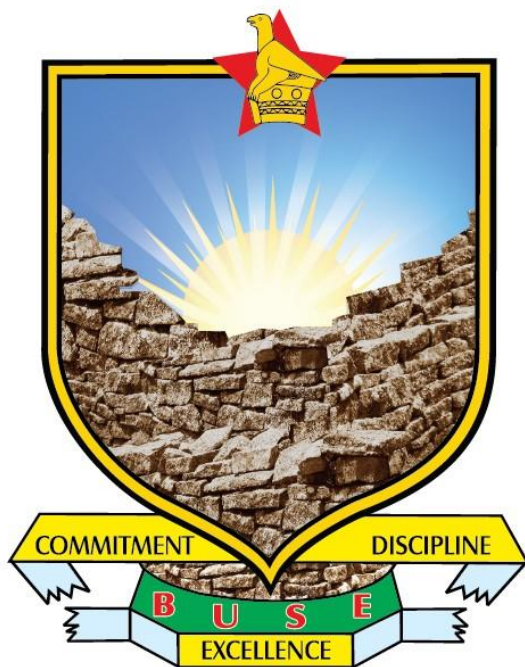


Bindura University of Science Education



The efficacy of Pydiflumetofon and Difenconazole for the control of *Gibberella* and *Diplodia* ear rot in seed maize (*Zea mays*).

By

MUDZIMU CHARITY JOYCE

B212788B

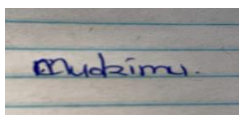
A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Biological Sciences.

June 2025

Approval form

The undersigned certify that they have read the dissertation titled 'The efficacy of Pydiflumetofon and Difenconazole for the control of *Gibberella* and *Diplodia* ear rot in seed maize (*Zea mays*)' and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

Signature of student:



Date: 15/06/25

Signature of Supervisor:



Date: 15/09/2025

Signature of Chairperson of Department:

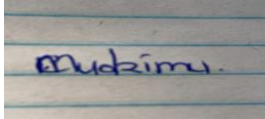


Date: 15/09/25

Declaration

I, Charity Joyce Mudzimu (B212788B) declare that this research herein is my own work and has not been plagiarized from another source(s) without acknowledgement of the concerned author(s) either electronically or otherwise

Signature:

A photograph of a handwritten signature 'Mudzimu.' in blue ink on a piece of lined paper. The signature is written in a cursive style.

Date: 15/06/25

Dedication

The present work is dedicated to my family and friends for their unwavering support and love which I greatly cherished.

Acknowledgements

Above all, I would like to thank God for the strength and determination that he blessed me with. I would like to express my deepest gratitude to my supervisor, Dr P Manyanga, for his unwavering guidance and support throughout this research project. I would also like to extend my sincere thankfulness to Seed Co Limited pathologist Dr N Chiurayise for his technical advice and financial support to undertake this study.

List of abbreviations

GER.....	<i>Gibberella</i> Ear Rot
SDHI.....	Succinate Dehydrogenase Inhibitor
DON.....	Deoxynivalenol
IPM.....	Integrated Pest Management
SRC.....	Stapleford Research Centre
AUDPC.....	Area under Disease Progress Curve
LSD.....	Least Significant Difference

List of figures

Figure 1 : Maize affected by Gibberella ear rot	9
Figure 2 :Showing maize affected by Diplodia ear rot	10
Figure 3 :Graph showing the mean values of disease incidence between treated and untreated..	16
Figure 4 :Bar graph illustrating the severity scores of treated and untreated experiments.....	17
Figure 5 :Graph showing grain yield of treated and untreated experiments.....	18

Abstract

The fungal diseases Gibberella ear rot and Diplodia are brought on by *Stenocarpella maydis* and *Fusarium graminearum*, respectively. Due to their substantial effects on seed quality and quantity, these diseases are economically significant in the production of maize. The purpose of this study was to evaluate how well the fungicides Pydiflumetofen and Difenoconazole affected the incidence, severity, and grain yield of the disease. Two blocks for each treatment in a field experiment. Two replications of twelve maize lines. At the flowering stage, the emerging silks were sprayed with all of the maize lines. The findings supported the hypothesis, showing that fungicide application significantly decreased the incidence and severity of disease in susceptible maize lines. The growth and development of the pathogens that cause Diplodia and Gibberella ear rot were successfully inhibited by the fungicides. The findings demonstrated that the incidence and severity of the disease varied significantly ($p < 0.05$) amongst maize lines. The results demonstrate the fungicide's potential as an effective instrument for managing disease in the production of seed maize.

Contents

Table of Contents

Approval form.....	i
Declaration.....	ii
Dedication	iii
Acknowledgements.....	iv
List of abbreviations	v
List of figures.....	vi
Abstract	vii
Chapter 1	1
Introduction.....	1
1.2 PROBLEM STATEMENT	3
1.3 SIGNIFICANCE OF STUDY.....	4
1.4 AIM OF THE STUDY	4
1.5 OBJECTIVES	4
1.6 RESEARCH QUESTIONS.....	5
1.7 HYPOTHESIS	5
Chapter 2	6
Literature review	6
2.4.1 PYDIFLUMETOFEN	11
2.4.2 DIFENOCONAZOLE	11
Chapter 3.....	13
Materials and methods	13
3.1 THE STUDY AREA.....	13
3.2 SOURCE OF RESEARCH MATERIAL	13
3.3 EXPERIMENTAL DESIGN.....	13
3.4 EXPERIMENT PROCEDURE:.....	13
3.4.1 LAND PREPARATION AND SEED PLANTING	13

3.4.2 FERTILIZER AND IRRIGATION MANAGEMENT	14
3.4.3 WEED AND PEST MANAGEMENT	14
3.5 DATA COLLECTION.....	15
3.6 DATA ANALYSIS	15
Chapter 4.....	16
Results	16
4.1 DISEASE INCIDENCE	16
4.2 DISEASE SEVERITY	17
4.3 GRAIN YIELD	17
Chapter 5.....	19
Discussion, summary, recommendations and conclusions	19
5.1 Discussion	19
5.1.1 DISEASE INCIDENCE	19
5.1.3 GRAIN YIELD.....	21
5.2 Summary	22
5.3 Recommendations	22
5.4 Conclusions	22
References.....	24

Chapter 1

Introduction

1.1 Background

A major cereal crop in many parts of the world, maize (*Zea mays*) is used as a main source of food and feed. Fungal diseases have the potential to negatively impact maize productivity by compromising seed quality, plant health, and ultimately grain yield. *Gibberella* and *Diplodia* are two well-known fungus pathogens that can damage maize seeds, resulting in decreased germination rates and stunted plant growth (Sutton, 1982). For instance, *Aspergillus* species contribute to *Aspergillus* ear rot, whereas *Fusarium* species are known to cause *Fusarium* ear rot and seedling blight. *Gibberella* ear rot, which is caused by *Fusarium graminearum*, and *Diplodia* ear rot, which is caused by *Stenocarpella maydis*, are the two main ear rot diseases that affect maize and can both drastically lower maize yields. Temperature and humidity levels have an impact on the incidence, spread, and severity of the disease. Relative humidity of 80% or higher increases the severity of *Gibberella* ear rot (Phad et al., 2023). Moderately severe ear rot symptoms in susceptible maize hybrids reduced yield by up to 48% in such favorable conditions (Vigier et al., 2001). During the reproductive stage, disease severity rises when humid conditions persist. The *Gibberella* ear rot fungus spreads through the silk channel. In addition, the infection process occurs when insects or birds damage the husk, which enables the fungus to directly access the kernels, or when the fungus lands on silks and uses the silk as a substrate to enter the ear through the silk channel (Vigier et al., 2001).

