MORPHOLOGICAL CHARACTERIZATION OF *Cercospora coffiecola* ON ROBUSTA COFFEE BERRIES IN UGANDA

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DECLARATION

I**, ANYIJUKA MARK,** do hereby declare that this work is original and has not been presented to any university or institution for any award.

Signature: …… Date: …….

APPROVAL

This dissertation has been supervised by us and it is hereby approved to be submitted for examination.

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Signed: …… Date: ……..

Dr. KAGEZI GODFREY

DEDICATION

This piece of work is dedicated to my family, friends, and workmates for their unwavering professional financial, and moral support which motivated pursuit of this degree.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
BES	Brown Eye Spot
CBD	Coffee Berry Disease
CLR	Coffee Leaf Rust
CRD	Completely Randomized Design
CWD	Coffee Wilt Disease
DDP	District Development Plan
DI	Disease Index
DNA	Deoxy ribonucleic acid
GDP	Gross Domestic Product
IAC	Agronomic Institute of Campinas, Brazil
ICO	International Coffee Organisation
ICPP	Inter-Government Panel on Climate Change
KR	Kituuza Robusta
MT	Metrix Tonne
NaCORI	National Coffee and Cocoa Research Institute
NARO	National Agricultural Research Organisation
PDA	Potato Dextrose Agar
PhD	Degree of Philosophy
RBD	Red Blister Disease
SM	Symptom Morphotype
TWA	Tap Water Agar

UCDA	Uganda Coffee Development Authority
UFLA	University Federal de Lavras
USA	United States of America
USAID	United States Agency for International Development
VCG	Vegetative Compatibility Groups

ABSTRACT

Coffee contributes enormously to Uganda's economy; however, farmers currently lose over 50% yield of the crop to coffee red blister disease (CRBD), a fungal infection caused by *Cercospora coffeicola*. Currently, the pathogen poses the greatest challenge to Robusta coffee production though its ecology is not fully studied. This study aimed at characterizing *C. coffeicola* on berries of Robusta coffee in Uganda. A total of 47 Robusta coffee farms were randomly sampled from seven (7) districts which were purposively selected from three (3) Robusta coffee growing regions. Fifty (50) coffee berries were sampled along a transect at each farm and assessed for RBD symptomatology variability. The Coffee Red Blister Disease (CRBD) was found in all the sampled regions and districts of Uganda with a prevalence rate of 38%. The highest CRBD prevalence (47%) was observed in central Uganda and was significantly different from that of Southwestern region (16%) ($P < 0.0001$). On average, the lesion incidence on diseased Robusta coffee berries was 10.6%. The highest lesion incidence was recorded in central region (13.1%) followed by mid-eastern region (12.0%) and least in the southwestern region (6.1%). CRBD lesion area coverage on the coffee berry averaged at 27.3% but varied significantly across the regions ($P=0.0069$) and districts ($P<0.0001$). Berries collected from mid-eastern and central regions recorded a statistically higher lesion area coverage (30%) than southwestern region (21.8%). The average number of CRBD lesions per coffee berry ranged from 4.4 to 8.4. Luuka district with 10 lesions per berry registered the highest number of lesions and lowest Isingiro district (0.5). The pathogen, *C. coffeicola*, was isolated from infected berries and the isolates assessed for morphological variability. Based on the isolates' pigmentation, radial growth, colony elevation, sporulation ability, and shape, the assessment resulted into six symptom morphotypes (SM) that were randomly assigned the capital letters of the alphabets "A" to "F". Isolated *C. coffeicola* fungi grew linearly on PDA at a rate of 2.9 to 3.6 mm/day to attain a diameter of 27 to 70 mm in 5-14 days. Morphotype IV (colony radius = 44mm/day) had the highest growth rate, while morphotype V had the slowest growth rate (colony radius = 35mm. Morphologically, the morphotype colonies were peripherally flat with slightly raised front view and umbonate to radially folded centers. Pathogenicity tests that were conducted on one susceptible Robusta coffee variety (KR6) and two tolerant varieties (KR3 and KR10) using three distinct morphotypes, L1C (Morphotype II), M1C (Morphtype III) N1C (Morphotype IV) revealed that all the isolates were pathogenic to the three selected coffee varieties. L1C caused the most cercosporiosis to KR 6 (DI = 41%) while M1C caused the least disease (DI=1.3%) on KR3. N1C did not infect KR10. KR6 had the highest disease index of 41.2%, while the resistant variety, KR10 had the lowest (6.8%). In conclusion, there is variability in the *C. coffeicola* pathogen complex across the Robusta coffee growing regions, as exhibited through symptomatology of the disease on berries. This study provides baseline information on CRBD symptomatology and pathogenicity to inform further research on the disease. This information can guide target CRBD management strategies as well as development of new CRBD resistant varieties.

CHAPTER ONE: INTRODUCTION

1.1 Background

Coffee remains Uganda's most important cash crop, contributing about 18% of the country's export earnings, which translates to US$ 1.50 billion from coffee exports annually (December 2023-November 2024) (UCDA, 2024). The entire production is carried out by approximately 1.9 million households, nearly 7.5% of the population that are spread across 108 districts in Central, Western, South-Western, Northern, and Eastern parts of the country (UCDA, 2019). The Busoga and mid-Northern regions are conducive for Robusta production while the Mountain Elgon region is exclusively for the Arabica coffee. However, both coffee types are grown in the North-Western, South-Western and Western regions (Fig. 1). Currently, Uganda is the World's 4th largest producer of Robusta coffee, which accounts for up to 80% of Uganda's total coffee produce. Apart from the economic importance of the coffee berries, the coffee by-products can have several applications including use as an organic fertilizer (Hoseini et al., 2021).

Despite Robusta coffee's relevance to Uganda's economy, its production fluctuates around 0.6 t/ha, close to ten times lower than its potential of 5 t/ha (Musooli, 2017). This subsequently signifies that the sector is underperforming and thus unable to meet the overwhelming current global market demand. At 6.87 million 60kg bags per year (April 2024 – March 2025 coffee year), the production is way below the 20 million 60 kg bags that is currently required by end of 2025 under the Presidential directive of 2015 (UCDA, 2021). This low production could be attributed to soil fertility decline, climate change, poor agronomic practices, high incidence of pests and diseases, and poor methods of

post-harvest handling of the crop produce for utmost quality, among others (Kobusinge et al., 2018). Fungal diseases pose one of the greatest challenges to coffee production, claiming over 50% loss of total coffee production annually (Kyalo et al., 2024). Among the fungal pathogens, *Cercospora coffeicola* (B. & Cke.), the causal agent of cercosporiosis, is one of the economically important fungal pathogen that is known to cause brown eye spot (BES) disease on the leaves of Arabica (*Coffea arabica* L.) and Robusta coffee, and red blister disease (RBD) on berries of Robusta coffee (Martins et al., 2008).

