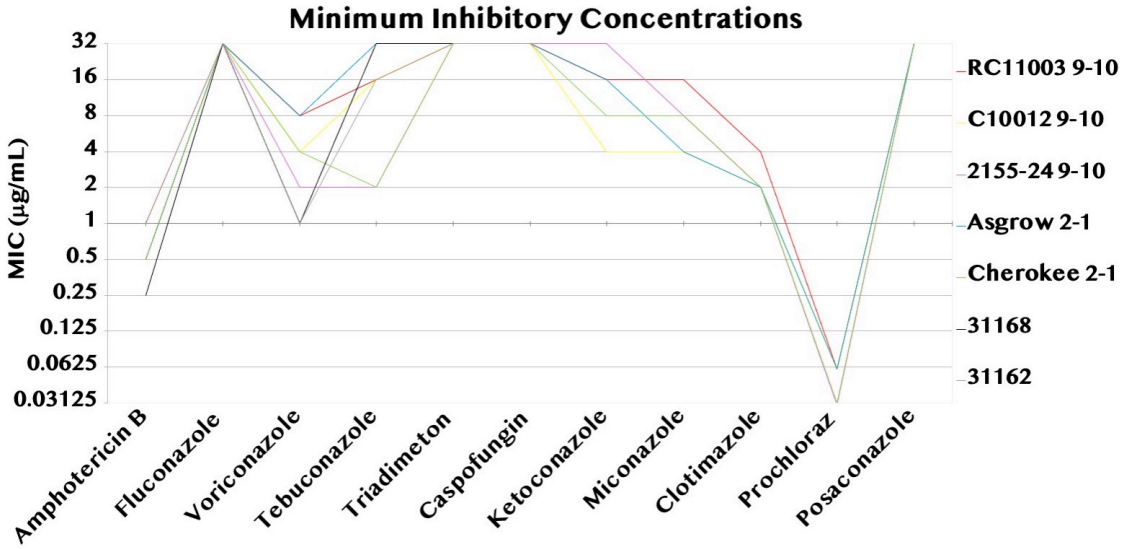

Fig. 1 demonstrates the minimum inhibitory concentrations (MICs) of all tested Fusarium solani species complex isolates and antifungal agents investigated in this study. The scale ranges from 0.03 µg/mL, denoting the lowest concentration tested and the minimum amount of antifungal required to inhibit the isolate, to >32 µg/mL, indicating a concentration beyond visual determination in this assay. An MIC of 32 µg/mL signifies observable growth of fungal spores in all wells, while an MIC of 0.03µg/mL indicates the absence of fungal growth across all wells. The distinct lines in the figure correspond to different isolates tested. Variability is observed among isolates and antifungals, suggesting potential resistance, while no variability is observed among others indicating uniform effectiveness and lack of variation in pathogenicity.

The examination of agricultural and clinical isolates against various *Fusarium solani* species complex strains revealed varying effectiveness in inhibiting fungal growth among different antifungal agents. Prochloraz demonstrated exceptional efficacy even at low concentrations, indicating minimal antifungal resistance and a potential for treating Fusarium infections. In comparison, several antifungal agents assessed in this study exhibited an inability to hinder fungal growth even at the highest concentration tested, 32 µg/mL. Fluconazole, triamcinolone, caspofungin, and posaconazole fell into this category, suggesting limited effectiveness in treating FSSC infections. Higher concentrations of antifungals can lead to more severe side effects. Therefore, prescribing the lowest effective concentration capable of eliminating the infection is crucial. Among the clinical antifungals evaluated, amphotericin B, voriconazole, and clotrimazole emerged with the lowest MICs, indicating their potential as preferred options for treating human mycoses involving *Fusarium*.

Statement of Research Advisor

Members of the FSSC have a broad host range and are encountered under field conditions and in hospitals. Management of FSSC infections may involve the use of antifungal compounds in a class termed the triazoles. As *Fusarium* persists in such broad environments, their exposer to antifungal agents used in one setting can potentially confer cross resistance to treatment options in the other environment. Amelia's research has indicated FSSC isolates exposed to fungicides used in agriculture, may potentially be selecting for resistance to antifungals used in the medical setting for people infected with isolates of the FSSC.

- Jeff Coleman, Entomology and Plant Pathology, College of Agriculture

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Authors Biography

Amelia Flesner is a senior-year student pursuing a B.S. degree in Clinical Laboratory Science within the Biochemistry Department at Auburn University. She has played a key role in the research of clinical and agricultural antifungal agents against a fungal species complex of particular concern.

Jeffrey J. Coleman is an Associate Professor in the Department of Entomology and Plant Pathology at Auburn University. After obtaining his Ph.D. in Plant Pathology at the University of Arizona, Dr. Coleman conducted postdoctoral research in medical mycology at Harvard Medical School. Since joining the faculty at Auburn University in 2014, he has developed a laboratory focused on investigating fungal pathogenesis using advanced molecular techniques.