Diplodia ear rot infection occurs during the silk growth stage, starting at the base of the ear and moving to the tip. Discolored kernels that can turn pink, red, or white are a hallmark of *Gibberella* ear rot. Consequently, hand-picking maize seed to get it ready for seeding reduced its viability and raised labor costs. The main source of the inoculum that infects maize kernels are residues of maize that are left in the field. Mycelium or other survival structures are the inoculum's means of survival. Chlamydospores produced by *Fusarium graminearum* are able to

endure time between host crops. Additionally, *Fusarium graminearum* produces perithecia on plant residue, which are then violently released into the atmosphere as ascospores.

Grain seed quality reduction, yield losses, livestock issues, and possible human toxicity are the outcomes of *Gibberella* and *Diplodia* ear rot (Flett et al., 1998). Infections with *Gibberella* and *Diplodia* can cause ears to become light, shriveled, or discolored, rendering the grain unfit for sale or planting. In the seed production sector, where quality standards are strict and even a small percentage of infected ears can result in widespread seed rejection and financial losses, this is particularly expensive. These ear rots seriously jeopardize crop yield and financial returns in addition to seed quality. *Gibberella* and *Diplodia* ear rot in seed maize have a major economic impact. Yield losses of up to 40% have been documented in certain instances.

These diseases can result in quality problems like mycotoxin contamination and higher seed production costs during hand picking, in addition to reducing yield (Chen et al., 2020). This may result in low germination rates, higher grain treatment expenses, and diminished marketability. These illnesses have been found to be persistent issues in Zimbabwe and other maize-producing regions. These characteristics can be seriously jeopardized by ear rots brought on by *Gibberella* and *Diplodia*, which lower overall seedling vigour, reduce germination rates, and physically damage kernels. The generation of dangerous mycotoxins, like deoxynivalenol (DON), which can contaminate maize grain, is another issue, especially with *Gibberella*. Food safety and regulatory compliance are crucial issues because these toxins pose serious risks to the health of both humans and animals.

Tillage, residue management, and crop rotation are general techniques that lessen the burden of disease. This isn't true for every farming scenario, though, because of farmers' conservation farming methods. Since seed maize lays the groundwork for the following planting season, it must meet especially high requirements for purity, viability, and germination potential. The purpose of fungicide treatments is to stop or manage these infections.

It is common practice to treat seeds with fungicides in order to reduce these risks; however, the efficacy of various fungicides can differ depending on a number of factors, such as formulation, application technique, and environmental circumstances (Munkvold & Brown, 2020). Treatment of the seed either increases the seed's resistance to pathogens or inhibits the growth of fungi. As

a result, fungicides are a crucial tool for managing these illnesses however, highly effective chemicals must be used. Among the many advantages of fungicides are higher yields, lower rates of disease, and better grain quality. The purpose of this study is to assess the effectiveness of pydiflumetofen and difenoconazole, which are used by SeedCo Company, a significant producer of maize seeds, in order to ascertain their effects on the rates of fungal infection, the severity of the disease, and grain yield.

1.2 PROBLEM STATEMENT

Currently, general management guidelines include planting less susceptible hybrids, crop rotation and tillage. However, these guidelines are not sufficed in maize seed production therefore use of fungicides remain the key in controlling ear rots but there is need to use effective fungicides. The use of fungicide can help to reduce the economic impact of these diseases by reducing disease incidence and improving grain quality. However, their effectiveness can vary significantly depending on factors like formulation, application method, and environmental conditions. Pydiflumetofen and Difenconazole are used for the control of *Gibberella* and *Diplodia* ear rot in seed maize (*Zea mays*) but their effectiveness has not been established. Evaluating their efficacy is crucial for several reasons that relate to crop health, seed quality, economic value, and sustainable agriculture. Effective fungicidal control is necessary to preserve the health and viability of seed maize. If fungicides can effectively suppress *Gibberella* growth and reduce mycotoxin levels, they contribute not only to crop protection but also to public health and market acceptability.

Moreover, because Pydiflumetofen and Difenconazole belong to different classes of fungicides succinate dehydrogenase inhibitors (SDHIs) and demethylation inhibitors (DMIs), respectively it is essential to evaluate their individual and combined performance.

Finally, environmental factors such as humidity, temperature, maize variety, and field practices can influence how well a fungicide performs. Conducting local efficacy trials ensures that recommendations are based on real-world performance in the target region, making the results more relevant and actionable for farmers and seed producers.

1.3 SIGNIFICANCE OF STUDY

Evaluating the efficacy of fungicides like Pydiflumetofon and Difenoconazole helps determine their ability to protect crops from such losses under specific field conditions. Evaluating the efficacy of fungicides in maize seed treatment is crucial for improving both seed quality and crop yield, making it essential to address gaps in current knowledge. This project is justified because it provides a rigorous, empirical assessment of specific fungicides used by SeedCo Company, offering critical insights into its performance under real-world conditions. By quantifying its impact on fungal incidence, disease severity, and grain yield, the study will inform better seed treatment practices and enhance overall maize production efficiency. Furthermore, these findings will help SeedCo refine their protocols, potentially leading to improved crop outcomes and economic benefits for farmers, thereby contributing to the broader agricultural sector's productivity and sustainability.

Understanding their effectiveness supports proper fungicide selection, application timing, and dosage decisions, leading to more precise and efficient disease control. This also helps avoid unnecessary chemical applications and supports the principles of integrated pest management (IPM). Evaluating fungicide efficacy also plays a role in monitoring and managing fungicide resistance. Over time, fungal pathogens can develop resistance if fungicides are used excessively or without rotation. Regular testing helps identify any decline in effectiveness early on, allowing for the adjustment of disease management strategies to preserve the long-term utility of these products.

1.4 AIM OF THE STUDY

The aim of this research was to find effective ways to control fungal infection in maize seed and to prevent disease outbreak throughout the growing season. The research evaluated the effectiveness of fungicides, Pydiflumetofen and Difenoconazole, in controlling fungal infections' incidence, severity and their impact on maize seed health and grain yield.