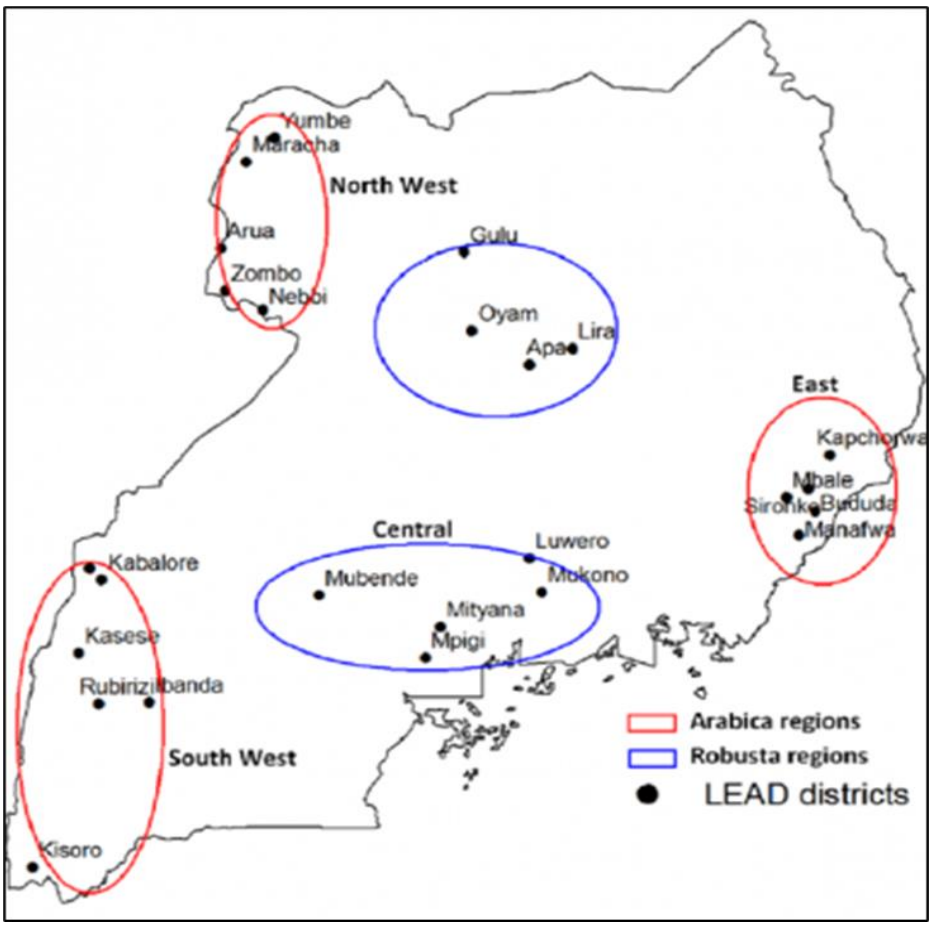

Figure 1: The top coffee producing districts in Uganda.
(Source: *https://www.coffeebeansafrica.com/about/coffee-farming-facts/.)*

For instance, cercosporiosis caused yield losses exceeding 30% during severe

endemics in Brazil across coffee growing areas (Zambolim et al. 1997). The disease remained detrimental to the crop with over 15% yield loss when effective fungicide-based alternatives (Silva, 2018; Martinez et al., 2023), as well as integrated disease management such as enhanced plant nutrition (Cardoso et al. 2013) and biological control (Cardoso et al., 2013) were deployed. Nonetheless, breeding for resistance against cercosporiosis has yielded promising results in Brazil (Botelho et al., 2017) even though no resistant coffee materials are available yet. Also, severe epidemics of cercosporiosis have been reported across various Robusta coffee growing tracts, for example, in Vietnam (Souza et al., 2012; Martinez et al., 2023, Hartati et al., 2024), and registered a constant threat to the global yield and quality of Robusta coffee. Moreover, the distribution, severity levels and population dynamics of the disease has received little attention in Uganda until 2021 when over 40% loss of the Robusta coffee in the central region of Uganda were attributed to cercosporiosis.

Cercospora coffeicola infection manifests as necrotic lesions on leaves, characterized by a light-colored center surrounded by a purplish-brown ring with yellowish edges (Nelson, 2008). On Robusta coffee berries, the pathogen is virulent at all developmental stages, initially appearing as slightly raised red spots with an orange-yellow halo. These spots gradually enlarge and coalesce into dark patches that may be raised or flattened with a sunken center, eventually drying out and consuming the entire fruit (Souza et al., 2012). Under favorable meteorological conditions, lesions spread to neighboring berries and clusters, leading to premature leaf drop, yield loss, and reduced grain quality (Lima et al., 2012). Despite this, symptom expression of *C.*

coffeicola remains variable and lacks conclusive description. Variability is largely shaped by meteorological factors (Durand-Bessart et al., 2020; Goulart et al., 2016; Lorenzetti et al., 2015), as well as ecological factors such as host and pathogen genetic variability (Botelho et al., 2017; Schumann and D'Arcy, 2012). These factors influence pathogen dynamics by affecting dispersion, sporulation, conidial germination, mycelial growth, cercosporin production, and host colonization (Durand-Bessart et al., 2020; Goulart et al., 2016; Lorenzetti et al., 2015). Symptom variability of *C. coffeicola* leaf spots has been extensively studied in Brazil, where two distinct forms—black spot and brown spot—were reported (Botelho, 2019). A similar scenario occurs with red blister disease on Robusta coffee in Uganda, which remains poorly described. To date, no detailed study has addressed the delimitation of *C. coffeicola* symptoms on Robusta coffee berries in Uganda, despite the pathogen's devastating impact on this national crop (Kyalo et al., 2024).

1.2 Problem statement

Cercospora coffeicola currently presents the most devastating berry disease to Robusta coffee in Uganda (Olango et al., 2024). The disease limits the quality and yield of the crop by 15 – 50% under severe infection (Souza, 2014). *Cercospora coffeicola* infects coffee on both the leaves and berries (Siddiqi, 1970; Nelson, 2008; Souza et al., 2012). Symptomology of the disease is well defined on the coffee leaves, with two key and distinctive symptoms - brown and black spots (Souza et al., 2015; Botelho et al., 2019; Vale et al., 2021). Contrary to this, information on symptomology of the disease on the coffee berries is very scanty (Nelson, 2008; Souza et al., 2012), though several field observations made on Robusta coffee in Uganda reveal that Cercosporiosis probably

exhibits a variety of symptoms (e.g., Kagezi et al., 2018; Kyalo et al., 2025). There is therefore a need to conduct detailed scientific studies to confirm and document the different symptoms since they have implications to the diagnosis (Bock et al., 2008) and thus management of the disease (Burgess et al., 2008; Radhakrishnan et al., 2019).

Beyond symptomatology, there is also limited information on the virulence variability of the pathogen (Tuggle et al., 1999; Gu et al., 2020). This can have significant impacts on disease management strategies as a highly virulent pathogen will cause more severe damage to the host plant, and/or require more aggressive control methods (Sacristán and García-Arenal, 2008; Zamani-Noor et al., 2008). However, there is limited information on the virulence of the various symptoms of *C. coffeicola* on berries of Robusta coffee which complicates its management. Understanding the virulence variability of *C. coffeicola* could therefore play a major role in the development of effective management strategies for this important disease of coffee (Nega et al., 2020). This study aimed at investigating the symptomatology and virulence of *C. coffeicola* on berries of Robusta coffee. This is crucial for informing future research as well as the development of management strategies for the disease in Uganda and beyond.

1.3 Objectives of the Study

1.3.1 General Objective

To investigate the variability of *Cercospora coffeicola* pathogen on berries of Robusta coffee for sustainable management of the Coffee Red Blister Disease (CRBD) in Uganda.

1.3.2 Specific Objectives

(i) To identify *Cercospora coffeicola* morphotypes causing Coffee Red Blister Disease (CRBD) on berries of Robusta coffee in Uganda.

(ii) To determine the virulence of the *C. coffeicola* complex on berries of Robusta coffee in Uganda.

1.3.3 Hypotheses

(i) There are no variable morphotypes of symptoms of CRBD and resultant isolates of *Cercospora coffeicola* on berries of Robusta coffee

(ii) *Cercospora coffeicola* morphotypes infect berries of Robusta coffee with the same magnitude.

1.4 Significance of the study

The study reveals the variability of *Cercospora coffeicola*, the causative agent of Red Blister Disease (RBD) on Robusta coffee through identification of morphotypes therein as regards isolate colony morphology and pathogenicity.

This study also enables precise extrapolation of the distribution of *C. coffeicola* using updated RBD scoring methods in the region, and facilitates accurate prediction of future disease magnitudes on Robusta coffee. Virulence reputations were determined for dominant pathogen morphotypes and highly virulent isolates were identified for reliable breeding of resistant material to the Red Blister Disease. In addition, tolerant Robusta coffee varieties were identified among the tested varieties in the study important for farmers to better decide on the varieties to incorporate in their prospective farming systems.

1.5 Justification

Cercospora coffeicola presents a great challenge to Uganda's production of Robusta coffee in that it has proven virulent to the crop at various phenological stages and on different plant parts: leaves, fruits, and periderm to a certain extent.

Continued reliance on the unclear identity of the pathogen *C. coffeicola* on Robusta coffee in Uganda exposes the crop to unpredictable epidemics, thereby threatening the livelihoods of over 1.9 million coffee farmers and other stakeholders who depend on it. *Cercospora coffeicola* has caused enormous damage to the nation by destroying susceptible varieties hence a threat to the Robusta coffee germplasm as a regrettable risk to contain. Based on this background therefore, a study was conducted to determine the symptomatology and virulence of *Cercospora coffeicola* on berries of Robusta coffee (*Coffea canephora*) so as to inform development of sustainable management strategies for Coffee Red Blister Disease (CRBD) in Uganda.