1.5 OBJECTIVES

The objectives of this research are;

1. To determine the severity and incidence of *Gibberella* and *Diplodia* ear rot in treated maize seed and untreated.
2. To compare the incidence and severity of *Gibberella* and *Diplodia* fungal infections between treated (Pydiflumetofen and Difenconazole) and untreated maize.
3. To determine and compare the grain yield of maize treated with Pydiflumetofen and Difenconazole and untreated maize.

1.6 RESEARCH QUESTIONS

The research answered the following questions:

1. What is the severity and incidence of *Gibberella* and *Diplodia* ear rot in treated maize seed and untreated?
2. How does seed treatment with pydiflumetofen and difenconazole affect the incidence and severity of *Gibberella* and *Diplodia* ear rot in treated maize and untreated seeds?
3. What is the grain yield of treated maize and untreated maize?

1.7 HYPOTHESIS

H₀: there is no significant difference in the incidence and severity of fungal infections and grain yield between treated and untreated maize.

H₁: There is significant difference in the incidence and severity of fungal infections and grain yield between treated and untreated maize.

Chapter 2

Literature review

2.1 INTRODUCTION

The energy-dense carbohydrates found in maize, a cereal crop, are used in both human and animal diets. Gibberella and Diplodia ear rot are two severe fungal diseases that can damage maize crops and cause significant yield and quality losses (Munkvold, 2003). Grain weight per ear and ear diameter decrease with increasing Gibberella ear rot (GER) severity. The hybrid maize's resistance level determines the extent of reduction. In susceptible hybrids, ear diameter and grain weight per ear are decreased more than in less susceptible hybrids. The severity of GER is 77% for less susceptible hybrids and 62% for susceptible hybrids, which results in an estimated 50% reduction in grain weight. Decisions regarding grain handling, sorting, and marketing can be guided by the use of GER severity as a stand-in for early estimation of deoxynivalenol contamination and yield loss in maize (Lana et al., 2022).

2.2 GIBBERELLA EAR ROT (GER)

The fungus *Gibberella* causes the maize ear to rot, decay, and produce mold-discolored, shriveled kernels, which renders the seed unfit for use as seed. Additionally, the contaminated maize kernels are unfit for use as human or animal feed. The impacted maize plant yields less than the desired amount and quality. This is due to the fact that the fungus produces mycotoxins, which contaminate grain with harmful substances, particularly deoxynivalenol (Chen et al., 2019). During the growing production period, the fungus causes a disease known as wheat head blight that affects both maize and wheat (Sutton, 1982). Furthermore, poor plant vigour and germination occur when the contaminated seed is used as planting material during the seed production process.

Environmental factors and pest damage have an impact on the incidence of these diseases. According to a study conducted in India, the severity of *Fusarium graminearum* increases when

the bean cutworm damages maize ears (Parker et al., 2017). When birds and armyworms eat maize kernels in the field, they can also spread these opportunistic infections, which can lead to injuries (Munkvold, 2003). The infection caused by *Gibberella* starts at the top of the ear and moves down the maize ear. There are currently only a few substances that exhibit some degree of efficacy in managing *Fusarium graminearum*. According to a study, prothioconazole and thiophanate-methyl have respective effects of 52% and 48% in disease control (Masiello et al., 2019).

Plant ear physical barriers are preventing some currently registered fungicides from successfully controlling Diplodia ear rot in maize in field settings (Luna and Wise, 2015). The fungicide is rendered ineffective since it cannot reach the pathogen. In recent years, fungicides like Difenoconazole and Pydiflumetofen have drawn notice for their broad-spectrum ability to combat a range of fungal diseases that impact cereal crops. In crops like soybean, wheat, and maize, pyriflumetofen, a more recent generation of succinate dehydrogenase inhibitor (SDHI) fungicide, has shown effectiveness against foliar and soil-borne diseases. It effectively halts fungal growth at the cellular level by blocking the mitochondrial respiratory chain. According to research by Forrer et al. (2019) and Franz et al. (2021), pydiflumetofen can lessen the severity of *diplodia* and *gibberella* ear rot.

Similarly, Difenoconazole, a widely used demethylation inhibitor (DMI), has been utilized globally for the management of *Fusarium* and *Alternaria* species in cereals, fruits, and vegetables. Its mechanism of action involves inhibiting sterol biosynthesis, thus weakening the fungal cell membrane (Franz et al., 2021).

Despite these uses, the majority of current research concentrates on foliar diseases or infections that affect seedlings rather than the specific pathogens, *Gibberella zeae* and *Diplodia maydis*, that cause ear rot in mature maize ears, especially in systems that produce seed maize. Studies by Munkvold and White (2016), for instance, point out that although fungicides are frequently used during the silking stage to manage foliar pathogens or lessen the build-up of mycotoxin, there is little proof of the direct effectiveness of particular active ingredients, such as Pydiflumetofen or Difenoconazole, against *Gibberella* or *Diplodia* ear rot pathogens. Furthermore, Paul et al.

(2018) point out that while triazole fungicides, such as Difenoconazole, do have some effect on *Fusarium graminearum*, their effectiveness in suppressing ear rot varies and is frequently impacted by the genotype of the maize, application timing, and weather.

Furthermore, according to Tacke and Casper (2020), fungicide trials in maize have traditionally concentrated on improving yield and suppressing general diseases rather than differentiating between commercial grain and seed maize. Existing data from general maize production cannot be directly translated due to the special requirements for seed maize, which include high germination, low disease tolerance, and the lack of a seed-borne inoculum. Peer-reviewed research explicitly examining the combined application or synergistic effects of SDHI and DMI fungicides (such as Pydiflumetofen and Difenoconazole) on ear rots in relation to seed production is also lacking.

Additionally, even though Pydiflumetofen is a component of commercial premixes, diseases like southern rust, northern corn leaf blight, and grey leaf spot are typically the focus of current product registrations and field trials rather than ear rots. Manufacturers like Syngenta provide regulatory and field data that validate effectiveness against specific foliar pathogens, but they do not provide specific results regarding the suppression of *Gibberella* or *Diplodia* ear rot in physiologically mature maize ears.

In conclusion, despite the fact that both pyriflumetofen and difenoconazole have been extensively used in maize production to control disease, there is a glaring lack of information in the scientific literature about their specific assessment against *Gibberella* and *Diplodia* ear rot, especially in seed maize systems.

The absence of such focused studies justifies the need for research to determine whether these fungicides can effectively reduce ear rot incidence and protect seed viability under local agro-ecological conditions.



Figure 1: maize affected by Gibberella ear rot

2.3 DIPLODIA EAR ROT

The infection known as Diplodia ear rot starts in the lower ear and moves up the maize cob. For nearly a year, *Stenocarpella maydis* remains viable on the surface of crop debris. The fungus *Stenocarpella maydis* can grow year-round as pycnidia in crop residue and in the soil, where it can remain dormant. According to Alvarez-Cervantes et al. (2016), *Stenocarpella maydis* naturally infects people one to three weeks after pollination when there is rain and temperatures between 28 and 30 °C.

At high disease severity, yield reductions of roughly 48% have been documented under such favourable environmental conditions (Vigier et al., 2001). Because the fungus' spores are released and spread by splashing raindrops, wet weather during the silk growth stage exacerbates the disease. Furthermore, the disease is more common in areas where insects, like earworms, injure the ears during the silk growth stage of maize production (Mabuza et al., 2018). It doesn't matter whether land is ploughed or zero tillage is used because *Diplodia maydis* can live in soil and debris and is easily spread by insects, wind, and splashing water (Flett et al., 1998).

The accumulation of these toxic fungi in maize grain was not significantly altered by trials conducted on conventional tillage farming land, three-year crop rotation, or no-till (Mabuza et al., 2018). Different strains of the *Diplodia* ear rot pathogen can cause varying symptoms in maize plants. As a result, it is challenging to create a single maize variety that is immune to every pathogen strain. Furthermore, the pathogen can spread to other crops, like wheat, which makes field control more challenging.

According to Mesterházy et al. (2012), breeding for resistance to *Diplodia* ear rot is additively inherited, which means that the traits are transferred from parents to children in a way that is directly proportional to the number of genes. As a result, their dominance and interaction effects are low. According to research conducted in South Africa, additive gene effects with low dominance and interaction effects govern the inheritance of resistance to ear rot, which is caused by *Stenocarpella maydis* (Rossouw et al., 2002).



Figure 2: showing maize affected by *Diplodia* ear rot

2.4 CHARACTERISTICS OF FUNGICIDES

Difenoconazole is a fungicide used as a seed dressing to protect crop plants from diseases spread by seeds and soil. Difenoconazole's active ingredients are mefenoxam and fludioxonil, a broad-spectrum seed treatment fungicide. The chemical class known as phenylpyrroles, which includes fludioxonil, was created from a naturally occurring antimycotic compound that was isolated from a soil bacterium. It interferes with the transport system in fungal cells, which interacts with the germ tube, mycelia, and conidial germination, among other stages of the fungus' life cycle (Mabuza, 2018). In cucurbits, maize, and sorghum, difenoconazole is a fungicide used as a seed dressing to control soil-borne and seedborne disease-causing pathogens like *Pythium* spp., *Rhizoctonia* spp., and *Fusarium* spp. (Syngenta, 2014).

2.4.1 PYDIFLUMETOFEN

Pydiflumetofen is a new-generation succinate dehydrogenase inhibitor (SDHI) fungicide used to prevent plant rot (Huang et al., 2019). This Syngenta-developed fungicide works wonders against hard-to-control diseases like fusarium and other fungi that seriously harm crops. This product combines the effects of difenoconazole and Adepidyn, a novel fungicide molecule with an SDHI mode of action that belongs to the carboxamide chemical class. Under conditions of high disease pressure, the application of pydiflumetofen resulted in a decrease in the accumulation of mycotoxin in maize grain, demonstrating its efficacy in preserving grain quality. (Eli et al., 2021).

2.4.2 DIFENOCONAZOLE

A common systemic triazole fungicide in agriculture, difenoconazole prevents fungal diseases like powdery mildew, rusts, leaf spots, and anthracnose in crops like cereals, fruits, and vegetables. It stops fungal growth and infection by preventing the biosynthesis of ergosterol, which is essential for fungal cell membranes. Depending on the severity of the disease and the surrounding circumstances, it is usually used every 7–14 days either as a preventative measure or as a treatment (Huang et al., 2019). Companies like Syngenta Zimbabwe, Agricura Zimbabwe, and Windmill Zimbabwe produce and distribute fungicides based on difenoconazole in Zimbabwe (Santiago, 2020).

These businesses offer formulations that can be used alone or in conjunction with other fungicides to increase efficacy. Despite the low toxicity of difenoconazole to humans, it is still

advised to wear appropriate protective gear when applying it (Vigier et al, 2001). Additionally, it must be used as part of an Integrated Pest Management (IPM) strategy to stop fungal populations from developing resistance.

A systemic fungicide called Miravis Duo is used to prevent foliar diseases in a variety of crops. It combines difenoconazole, from the chemical group of triazoles, with the action of a novel active ingredient (Pydiflumetofen), from the chemical group of carboxamides (Syngenta, 2020). The combination of the two active ingredients results in a combined action that inhibits the fungus's ergosterol synthesis and respiratory process. These characteristics guarantee a wide range of control, lowering the possibility of resistant strains emerging. Nevertheless, this fungicide field trial was conducted because all of these empirical studies were conducted under particular circumstances, and the findings might not be generalizable to all areas where maize is grown. In addition to combining two distinct modes of action chemistries to reduce the risk of resistance, the duo mixture provides a step-change in the control of leaf spots. Despite these uses, the majority of current research concentrates on foliar diseases or infections that affect seedlings rather than the specific pathogens, *Gibberella zea* and *Diplodia maydis*, that cause ear rot in mature maize ears, especially in systems that produce seed maize. Studies by Munkvold and White (2016), for instance, point out that although fungicides are frequently used during the silking stage to manage foliar pathogens or lessen the buildup of mycotoxin, there is little proof of the direct effectiveness of particular active ingredients, such as Pydiflumetofen or Difenoconazole, against *Gibberella* or *Diplodia* ear rot pathogens. Furthermore, Paul et al. (2018) point out that while triazole fungicides, such as Difenoconazole, do have some effect on *Fusarium graminearum*, their effectiveness in suppressing ear rot varies and is frequently impacted by the genotype of the maize, application timing, and weather.

Chapter 3

Materials and methods

3.1 THE STUDY AREA

The experiment was conducted at Seed-Co Stapleford Research Centre (SRC), which a natural occurring environment and lie in natural region IIa, in Harare province Zimbabwe (17°42'44" S 30°53'48" E). The area receives annual rainfall of 750 - 900 mm per annum at an altitude of 1483 above sea level, with average temperature of 16°C in winter and 26°C in summer. The soils are red loam classified as fersialitic which are formed from granite rock.

3.2 SOURCE OF RESEARCH MATERIAL

The maize seeds were derived from Seed Co Limited. The variety used is susceptible to *Gibberella* and *Diplodia* ear rot. Difenconazole and pydiflumetofen were also taken from the company.