CHAPTER TWO: LITERATURE REVIEW

2.1 Importance and description of coffee

Coffee is an important commodity on the global market with an estimated value of around $200M (Simon-Gruita et al., 2019). The Uganda's economy thrives enormously on agriculture, ranking the enterprise in the top earners for the country's GDP and foreign exchange. The crop is consumed mostly as a beverage (ICO, 2021). Based on its diverse applicability, the crop employs a substantial amount of stakeholders spread across the numerous value chain. For example, about 1.9 households are involved in the production of coffee in Uganda alone (UCDA, 2021).

Coffea contains over 40 species, among which three: *Coffea robusta, C. liberica,* and *C. arabica* are the most commercially produced for consumption. The *Coffea* species vary enormously from phenotypical, biochemical, physiological as modulated from the diverse genetic markup among the plant species (Davis et al., 2023). For example, *C. robusta* is an ever-green broad-leaved plant that grows robustly to reach canopy diameter of around 3m and height of ~ 5m. The plant species grows best at altitudes below 1400 masl (Musoli et al., 2013). The species is the second most grown after *C. Arabica* on the globe (Madrid-Casaca et al., 2021), with one third of it grown in Brazil (Veloso et al., 2023). While Robusta coffee accounts for nearly 80% of the country's production, Arabica coffee constitutes about 20%. The crop covers an approximate 200 sq miles which translates to over 20% on the country's arable land. Uganda comes in 4th as the biggest producer of the crop globally (UCDA, 2021), and produces over 30 MT of the crop annually. Given over 70% of coffee growers in the country rely on a rain-fed production system, this

exposes the production systems of the crop to the changing climatic conditions.

2.2 Constraints to coffee production

Among other challenges, coffee production is limited by biotic (pests and diseases), and abiotic (i.e. reduced soil fertility, drought, excessive precipitation, poor post-harvest handling methods, and poor crop management practices) factors (Cardoso et al., 2013; Wang et al., 2015). The pests limiting the production of the crop range from leaf eaters, fruit borers, branch and stem borers to root infesters (Wang et al., 2015). Among the coffee production biotic constraints, diseases caused by fungal agents take a lion's share of the yield and/or quality limiting factors often causing reductions exceeding 50% annually.

2.3 Fungal diseases constraining Robusta coffee production

Fungal pathogens dominate the constraints to coffee production across the coffee growing regions (Durand-Bessart et al., 2020). These include Coffee Wilt Disease (CWD), Coffee Leaf Rust (CLR), Brown Eye Spot (BES), Coffee Red Blister Disease (CRBD), and Coffee Berry Disease (CBD) (Muller et al., 2009). CWD caused by a soil borne fungus, *Fusarium xylarioides*, has been reported as the most important disease of coffee. The pathogen causes wilting of susceptible varieties at any stage through infection via the roots or injury on the stem of the plant (Geiser et al., 2005; Peck, 2024). The second most important disease is CLR, caused by *Hemileia vastatrix*, followed by BES that is attributed to *Cercospora coffeicola*. Both pathogens cause leaf diseases that reduce the photosynthetic area of the plant through induction of necrosis and/or defoliation of the plant (Liebig, 2017; Soliman et al. 2019). Berry-infecting diseases such as RBD and CBD cause the most noticeable reduction

of the crop productivity since they infect the most economically important part of the plant. RBD caused by *Cercospora coffeicola*, is most important on Robusta coffee where it causes substantial crop yield reduction that can go beyond 50% in severe epidemics (Matovu et al., 2013; Vale et al., 2020).

2.4 Taxonomic Insights and Agricultural Importance of Cercosporoid Fungi

Cercospora coffeicola, the focal subject of this study, belongs to one of the oldest, largest and heterogenous group of hypomycetes genera (Groenewald et al., 2013). The name Cercospora which is applied from the combination of Greek "kerkok" meaning tail, and "sporos" meaning seed, determines the filiform conidia of the fungus. The genus has been treated as an anamorphic genus, linked to the teleomorph genus Mycosphaerella (Capnodiles, Mycosphaerellaceae; Stewart et al., 1999, Crous et al., 2004). Cercospora genus is estimated to contain over 3000 species names as suggested by Chupp (1954) and Pollack (1987). Cercosporoid fungi form one of the most important phytopathological agents (Groenewald et al., 2013). The pathogens are highly incident in production fields on various plant parts, except in the roots.

2.5 Economic importance of genus Cercospora to Agriculture

The genus has been reported on a multitude of plant species including cereals, vegetables, ornamentals, forest trees, and grasses, among others. The genus contains species that identify as highly host-specific, while others have a broad host range; for example, the *Cercospora apii* complex (Groenewald et al., 2005, 2006).

The Cercospora genus causes important diseases that compromise the productivity of host plants essential for human survival. Hence the genus

undermines efforts towards better food security and the economic well-being of masses dependent on the affected crops. Cercosporoid fungi may occur on the plants as: i) saprobic organisms, consuming dead matter on the plant; ii) secondary invaders, establishing at localities after activity of the primary pathogen; and iii) destructive pathogens, infecting healthy tissue of plants to cause symptoms that minimize plant vigor and productivity that translates to substantial economic loss. Plant parts preferred by the genus include leaves, fruits, flowers, bracts, seeds, pedicels, branches and stems. The cercosporoid fungi are associated with fruit rots, necrotic lesions on fruits, flowers, bracts, seeds and pedicels, as well as fruit and leaf spots (Silva et al., 2016). Due to Cercosporoid fungi pathogenicity to plants, they have equally been utilized as biological agents of fungal diseases and mycoherbicide agents (Mutebi, 2021).

2.6 Distribution of Cercosporoid fungi

Cercosporoide fungi have a wide coverage and distribution worldwide (Agrios, 2005; To-Anun et al., 2011; Crous et al., 2013; Groenwald et al., 2013). This has been enabled by the genus' ability to infect a wide variety of plant species across a wide range of climatic conditions, i.e. from cool temperate to tropical regions (Crous et al., 2013).

2.7 Phylogeny of the Genus Cercospora

Characterization of Cercospora as a genus has been approached with a number of methods:

1. Based on morphological criteria as described by Chupp (1954) in his monograph, a broad concept of the genus Cercospora was established. Other authors taxonomyically gave thename "Cercospora" based on host plant

association (Ellis, 1971). The morphological features' analysis strategy was limited to hila thickening, pigmented/melanated conidia, and style of conidial production (i.e. borne singly or in chains). This work created awareness and paved way for subsequent mycologists in an attempt to separate the genera within the Cercospora complex. Different combinations of fungal features like: i) conidiomatal structure,ii) sporodochia or synnemata, iii) presence or absence of superficial mycelium as well as their texture, iv) conidiophores branching, arrangement, ornamentation and pigmentation, v) conidiogenous cells (proliferation, placement and scar/hilum type), and vi) conidial shape, formation, ornamentation, septation, catenulation and pigmentation, were deployed. On the other hand, Deighton and Braun took a similar approach in their series of papers (Deighton, 1971, 1976, 1979; Braun 1996, 1999) came up with genera like Cercosporella, Cercosporidium, Paracercospora, Pseudocercospora, Pseudocercosporella, Passalora, Stenella and Stigmina. Crous and Braun (2003) scrutinized the morphologically distinguishable species associated with Cercospora s. lat in reference to morphological features that included: structure of conidiogenous loci (scars) and hila, whether thickened or unthickened, pigmentation in conidiophores, and conidia whether present or absent. The tasked proved highly problematic since morphological characters of the species were more uniform giving rise to a high-level intraspecific variation (Groenewald et al., 2013; Bakhshi et al.. 2015).

2. Based on gene sequence data: this has become the most reliable method to solve taxonomic problems around Cercospora species delimitation (Groenewald et al., 2013; Bakhshi et al., 2015, 2018). Although a substantial

number of Cercospora species lack adequate sequence data as noted by Groenewald et al. (2013), the genus Cercospora has attracted adequate attention in the recent past.

2.8 Distribution and host range of *Cercospora coffeicola*

The pathogen was first reported in Thailand by Sontirat et al. (1980). Currently, it widely distributed throughout all Coffee growing tracts in the tropics and subtropics (Nelson 2008) as well as where the plant species flourishes wildly including islands within the Pacific and Actic (Chand et al., 1959).

Crous and Braun (2003) reported the occurrence of *C. coffeicola* in nearly 100 countries spread across the seven continents of Africa, Asia. North, Central, South America, the Caribbean, and Oceania.