3.3 EXPERIMENTAL DESIGN

This study was a field experiment with two treatments, treated and untreated seeds and two plots were used for each treatment. Each plot had 12 lines of maize and each line had 8 planting stations. Data was collected per line making 24 replications for each treatment. Buffer zones, untreated areas of 3 meters wide were maintained between treated and control plots. Pydiflumetofen and Difenconazole were the fungicides used to treat the maize and applied at different stages. Maize seed was treated before planting with Difenconazole and at silking stage with Pydiflumetofen. SC555 was the variety which was used.

3.4 EXPERIMENT PROCEDURE:

3.4.1 LAND PREPARATION AND SEED PLANTING

Land preparation was done using conventional tillage methods, which use both primary and secondary tillage implements. Firstly, the land was ripped to break the soil pan followed by ploughing with a disc plough, disc harrow, and roller to break large clods and have fine tilth.

The seeds were treated with difenoconazole, 0.03 grams of active ingredient per kilogram of seed, which corresponds to approximately 10 to 30 grams per metric ton of maize seed. Maize lines were sown 2 seeds per station at 0.25 m in a row by 0.75 m interrow spacing, with each row consisting of eight planting stations.

3.4.2 FERTILIZER AND IRRIGATION MANAGEMENT

Prior to planting, 400 kg ha⁻¹ of Compound D (7:14:7) fertilizer was applied. Seven days after the crop appeared, it was thinned, leaving one plant per station, with each plot having ten rows. To reduce leaching losses, a top dressing of ammonium nitrate (34.5%) N was applied when the maize had 10 leaves at a rate of 400 kg ha⁻¹, divided into 2 at 200 kg/ha each of the first and second split. Four weeks after planting, the first top dressing application was made, and eight weeks later, the second. From planting to harvest, the experiment was managed as dry land with no additional irrigation.

3.4.3 WEED AND PEST MANAGEMENT

Herbicide was used before an emergency and Immediately following planting, 3.5 liters of metholachlor were applied per hectare. To keep the field free of weeds, spot hand weeding was done. Lambda was sprayed to control cutworms shortly after the seed emergency. Using a hand knapsack, 300 ml/ha⁻¹ of emamectin benzoate was applied to control fall armyworms (*Spodoptera frugiperda*). During the flowering stage, the emerging silks were sprayed with all of the maize lines. Using a hand sprayer, pydiflumetofen and difenoconazole were sprayed three times at 5-day intervals at a ratio of 10 ml/125 ml of water to ensure that all the plants were sprayed equally. Between the treated and control plots, buffer zones—untreated areas at least three meters wide—were kept to reduce spray drift. Spraying was done when wind speeds were low (<5 km/h) to prevent drift. Applications were made downwind of the control plots to ensure that spray does not reach untreated areas. Shields or controlled nozzles were also used to direct spray only within the designated plot and prevent drift. Low-drift nozzles and coarse droplet size settings can reduce airborne movement of the fungicide.

3.5 DATA COLLECTION

Maize lines were evaluated visually for ear rot disease in the field at physiological maturity. Parameters recorded include: Disease incidence, severity and grain yield. Number of infected plants in each line were counted and the incidence of disease was scored as number of infected cobs per line.

Disease Severity (%) this measures the extent of disease symptoms on affected plants based on a disease rating scale. Visual disease rating based on a standardized scale 1-9 % and Calculation (using a scale-based assessment). Severity scores are based on the percentage of the ear affected by the disease rating on a scale of 1-9, where 1=0% infection, 2= 1-10%, 3=11-20%, 4=21-30%, 5=31-40%, 6= 41-50%, 7=51-70%, 8=71-90%, 9=91-100%. Scores 1-3 shows resistance, 4-6 shows moderate susceptibility and 7-9 shows highly susceptible. Data was analyzed as averages severity per line. Data collection on yield was recorded only on cobs that were not infected by the fungal diseases at all. The uninfected cobs from the treated experiment were shelled differently from the cobs from the untreated so as to compare the grain yield. The uninfected cobs were shelled to weigh grain mass of treated and untreated. Grain yield was calculated considering grain weight in kilograms per 24 lines.

3.6 DATA ANALYSIS

All data collected was analyzed using GenStat software 18th edition. Assumptions of two sample T-test and descriptive statistics were tested. Two sample T test was done to compare disease incidence, disease severity and grain yield between treated and untreated maize. Data analyzed on incidence was the count of infected cobs per line between treated and untreated. On disease severity data analyzed was mean severity scores of the lines. Grain yield was weight of uninfected grain. Grain yield was recorded separately for treated and untreated. Fisher's LSD Protected method was used to separate means at a 5% significance level when differences were detected from the T-test when $p < 0.05$. Bar graphs were used to present the results.

Chapter 4

Results

4.1 DISEASE INCIDENCE

One cob per line in both the treated and untreated experiments was the smallest number of cobs observed in the disease incidence results. For the treated, the maximum number of cobs per line was two, and for the untreated, it was three. The treated and untreated plants had mean disease incidence values of 0.67 and 1.04, respectively. From the T-test $T=0.000131$ and the F-test $F=0.015$. A highly significant difference between the treated and untreated is indicated by the p-value, which is less than 0.05 ($p<0.05$).