In terms of host range, which denotes the behavior of a pathogen and its ability to infect members of a particular specie or a group of related species, the pathogen is known to be non-host specific. According to Burnet (2003), phytopathological fungi exhibit host specificity due to their ability to infect particular living plants. Howbeit, there is limited documentation of speciation and host specificity of Cercospora. *Cercospora coffeicola* exhibits mild host specificity to coffea spp (Nelson, 2008) since it causes disease to majority of the species within the genus. Just like other species of Cercospora, the pathogen specifies its occurrence on coffee due to particular environmental conditions that coincides with the flourishment of the plant and the ability of the pathogen to invade and derive its nourishment thereof (Zhou and Hyde, 2001; Groenewald et al., 2006). *C. coffeicola* has been reported on various host

species, majority of which belong to the family Rubiaceae. The family contains approximately 40 Coffea species including *Coffea arabica, C. canephora, C. excelsa, C. laurina, C. liberica, C. Robusta, and C. stenophylla*, among others (Crous and Braun, 2003).

2.9 Symptom variability of Cercosporiosis in Robusta coffee

Coffee coffeicola has been able to establish infection on various parts of the coffee plant. However, symptom identification has only been given adequate attention on infection caused. For instance, on the leaves, the disease has been identified in the field by symptoms of Brown Eye Spot (BES). BES infection often manifests as circular or irregular spots with tan, or gray centers surrounded by reddish brown distinct ring that is visible on the upper and lower surface (Souza, 2012). Lesions range from 2 to 6 cm in diameter (Nelson, 2008). BES limits crop productivity by heightening defoliation that reduces the overall photosynthetic area of the plant (Nelson, 2008; Souza, 2012, 2016).

Botelho (2019) reported a new symptom caused by the pathogen on the leaves as a darker and larger lesion, named “black spot”. Variability between the two symptoms has been investigated to define morphological, molecular and virulence distinctness. Symptom identification on the stem parts has hardly been reported, even though the pathogen is known to establish an infection on the plant part.

On coffee fruits, the pathogen is documented to be infectious to all stages of development. The disease lesions start as light to deep brown or reddish brown raised, small, circular or irregular lesions (4-10 mm) surrounded by a purplish

halo that yields a prematurely ready tissue on the fruit. These later enlarge to form sunken ovoid darkened centers with a greyish layer of presumed fungal spores. The fungal lesions penetrate to bind the pulp to parchment and seed complicating the post-harvest handling methods of the crop produce (Nelson, 2008). Given that symptom manifestation is as a result of particular infection by a plant pathogen, more species can occur since one ecological requirement may be favorable to another (Ngugi et al., 2001).

Moreover, the interaction across various kinds of microbes such as mutualistic, antagonistic, neutral, etc or among fungi is known to occur, often resulting into symptoms that differ from those produced by the pathogens in singular occurrences (Madaraiga and Scharen, 1986; Davidson et al., 2012). Symptomatology of established by *C. coffeicola* has been commonly confused with of *Colletotrichum kahawae*, a fungal pathogen that causes anthracnose disease on *Coffea arabica*. Studies to find out whether *C. kahawae* causes anthracnose disease on *C. robusta* or other varieties of coffee have not been investigated thoroughly. Red blister disease, also known as brown eye spot, of the fruits has earned attention in Uganda. The disease has been reported to upsurge in Robusta coffee growing parts of the country (UCDA, 2023). There has been a lot of mis-identification of the disease on the fruits of the crop across the coffee growing regions in the world. A diversity of symptoms associated with RBD on berries of Robusta coffee has been observed on various locations across the Robusta coffee growing tracts in Uganda. Variability in symptom manifestation has been attributed to the variability in the environmental factors that enable successful infection of the crop by the pathogen. These factors may include air temperature, humidity, soil fertility,

and climate change, among others. While RBD is known for its adverse impact on the productivity and quality of Robusta coffee, little is known about the infection process and factors involved in symptom formation and appearance following *C. coffeicola* infection of Robusta coffee berries (Godoy et al., 1997; Pozza et al., 2010; Dell' Acqua et al., 2011; Souza et al., 2011, 2012; Delima et al., 2012).

2.10 Infection process and symptom expression of Cercosporiosis in Robusta coffee

Host infection by the pathogen is often enabled by conidia. These are transmitted to the host wind and rain water dispersion from infected plant parts or wintering spore depositions to healthy plant parts. The pathogen affects all the aerial parts of the plant including leaves, fruits and fresh green sections of the stem (Godoy et al., 1997). The *C. coffeicola* disease cycle has limited literature. Although it has been reported that most species of Cercospora demonstrate a hemibiotrophic life style, *C. coffeicola* routinely deploys a necrotrophic colonization strategy to obtain nourishment. Such a strategy, kills host cells when the pathogen digests host cells using cercosporin and oxygen reactive particles (Daub et al., 2013)

Infection of the leaf

Cercosporoid pathogens present variability through germination, penetration, and sporulation. Penetration through the stomata has been observed for *C. moricola* and *C. caricis* during infection of mulberry and cassava, respectively (Gupta et al., 1995; Neto et al., 1998), while direct penetration through the epidermal cells and stomata has been demonstrated in *C. arachidicola* infection of peanut foliage (Smith et al., 1992). Whereas conidia of *C.*

moricola develop numerous germ tubes, *C. henningsii* conidia has been reported to bug and fragment into many microconidia (Gupta et al., 1995; Ayesu-Offei and Antwi-Boasiako, 1996).

The germ tubes elongate toward the stomatal opening where the pathogen penetrates the host. The invader then grows within the intercellular space before progressing to the spongy parenchyma cells. At seven days after conidia landing, symptoms of the disease start to manifest. The pathogen proceeds to devour cells within the leaf using hyphal extensions as the lesion on the leaf enlarge. At about 20 days post conidial landing, symptoms are observed as circular necrotic lesions encircled by chlorotic halos. Conidiophore emerge in groups on the abaxial surface through the stomata.

Infection of the fruit

An investigation was made to study the infection process of *C. coffeicola* in immature berries of Robusta coffee. The pathogen conidia germinated around 4 hours after deposition to show two germ tubes, in contrast with the BES *C. coffeicola* that produced one germ tube. The germ tubes grew towards the wounds on the fruit and not the stomatal openings though the fungal structures grew over the natural openings, also differing from the leaf infection process. There internal mycelial development though appressoria was not made by the pathogen. However, this investigation did not cover development of lesions on the fruit. Nevertheless, lesion development on coffee berry was investigated by Paula et al. (2019). The authors reported progress of the infection on the yellow and red fruit of the crop. Scarcity of information about expression of Cercospora infection on berries of Robusta coffee prevails since the pathogen has not been of economic importance on the plant part. Hence scientists

focusing on the pathogen's infection on the leaves where the caused disease greatly limits the productivity of coffee. More studies are encouraged to understand the variability of *C. coffeicola* infection across the ten Robusta coffee lines in Uganda.

2.11 Identification and differentiation of *Cercospora coffeicola*

Cercospora coffeicola, having stemmed from the traditional nomenclature system based on host -pathogen association, the species is not exception to the prevailing challenges associated with the method's reliability. Host specificity attributed to taxonomic resolution of Cercosporoid fungi has been disregarded due to multiple reports of the pathogens infecting and sporulating on different species of hosts (Vaghefi et al., 2017, 2018; Knight et al., 2019). For example, *C. coffeicola* does not only infect various species of *Coffea* including *C. canephora* and *C. arabica* but also distant species like castor beans (*Rucinus communis*) (Sauza et al., 2011). The fungus has a number of names that include *Ramularia goeldiana, Cercospora coffeae, Cercospora herrerana,* and *Mycosphaerella coffeicola* as sexual morph. Crous and Braun (2003), emphasized that species of Cercospora genus contain morphologically confusable species. This has in the long run translated to a highly interspecifically variant taxon as noted by Groenewald et al. (2013) and Bakhshi et al. (2015).

Various approaches have been put to the task of characterizing *C. coffeicola* population structure among which the use of nitrogen non-utilizing mutants with vegetative compatibility groups (VCG) was deployed to define the population structure of the pathosystem in variable cropping systems of Brazil (Martins et al., 2008). With this method Martins et al (2008) found high

genetic variability across all hierarchical levels checked finding a high number of VCGS in populations of *C. coffeicola*.