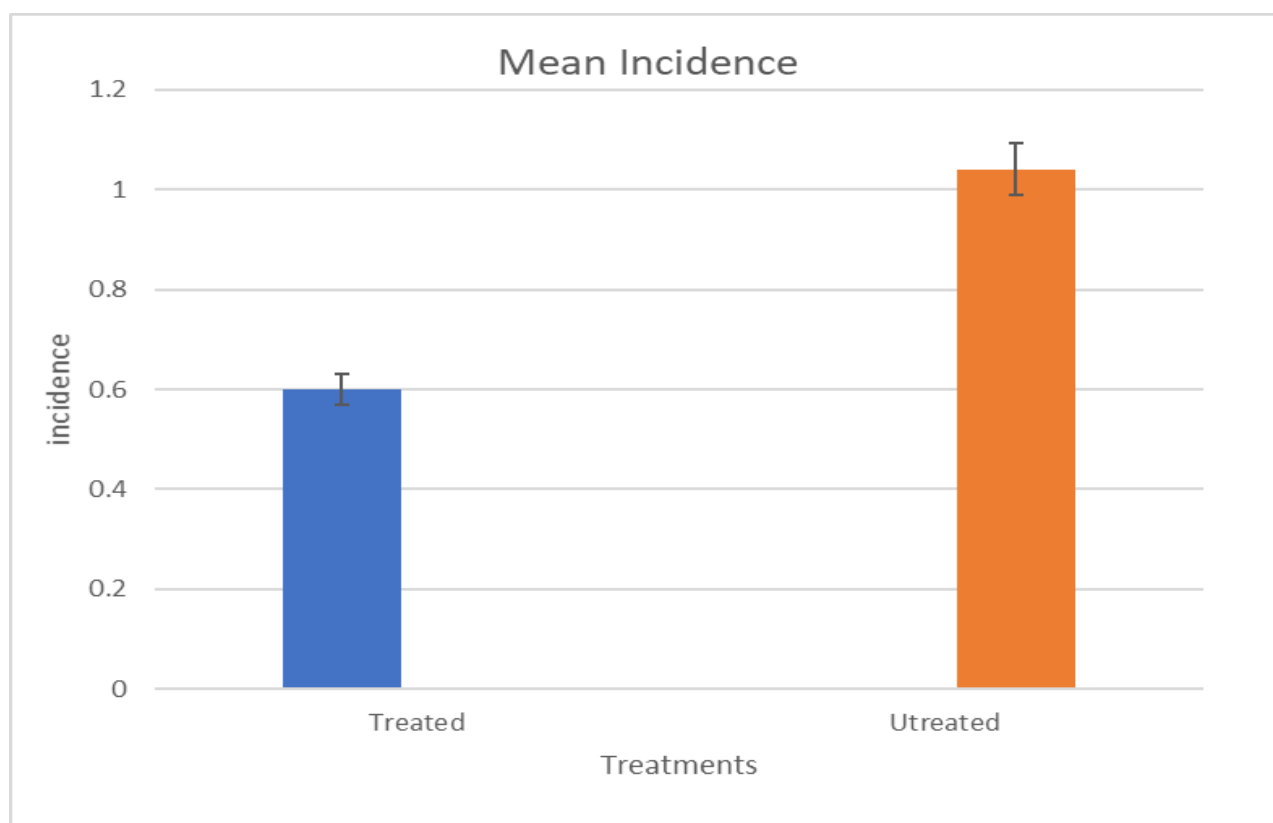


Figure 3 :Bar graph showing the mean values of disease incidence between treated and untreated.

4.2 DISEASE SEVERITY

The lowest severity score for both treated and untreated patients was 1, and the highest score for both untreated and treated patients was 5. The treated and untreated groups had mean values of 2.42 and 2.70, respectively. According to the t test, $P = 0.010$ ($P < 0.05$), and the F test, $F = 0.013$, the severity of the treated condition is significantly lower than that of the untreated condition.



Figure 4 :Bar graph illustrating the severity scores of treated and untreated experiments.

4.3 GRAIN YIELD

The average grain yield for the treated experiment was 43.8 kg per 24 lines, while the untreated experiment yielded 37.6 kg per 24 lines. Grain yield pairwise comparisons using the two-sample

t-test revealed statistically significant differences ($P < 0.05$) between the treated and untreated experiments. The conclusion that there was a significant difference in the mean grain yield between the two experimental groups was supported by the observed p-values, which were below the 0.05 significance threshold. These findings imply that although fungicide treatments have an impact on disease prevention, they also caused notable yield disparities between the treated and untreated experiment.

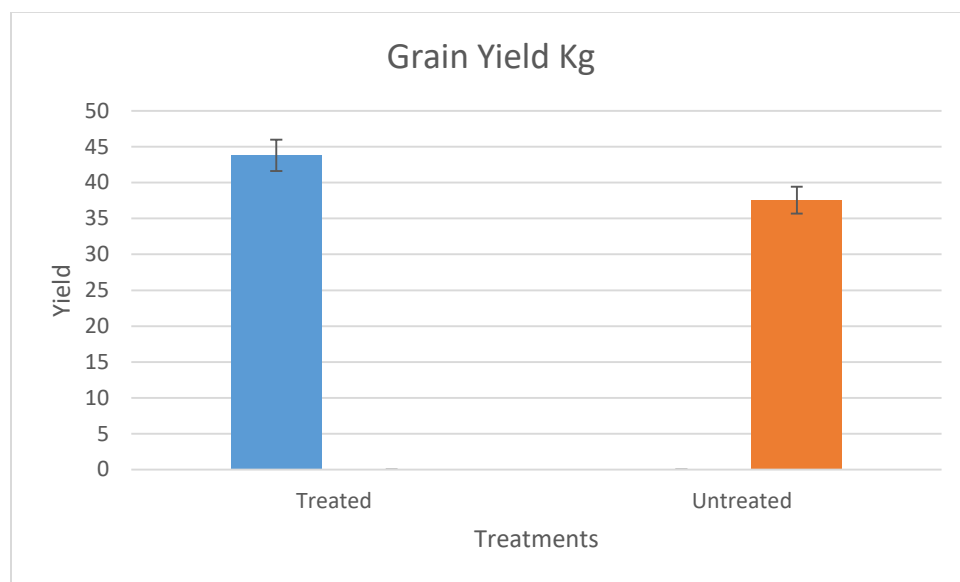


Figure 5 : Graph showing grain yield of treated and untreated experiments.

Chapter 5

Discussion, summary, recommendations and conclusions

5.1 Discussion

5.1.1 DISEASE INCIDENCE

The bar graph illustrates the mean disease incidence of treated and untreated of variety SC555, showing treated maize is less affected. The data suggest a decrease in incidence due to treatment with pydiflumetofen and Difenoconazole, with the least affected with 0 cobs and the most affected with 3 maize cobs. The mean disease incidence of the treated maize was 0.67 and the mean disease incidence of untreated maize was 1.04.

The fungicides Pydiflumetofen and Difenoconazole were used across all lines, but the extent of disease suppression may have varied due to application effectiveness, plant uptake, or initial disease load. Some lines might have received slightly better fungicide coverage, leading to lower incidence due to human error. Moreover, environmental conditions at the time of application for example, rainfall, humidity and temperature can influence fungicide retention and effectiveness (Paul et al., 2018).

The development of fungal diseases is significantly influenced by environmental factors like temperature, precipitation, and humidity. It's possible that lines placed in marginally different field microenvironments had differing infection levels. For instance, Gibberella ear rot is encouraged by high moisture levels, whereas Diplodia ear rot grows best in hot, dry conditions during pollination but in wet conditions during grain fill (Santiago et al., 2020). It's possible that not every line of maize had the same pathogen load in the field. Higher infection rates may have resulted from some lines being exposed to higher concentrations of fungal spores. Fungicide treatment and natural maize resistance may interact. The fungicide may be more effective because certain maize lines may naturally have lower infection rates. On the other hand,

excessive pathogen pressure may cause highly susceptible lines to continue exhibiting notable disease incidence even after treatment. This is consistent with research showing that applying fungicides in conjunction with genetic resistance is more successful than depending only on chemical control (Mesterházy et al., 2020). The graph's mean variations in disease incidence show the intricate interactions between pathogen pressure, environmental variables, fungicide efficacy, and genetic resistance. By being aware of these differences, disease management tactics like choosing resistant lines and enhancing fungicide application techniques can be improved. Molecular analyses to identify particular resistance genes in maize lines with the lowest disease incidence could be part of future research.