Vale (2021) used a multi-locus approach to screen 19 isolates of varying symptom for BES disease. Through this approach, five regions of the pathogen's genetic makeup were amplified and analyzed. The regions included the internal transcribed spacer regions and intervening 5.8S nrRNAs, actin, calmodulin, histone H3, and translation elongation factor, 1-alpha. The authors discovered that all the strains aligned with *C. coffeicola* strain from Japan without finding intraspecific variability.

Identifying symptom morphotypes and cultural morphotypes of *C. coffeicola* is essential for effective disease management and understanding the pathogen's variability. Symptom morphotypes reflect distinct disease manifestations on infected coffee berries, which can vary among isolates, aiding in accurate diagnosis and severity assessment. Conversely, cultural morphotypes pertain to growth characteristics observed in vitro, such as colony color and texture, providing insights into the pathogen's physiological diversity. This knowledge facilitates the differentiation of pathogenic races and informs breeding programs aimed at enhancing coffee resistance. Comprehensive characterization of both morphotypes is crucial for developing targeted management strategies to mitigate the impact of *C. coffeicola* on coffee production.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Identification of *Cercospora coffeicola* morphotypes on berries of Robusta coffee

3.1.1 Study Area

The study was conducted in the three major Robusta coffee growing regions of Uganda including central, southwestern and mid-eastern (Jassogne et al., 2013). At least two neighboring districts in each region were randomly selected for a survey. The selected districts included Mukono, Luweero, and Nakaseke in central region; Ntungamo and Insingiro in southwestern region; and Iganga and Luuka in mid-eastern Uganda (IIITA, 2024).

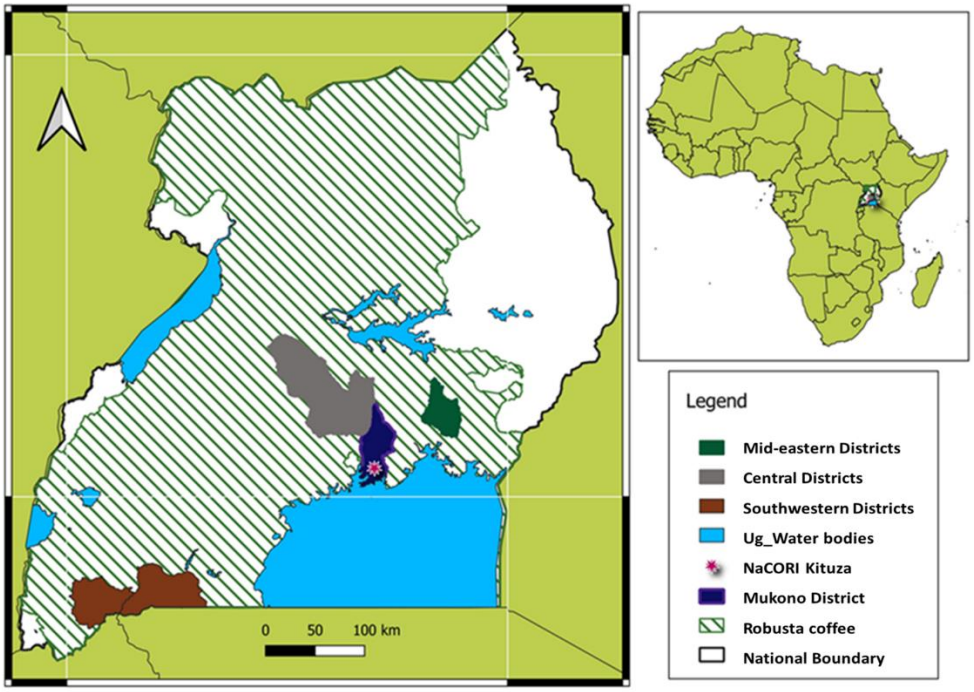

Figure 2: Study districts in the three major Robusta growing regions of Uganda

3.1.2 Study design and sampling technique

A multi-stage sampling technique was used to select well established Robusta coffee farms of more than 0.5 acres since over 80% of the coffee growers are

smallholder farmers. Sampling a farm of more than 0.5 acres of robusta coffee enables detection sensitivity and disease incidence and severity may follow aggregated or clustered patterns. In each of the seven (7) districts, two (2) subcounties were randomly selected and in each subcounty, 6 or 7 farms were sampled giving a total of 47 farms of Robusta coffee that were used in the study. At each coffee farm, 50 Robusta coffee berries were picked randomly from the Coffee Red Blister Disease (CRBD)- infected Robusta coffee trees and surface sterilized with 70% ethanol before they were packed in sterile air-tight ziplock bags (Guo et al., 2000). The samples were then transported to the pathology laboratory at NaCORI, Mukono, Uganda.

3.1.3 Symptom identification and assessment

The sampled coffee berries were assessed for CRBD incidence by recording presence or absence of the disease. Symptoms of the disease were then assessed visually and differentiated into symptom classifications based on their phenotypic variability, i.e. pigmentation, elevation, wetness, evenness and lesion diameter (mm) on the berry as described by Nelson (2008). Incidence of each symptom classification was recorded as presence or absence of the particular symptom classification on a coffee berry. Disease severity was recorded as the area of the particular lesion on the coffee berry's pericarp and expressed as a percentage. After that, the average number of lesions belonging to a particular symptom classification per coffee berry was computed.

3.1.4 Pathogen isolation and identification

Two thousand three hundred fifty (2,350) CRBD-infected coffee berries were surface sterilized with 70% ethanol, placed on sterile ethanol and left to air dry. *Cercospora coffeicola* was isolated from the lesions from the coffee ber-

ries by excising and plating on water agar as described by Tondok and Sa'adah (2023), and then incubated for five days under room temperature at photoperiod of 12hr/12hr light-dark period. The resultant fungal colonies, typical of *Cercospora coffeicola*, were sub-cultured on Potato Dextrose Agar (PDA; Sigma-Aldrich, St. Louis, MO, USA) and incubated under similar growth conditions described above for 14 days (Souza et al., 2012). Pure cultures were generated by transfer of hyphal tips on PDA according to Guo (2021). Prior to use, water agar and PDA were sterilized in an autoclave at 121°C for 15 minutes, cooled and then dispensed into sterile 90 mm diameter Petri dishes.

Colony characteristics, namely, colony pigmentation, texture, growth rate, sporulation ability, shape, and elevation were assessed on the cultures according to Chupp (1954). Pigmentation was visually observed on 12 day – old cultures on PDA. Texture was assessed by examining the culture morphology. Ability to sporulate was assessed by scraping the surface of the colony and examining the harvested hyphal mass for using a ZEISS Axio Lab.A1 optical microscope (Carl Zeiss Microscopy GmbH, Germany) at a magnification of X400. Lastly, growth rate was determined by measuring colony diameter (in mm) daily for 14 days. Colonies were maintained at room temperature at photoperiod of 12hr/12hr light-dark period.

3.1.5 Data analysis

The collected data on disease incidence and severity were entered into Microsoft Excel and exported to SAS for Windows 10 for all the analyses. These were analyzed using analysis of variance (ANOVA). Before running the ANOVA, datasets were tested for normal distribution using Shapiro-Wilk test.. In order to check if the variance of dependent variable was equal across

groups, Levene's test was used. Since the datasets met these two assumptions evidenced by P>0.05 for both Shapiro-Wilk test and Levene's test, they were not transformed. Thereafter, where significant differences were observed across group means, the means of incidence and severity of the symptom classification of CRBD were separated at a 95% confidence level. For the means' separation, Tukey's studentized range test was used (SAS, Version 9.4. Inc)

3.2 Determination of *Cercospora coffeicola* isolates virulence on Robusta coffee berries in Uganda

3.2.1 Study Area

The experiment was conducted on station in the NaCORI experimental fields. The institute is located at Kituuza village, Ssaayi Parish, Ntenjeru Sub-county, Mukono district (Fig. 2). Mukono district is described in sub-section 3.1.1 above.

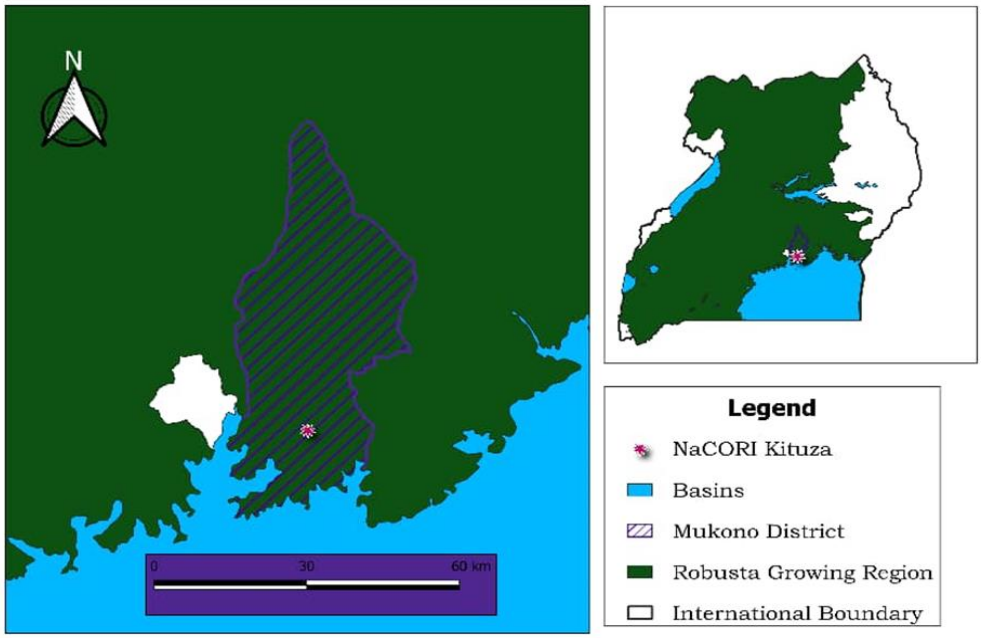

Figure 3: Location of National Coffee Research Institute (NaCORI), Kituuza, Mukono district, Uganda

3.2.2 Experimental design

The on-station experiment was laid in a completely randomized design (CRD)

in a screen house, with three replicates. The treatments were three (3) *C. coffeicola* isolates that were randomly selected from separate morphotypes obtained in the identified colony morphotypes. Three Coffee Wilt Disease resistant (CWD-r) Robusta coffee varieties, i.e. KR3, KR6, and KR10, were used in the study. A coffee cluster with more than 4 fruits at hardening acted as a sampling unit and this was replicated three times.

3.2.3 Establishment of experimental trial and selection of plant materials

The screen house experiment was set-up in the screen-house at NaCORI in February 2021. Three Robusta coffee varieties: KR3, KR6, and KR10 that have been observed to exhibit tolerance to the CRBD pathogen in descending order, i.e. KR10>KR3>KR6, (Godfrey Sseremba, Pers. Comm.) were selected for the study. Ten mature coffee trees for each variety (aged 2 years) with at least 3 actively bearing primaries (having 3 or more clusters each bearing at least four berries at hardening stage) were used for the study.

3.2.4 Selection of *C. coffeicola* isolates, preparation of inocula, and inoculation

Three morphotypes were purposively selected based on their pronounced pathological characters such as cercosporin production and growth rate. A single isolate was selected randomly from each of the 3 morphotypes and mass-produced for inoculum generation.

Each isolate was grown on sterile PDA media in 90mm Petri dishes for 12 days and then harvested to generate fungal inoculum according to Donzelle and Churchil (2007). The 12 day – old cultures were used for inoculum preparation by scraping off with a sterile scalpel, weighed, and fragmented in a blender (commercial blender, USA) at full speed for 3 min. Mycelium suspen-

sions were prepared each isolate to a final concentration of 15mg mL^{-1}.

A volume of 10 mL inoculum of a given isolate was smeared on to a cluster with 4 or more berries using sterilized cotton wool on the selected primary branch of a particular Robusta coffee variety and replicated 3 times.

3.2.5 Data collection

Latent period and incidence were captured for each new infection of the berries by the pathogen. Severity data were captured by estimating and computing the percentage area of each berry covered by disease symptoms every 2 days from the 15^{th} day after inoculation when the first symptom was observed. Data was collected for 6 weeks.

3.2.6 Data analysis

Disease progress curve was constructed and Area Under Disease Progress Curve (AUDPC) calculated to determine the pathogen morphotypes' infection severity on Robusta coffee varieties. Equally, datasets for lesion incidence, number of lesions per berry, lesion area per berry, disease index, colony diameter, and growth rate were subjected to analysis of variance (ANOVA). Prior to conducting the analyses, the data were tested for normality and homogeneity of variance as previously described in section 3.1.5. Similarly, where significant differences were observed, treatment means were separated by Tukey's studentized range test at a 5% alpha level. All the statistical analyses were done in SAS statistical software (Version 9.4).

CHAPTER FOUR: RESULTS

4.1 Morphological identification of coffee red blister disease (CRBD) pathogen, *Cercospora coffeicola* morphotypes on Robusta coffee in the three major Robusta coffee growing regions of Uganda

4.1.1 Coffee Red Blister Disease (CRBD) incidence in the three major Robusta coffee growing regions of Uganda

Coffee Red Blister Disease was prevalent in all the sampled districts and regions. The incidence of CRBD on the 47 coffee gardens assessed in the study area was 38%. This incidence varied significantly ($p < 0.001$) across the coffee growing regions, with the highest incidence (47%) registered in the central region and the lowest (16%) observed in the southwestern region.

4.1.2 Coffee Red Blister Disease (CRBD) metrics in the major Robusta coffee growing regions of Uganda

The metrics of the Coffee Red Blister Disease (CRBD) in the major Robusta coffee growing regions of Uganda are summarized in Table 1 and Fig. 3 and 4 below.

4.1.2.1 Lesion Incidence

Overall, mean lesion incidence of 11.9% was observed on diseased Robusta coffee berries and it varied significantly across the regions (P=0068) as well as across districts ($P<0.0001$). The highest incidence was recorded in the central region (13.38%), but this was not significantly ($p \geq 0.05$) different from the mid-eastern region (12.0%) while the lowest incidence was observed in the southwestern region (6.61%) (Table 1). At district level, the highest incidence was recorded in Mukono district (15.0%), but this was not significantly ($p \geq 0.05$) different from the other districts apart from Isingiro district, which recorded the lowest incidence (1.25%) (Fig.3).

Table 1: Coffee Red Blister Disease (CRBD) metrics across the three major Robusta coffee growing regions of Uganda.

Regions	**Disease metrics**		
	LI (%)	**LAB (%)**	**NLB**
Central	13.38 ± 1.05 a	30.33 ± 1.60 a	8.59 ± 0.77 a
Mid-eastern	12.00 ± 1.77 ab	30.20 ± 2.95 a	8.16 ± 1.29 ab
Southwestern	6.61 ± 1.50 b	21.79 ± 4.07 a	4.39 ± 1.31 a
Mean	11.94 ± 0.82	28.77 ± 1.38	7.76 ± 0.61
CV	85.02	59.92	97.21
F value	5.16	2.87	3.55
P value	0.0068	0.0596	0.0311

LI=Lesion incidence, LAB=Lesion area per berry, NLB=Number of lesions per berry, Means ±SE in columns followed by the same letters are not statistically different at a 5% probability level, Tukey's studentized range test.

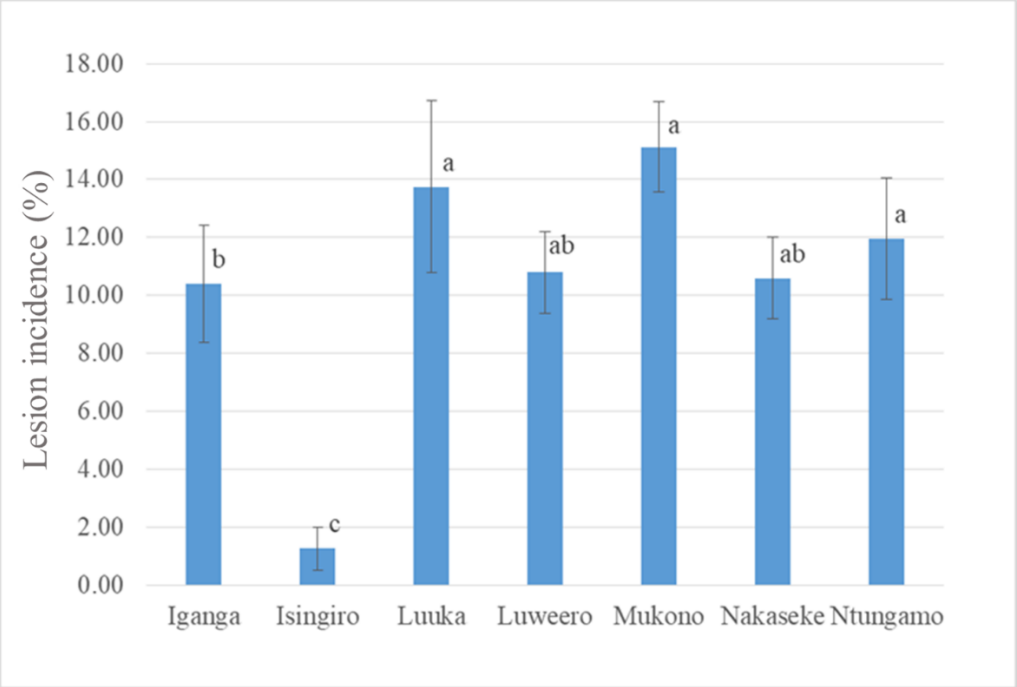

Figure 4: Coffee Red Blister Disease (CRBD) lesion incidence. Error bars represent the standard error of the mean. For each variable, bars bearing the same letters are not significantly different at a 5% probability level, Tukey's studentized range test.