5.1.2 DISEASE SEVERITY

There was a significant difference between treatments whereby the treated experimental units showed low disease severity than the untreated (control). The highest disease severity score was 5 for both the treated and untreated experiment.. The minimum disease severity score recorded was 1 for treated and untreated experiment. The mean disease severity was 2.42 and 2.70 for the treated and untreated respectively. This agree with experiment done by (Eli et al., 2021) whereby pydiflumetofen reduce *Gibberella* ear rot in maize under misted and non-misted field conditions. This illustrates how effective a chemical is in favourable disease-environment circumstances. In contrast, pydiflumetofen, a novel carboxamide fungicide, has demonstrated the highest efficacy in reducing *Gibberella* ear rot in maize. This was accomplished by considering the best time to spray, which is during the silk growth stage, when this experiment was conducted. Additionally, there was no discernible difference in any of the other maize lines, which was consistent with research on the management of black leg fungal disease in canola, which showed that seeds treated with pydiflumetofen had a significantly reduced severity of the disease (Padmathilake et al., 2022). However, the field's significance in reducing disease severity depended on the cultivar, which supported the current study's finding that only two lines of maize responded to fungicide more than other lines did. This suggests that these lines had more severe *Gibberella* and *Diplodia* ear rot symptoms than the other lines.

The differences in disease severity among the maize lines highlight the importance of genetic susceptibility and resistance in managing *Diplodia* and *Gibberella* ear rot. On the other hand, findings from a different study that applied Pydiflumetofen 7.5% + Difenoconazole 12.5% w/v to tomatoes to prevent powdery mildew (*Leveillulataurica*) and early blight (*Alterneriaspp.*) revealed that all treatments significantly reduced the severity of the diseases when compared to the untreated control (Ekabote, 2013).

Pydiflumetofen 7.5% + difenoconazole 12.5% w/v was found to be effective against powdery mildew and anthracnose in grapes in a related study conducted by Phad et al. (2023). The effectiveness of fungicides against maize ear rot in comparison to other horticultural crops has not been thoroughly studied up to this point.

5.1.3 GRAIN YIELD

One of the stated objectives of this study was to compare grain yield between fungicide-treated and untreated maize in the context of controlling *Gibberella* and *Diplodia* ear rots. The results clearly show that the plots treated with Pydiflumetofen and Difenoconazole yielded significantly more grain than untreated plots. On average, the treated group produced approximately 6 kg more grain, translating to a 16% increase in yield.

The statistical analysis confirms that this increase is not due to random chance. The two-sample t-test yielded a t-value of approximately 3.02, which is statistically significant at the 5% level. This supports the conclusion that fungicide application had a positive effect on maize productivity. As previously shown by the lower incidence and severity scores in the treated plots, the increase in yield can be ascribed to less disease pressure. The plants could devote more resources to kernel development and ultimately increase yield if there were fewer diseased ears and less severe infections (Xia et al, 2021). This increase is especially valuable in seed maize production because it guarantees better quality seeds, more consistent kernel development, and higher returns (Priyanto et al., 2023).

Overall, the grain yield data support the incidence and severity findings, all of which indicate that the fungicide treatment was effective. Pydiflumetofen and Difenoconazole are valuable in integrated disease management strategies for maize because they not only protect against fungal

pathogens but also provide quantifiable agronomic benefits, as evidenced by the consistent improvements across all parameters.

5.2 Summary

The results show that treating maize with difenoconazole and pydiflumetofen decreased the incidence of *Gibberella* and *Diplodia* ear rot . The effectiveness of the fungicide was demonstrated by the significant difference in the severity of *Gibberella* and *Diplodia* ear rot in maize between the treated and untreated experiments. The significant difference in grain yield between the treated and untreated experiments demonstrated how well Pydiflumetofen and Difenconazole worked to control *Gibberella* ear rot and *Diplodia* in maize.

5.3 Recommendations

The findings of this study have practical implications for farmers, agronomists and seed producers involved in maize cultivation.

- The use of Pydiflumetofen, Difenconazole can be recommended to farmers growing seed maize to prevent disease incidence and severity caused by *Gibberella* and *Diplodia* ear rot to susceptible lines.

The study can be practiced in multiple farming environments to evaluate the effects of different climatic conditions.

- There is a need to evaluate by integrating with pest management.

5.4 Conclusions

The results of the study showed that the incidence of *Gibberella* and *Diplodia* ear rot infections in maize is considerably reduced by the application of the fungicide Pydiflumetofen and

Difenoconazole. The data collected demonstrates the effectiveness of fungicides in lowering the incidence and severity of diseases in susceptible maize lines. This suggests that these fungicides were effective in preventing the pathogens that cause the diseases from growing and developing. According to the study's findings, applying fungicides like Pydiflumetofen and Difenoconazole to seed maize significantly reduces the incidence and severity of Gibberella and Diplodia ear rot, which ultimately saves time and money when hand-picking the crop.

References

- Alvarez-Cervantes, J., Hernandez-Dominguez, E.M., Tellez-Tellez, M., Mandujano Gonzalez, V., Mercado-Flores, Y., Diaz-Godinez, G., 2016. *Stenocarpella maydis* and *Sporisorium reilianum*: Two Pathogenic Fungi of Maize, in: Sultan, S. (Ed.), *Fungal Pathogenicity*. InTech. <https://doi.org/10.5772/62662>
- Bensch, M.J., 1995. *Stenocarpella maydis*(Berk.) Sutton Colonization of Maize Ears. J. Phytopathol. 143, 597–599. <https://doi.org/10.1111/j.1439-0434.1995.tb00207.x>
- Chen, Y., Kistler, H.C., Ma, Z., 2019. *Fusarium graminearum* Trichothecene Mycotoxins: Biosynthesis, Regulation, and Management. Annu. Rev. Phytopathol. 57, 15–39. <https://doi.org/10.1146/annurev-phyto-082718-100318>
- Ekabote, S.D., n.d., 2013. Evaluation of Pydiflumetofen 7.5% + Difenoconazole 12.5% w/v (200 SC) on tomato against early blight (*Alternaria* spp), and powdery mildew (*Leveillula taurica*) under field condition. Int. J. Chem. Stud.
- Eli, K., Schaafsma, A.W., Limay-Rios, V., Hooker, D.C., 2021. Effect of pydiflumetofen on Gibberella ear rot and Fusarium mycotoxin accumulation in maize grain. World Mycotoxin J. 14, 495–512. <https://doi.org/10.3920/WMJ2020.2638>
- Eli, K., Schaafsma, A.W., Limay-Rios, V., Hooker, D.C., 2021. Effect of pydiflumetofen on Gibberella ear rot and Fusarium mycotoxin accumulation in maize grain. World Mycotoxin J. 14, 495–512. <https://doi.org/10.3920/WMJ2020.2638>
- Flett, B.C., McLaren, N.W., Wehner, F.C., 1998. Incidence of Ear Rot Pathogens Under Alternating Corn Tillage Practices. Plant Dis. 82, 781–784. <https://doi.org/10.1094/PDIS.1998.82.7.781>
- Forrer, H. R., Musa, T., Messmer, M. M., & Studer, B. 2019. Fungicide use and resistance in cereals: Focus on SDHI and triazole efficacy. *Journal of Plant Protection Research*, 59(2), 118–125. <https://doi.org/10.24425/jppr.2019.129745>
- Franz, K., Vogelgsang, S., & Forrer, H. R. 2021. Efficacy of succinate dehydrogenase inhibitors (SDHIs) and demethylation inhibitors (DMIs) in controlling fungal pathogens in wheat and maize. *Crop Protection*, 144, 105631. <https://doi.org/10.1016/j.cropro.2021.105631>
- Huang, X., Luo, J., Li, B., Song, Y., Mu, W., Liu, F., 2019. Bioactivity, physiological characteristics and efficacy of the SDHI fungicide pydiflumetofen against *Sclerotinia sclerotiorum*. Pestic. Biochem. Physiol. 160, 70–78. <https://doi.org/10.1016/j.pestbp.2019.06.017>
- Lana, F.D., Madden, L.V., Carvalho, C.P., Paul, P.A., 2022. Impact of Gibberella Ear Rot on Grain Quality and Yield Components in Maize as Influenced by Hybrid Reaction. Plant Dis. 106, 3061–3075. <https://doi.org/10.1094/PDIS-01-22-0148-RE>