4.1.2.2 Lesion Relative Area per Berry

CRBD lesion area coverage on the coffee fruits sampled in the three major Robusta coffee growing regions of Uganda, averaged at 28.7% and did not varied significantly across the regions (P=0.0596) The highest lesion area coverage was recorded in the central region (30.3%); but this was not significantly ($p \geq 0.05$) different from mid-eastern region (30.2%), whereas the lowest percentage (21.8%) was in the southwestern region (Table 1). Across districts, the highest CRBD lesion area was recorded in Ntungamo district (37.1%), but this was not significantly ($p \geq 0.05$) different from other districts apart from Isingiro district, which had the lowest percentage (6.3 %) (Fig. 4).

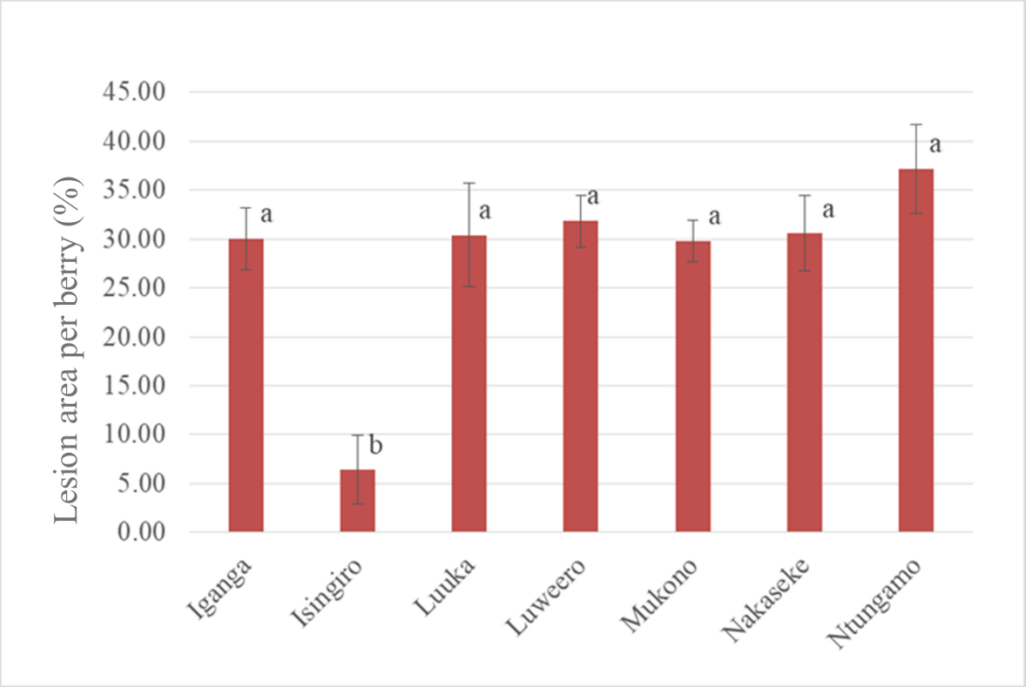

Figure 5: Coffee Red Blister Disease (CRBD) lesion area per berry. Error bars represent the standard error of the mean. For each variable, bars bearing the same letters are not significantly different at a 5% probability level, Tukey's studentized range test.

4.1.3 Coffee Red Blister Disease (CRBD) metrics

4.1.3.1 Number of lesions per berry

Overall, mean number of CRBD lesions per berry was 7.0 and was significantly (P=0.0311) different across the regions. Nevertheless, the highest mean number of CRBD lesions per berry (8.4) was observed in the central region and the lowest (4.4), in the southwestern region (Table 1).

On the other hand, the mean number of CRBD lesions per berry varied significantly (P=0.0056) across the sampled districts. The highest number of lesion per berry was recorded in Luuka district (10.0), but this was not significantly ($p \geq 0.05$) different from observations in Mukono (9.4), Ntungamo (8.3), Nakaseke (7.4) and Luweero (7.3) whereas, the lowest number was recorded in Isingiro district (0.5) (Fig. 4).

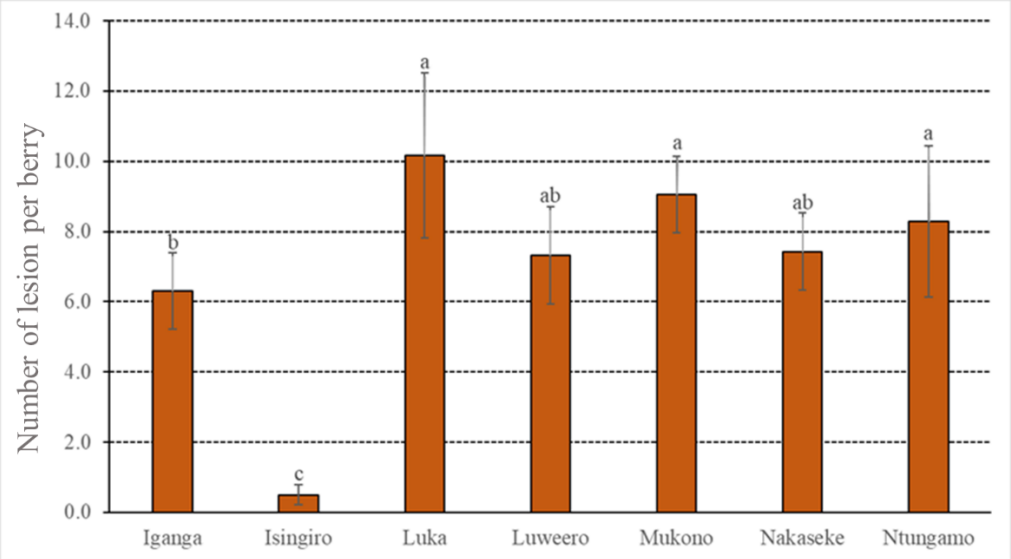

Figure 6: Number of Coffee Red Blister Disease (CRBD) lesion per berry. Error bars represent the standard error of the mean. For each variable, bars bearing the same letters are not significantly different at a 5% probability level, Tukey's studentized range test.

4.1.4 Morphological characterization of CRBD Symptoms on Robusta coffee berries

Based on the visual attributes of the observed CRBD symptoms, six (6) symptom classifications, i.e. A to F, emerged from the samples collected from the three major coffee growing regions of Uganda (Table 2). The symptom morphology classification of Coffee Red Blister Disease (CRBD) lesions which were observed on sample coffee berries in the three major Robusta coffee growing regions of Uganda is summarized in Table 3. All the disease metrices varied significantly across the regions; lesion incidence ($P<0.0001$), number of lesions per berry ($P<0.0001$), and lesion area coverage per berry ($P<0.0001$) among symptom morphotypes. The highest lesion incidence was recorded on symptom classification C (18.6%), but this was not significantly ($p\geq0.05$) different from classification A (12.6%) and E (11.5%). The lowest lesion incidence was observed on symptom classification D (5.3%). In addition, the high-

est number of lesions per berry was recorded on symptom classification A (11.5), but this was not significantly ($p \geq 0.05$) different from classifications C (9.9) and B (11.0). The lowest number of lesions per berry was recorded on symptom classifications D (2.7) and F (2.9). Whereas the highest percentage lesion area per berry was recorded on symptom classifications F (44.1%) and E (40.9), the lowest percentage was observed on classification B (18.8%).