- Luna, M.P.R., Wise, K.A., 2015. Timing and Efficacy of Fungicide Applications for Diplodia Ear Rot Management in Corn. *Plant Health Prog.* 16, 123–131. <https://doi.org/10.1094/PHP-RS-15-0010>
- Mabuza, L.M., Janse Van Rensburg, B., Flett, B.C., Rose, L.J., 2018. Accumulation of toxigenic *Fusarium* species and *Stenocarpella maydis* in maize grain grown under different cropping systems. *Eur. J. Plant Pathol.* 152, 297–308. <https://doi.org/10.1007/s10658-018-1475-y>
- Masiello, M., Somma, S., Ghionna, V., Logrieco, A.F., Moretti, A., 2019. In Vitro and in Field Response of Different Fungicides against *Aspergillus flavus* and *Fusarium* Species Causing Ear Rot Disease of Maize. *Toxins* 11, 11. <https://doi.org/10.3390/toxins11010011>
- Mesterházy, Á., Lemmens, M., Reid, L.M., 2012. Breeding for resistance to ear rots caused by *Fusarium* spp. in maize – a review. *Plant Breed.* 131, 1–19. <https://doi.org/10.1111/j.1439-0523.2011.01936.x>
- Mesterházy, Á., Oláh, J., & Popp, J. 2020. Resistance and control of *Fusarium* head blight in wheat: Role of fungicides, resistance genes, and breeding. *Frontiers in Microbiology*, 10, 2986. <https://doi.org/10.3389/fmicb.2019.02986>
- Munkvold, G. P. (2003). *Fusarium* diseases in maize. In: *Maize production and technology* (pp. 79-104).
- Munkvold, G. P. 2017. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *European Journal of Plant Pathology*, 147(1), 83–102. <https://doi.org/10.1007/s10658-016-0995-2>
- Munkvold, G. P., & Brown, D. W. 2020. Fungal pathogens and mycotoxins in maize. Parker, N.S., Anderson, N.R., Richmond, D.S., Long, E.Y., Wise, K.A., Krupke, C.H., 2017. Larval western bean cutworm feeding damage encourages the development of *Gibberella* ear rot on field corn. *Pest Manag. Sci.* 73, 546–553. <https://doi.org/10.1002/ps.4313>
- Padmathilake, K.R.E., Parks, P.S., Gulden, R.H., Rosset, J., Zhao, L., Fernando, W.G.D., 2022. Pydiflumetofen: An SDHI seed-applied fungicide, a potential tool for the canola-blackleg management toolbox. *Plant Pathol.* 71, 1992–2003. <https://doi.org/10.1111/ppa.13612>
- Parker, N.S., Anderson, N.R., Richmond, D.S., Long, E.Y., Wise, K.A., Krupke, C.H., 2017. Larval western bean cutworm feeding damage encourages the development of *Gibberella* ear rot on field corn. *Pest Manag. Sci.* 73, 546–553. <https://doi.org/10.1002/ps.4313>
- Paul, P. A., El-Allaf, S. M., Lipps, P. E., & Madden, L. V. 2018. Rainfastness of azoxystrobin, pyraclostrobin, and prothioconazole plus tebuconazole for control of wheat *Fusarium* head blight and deoxynivalenol. *Plant Disease*, 92(4), 611–618. <https://doi.org/10.1094/PDIS-92-4-0611>

- Phad, N., Shinde, D., Kabade, S., Pharate, S., Jamdade, R., Saha, S., 2023. Role of new fungicide Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) in the management of powdery mildew and anthracnose diseases in grapes. *J. ECOFRIENDLY Agric.* 18, 348–352. <https://doi.org/10.48165/jefa.2023.18.02.23>
- Priyanto, S.B., Efendi, R., Zainuddin, B. 2023. Genetic variability, heritability, and path analysis for agronomic characters in hybrid maize. *Kultivasi* 22. <https://doi.org/10.24198/kultivasi.v22i1.38807>
- Rossouw, J.D., Van Rensburg, J.B.J., Van Deventer, C.S. 2002. Breeding for resistance to ear rot of maize, caused by *Stenocarpella maydis* (Berk) Sutton. 2. Inheritance of resistance. *South Afr. J. Plant Soil* 19, 188–194. <https://doi.org/10.1080/02571862.2002.10634463>
- Santiago, R., Cao, A., & Butrón, A. 2020. Genetic resistance in maize to fumonisin contamination and Fusarium ear rot. *Toxins*, 12(5), 318. <https://doi.org/10.3390/toxins12050318>
- Sutton, J.C., 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. *Can. J. Plant Pathol.* 4, 195–209. <https://doi.org/10.1080/07060668209501326>
- Vigier, B., Reid, L.M., Dwyer, L.M., Stewart, D.W., Sinha, R.C., Arnason, J.T., Butler, G. 2001. Maize resistance to gibberella ear rot: symptoms, deoxynivalenol, and yield ¹. *Can. J. Plant Pathol.* 23, 99–105. <https://doi.org/10.1080/07060660109506915>
- Xia, R., Schaafsma, A.W., Limay-Rios, V., Hooker, D.C., 2021. Effectiveness of a novel fungicide pydiflumetofen against Fusarium head blight and mycotoxin accumulation in winter wheat. *World Mycotoxin J.* 14, 477–494. <https://doi.org/10.3920/WMJ2020.2637>