Table 2: Symptom classification description of Coffee Red Blister Disease (CRBD) on coffee berries sampled from the three major Robusta coffee growing regions of Uganda

Symptom classification	Symptom class description	Visual symptom progressive variability
A	Elevated, brownish wet blisters with yellowish surrounding (2-4 mm).	
B	Elevated, brownish wet blisters with yellowish surrounding (2-4 mm).	
C	Flat, reddish-tan wet blister (4 -20 mm)	
D	Elevated, reddish-tan wet blister (4-8 mm) with elevated brownish–yellowish concentric ring (6-10 mm).	
E	Sunken, dark dry blister (6 -15 mm)	
F	Indented, dark dry aspect with cracks (4 -16 mm)	

Table 3: Variability in symptom morphology classification of Coffee Red Blister Disease (CRBD) lesions on coffee berries sampled from the three major Robusta coffee growing regions of Uganda.

Symptom morphology	Disease metrics		
	Lesion incidence (%)	Number of lesions per Berry	Lesion area per berry (%)
A	12.59 ± 1.55 ab	11.50 ± 1.84 a	22.14 ± 2.65 c
B	11.92 ± 1.42 b	10.96 ± 1.66 a	18.81 ± 1.88 c
C	18.57 ± 2.03 a	9.86 ± 1.02 ab	37.26 ± 2.22 ab
D	5.33 ± 0.59 b	2.73 ± 0.30 c	26.67 ± 2.83 bc
E	11.48 ± 1.50 b	4.95 ± 0.75 bc	40.91 ± 2.64 a
F	6.82 ± 1.07 b	2.91 ± 0.44 c	44.09 ± 5.08 a
Mean±SE	11.94 ± 0.82	7.76 ± 0.61	28.77 ± 1.38
CV	85.02	97.21	59.92
F value	6.46	6.86	13.89
P value	<0.0001	<0.0001	<0.0001

For each variable, Means ±SE in column followed by the same letters are not statistically different at a 5% probability level, Tukey's studentized range test.

4.1.5 Colony characterization of Coffee Red Blister Disease (CRBD)

Based on morphological trait assessment, results of colony pigmentation, radial growth rate and colony architecture led to formation of six groups that were referred to as "morphotypes". The morphotypes were designated as I, II, III, IV, V and VI and expressed diverse symptom morphology classifications. All the other morphotypes contained at least one symptom morphology classification described in Table 3. For instance, morphotypes I and II expressed three (3) and five (5) symptom morpholo-

gies, respectively (Table 4). The other morphotypes, III, IV, V, and VI expressed two symptom morphologies.

Consequently, morphotype II with five symptom classifications emerged as the most diversely populated group (A, B, C, F, and E). Whereas symptom morphology classifications A and D were observed in two morphotypes, the symptom classifications B and C were highly prevalent and observed in all the morphotypes except for morphotypes V and VI. On the other hand, symptom morphology classifications E and F were rare and occurred in only one morphotype (Table 4).

Furthermore, results of morphological growth assessment revealed that the isolated fungi responsible for the CRBD on Robusta coffee berries grew linearly on PDA media over the 14-day assessment period. On average, the isolates grew at a rate of 2.87 to 3.59 mm/day to attain a diameter of 27 to 70 mm in 5-14 days, depending on the morphotype. At day 10, most colonies fully covered the plates although their resident morphotypes's growth did not vary significantly ($P \geq 0.05$). Morphotype IV at a colony radius of 44.1mm, had the highest growth rate followed by morphotypes II, VI, I, III, while morphotype V had the shortest radius of 34.6mm (Table 4). Morphologically, the morphotype colonies were peripherally flat on the media surface (front view) with slightly raised and umbonate to radially folded centers. For the reverse side, the colonies appeared velvety olivaceous-gray to brown and dark grayish. Irrespective of growth rate, all the colonies of all the different morphotypes were void of spores when cultured on PDA for the entire growth duration.

Table 4: Colony characteristics of *Cercospora coffeicola* fungal morphotypes after culturing on potato dextrose agar for 14 days at 28°C.

Morphotype	Morphotype description	Radial growth (mm) at day 10 post inoculation	Symptom classification Incident	Visual presentation (Front/Back)
I	Raised greyish-brown center to dark greyish circular filiform periphery with a dark greyish reverse	35.50 ± 1.32	B, C, and D	
II	Umbonate light greenish center to dark greenish brown circular entire periphery with a dark greenish to light greenish reverse	40.40 ± 1.72	A, B, C, F, and E	
III	Umbonate light green-greyish colony with a circular entire margin and a greenish to brown reverse	34.67 ± 2.91	B and C	
IV	Flat grey greenish center to a light greenish circular entire periphery and a dark to light greenish reverse	44.14 ± 3.53	A and B	
V	Umbonate whitish to light greyish and dark greenish periphery with a cracked blue greenish reverse	34.62 ± 3.35	C and D	
VI	Raised whitish fluffy colony with a filiform circular periphery with a dark brownish reverse having a light brownish periphery	37.00 ± 4.55	A	

4.2 Pathogenicity of the Coffee Red Blister Disease (CRBD) isolates

Results of pathogenicity assessment of selected fungal isolates (L1C- Morphotype II, M1C-Morphotype III and N1C - Morphotype VI) on Coffee Red Blister Disease (CRBD) susceptible Robusta coffee variety (KR6) and tolerant varieties (KR3 and KR10) revealed that the isolates were pathogenic to inoculated coffee berries of all these varieties.

4.2.1 Effects of selected *Cercospora coffeicola* isolates on berries of Coffee Wilt Disease resistant (CWD-r) Robusta coffee varieties

Table 5 shows that the disease index varied significantly ($p<0.0001$) across the *Cercospora coffeicola* isolates for the three selected Coffee Wilt Disease resistant (CWD-r) Robusta coffee varieties. The highest disease index was recorded on isolate L1C for all the selected CWD-r varieties. Among the varieties, the susceptible variety, KR6 had the highest index of 41.2%, while the resistant variety, KR10 had the lowest (6.8%). N1C did not cause disease on KR10 while control remained below the isolates treatment.

Table 5: Disease index (%) of *Cercospora coffeicola* isolates on the selected Coffee Wilt Disease resistant (CWD-r) Robusta coffee varieties

Treatment	CWD-r varieties		
	KR3	KR6	KR10
N1C	2.57 ± 0.41a	3.29 ± 0.35a	0.00 ± 0.00a
M1C	1.26 ± 0.22a	16.27 ± 1.13b	6.60 ± 0.59b
L1C	20.76 ± 1.02b	41.22 ± 2.58c	6.84 ± 1.01b
Control	0.90 ± 0.21a	1.96 ± 0.42a	0.45 ± 0.22a
Mean ± SE	6.37 ± 0.51	15.70 ± 1.07	3.47 ± 0.34
CV	158.04	135.35	193.74
F value	285.20	161.63	40.00
P value	<0.0001	<0.0001	<0.0001

4.2.2 Coffee Red Blister Disease (CRBD) progress on Coffee Wilt Disease resistant (CWD-r) Robusta coffee varieties

Table 6 shows that the rate of disease progress on the varieties varied significantly ($P<0.0001$) among the isolates. The highest rate of disease progress (8.87 logit per day) occurred on KR3 by isolate M1C while the lowest disease rate (0 logit per day) occurred on KR10 for isolate N1C.

For the control treatment, there were traces of disease infection on inoculated coffee berries, but the disease never progressed and remained at ≤0.01% (Fig. 6). However, disease progression and severity on the different Robusta coffee varieties depended on time ($P<0.0001$) and the interaction between time and

isolate (P=0.0233).

Generally, the disease severity index at 60 days post inoculation onwards was significantly (P≥0.05) higher than that at ≤15 days post inoculation. Although no statistical difference on disease severity existed across time from inoculation to the terminating the experiment for the control, M1C, and N1C treatments, the disease severity index recorded at ≤ 5 days post inoculation was significantly lower than that registered at 59 days post inoculation for the L1C treatment.

Table 6: Coffee Red Blister Disease (CRBD) progress rate (r) in logit per day and adjusted coefficient of determination (R^2) on selected Robusta coffee varieties

	KR10		KR6		KR3	
Isolates	Rate r	R^2	Rate r	R^2	Rate r	R^2
L1C	0.17	0.20	0.06	0.65	0.04	0.51
M1C	0.70	0.79	0.08	0.41	8.87	0.11
N1C	0	0	0.14	0.59	8.83	0.07
Control	0	0	0.22	0.17	8.46	0.20

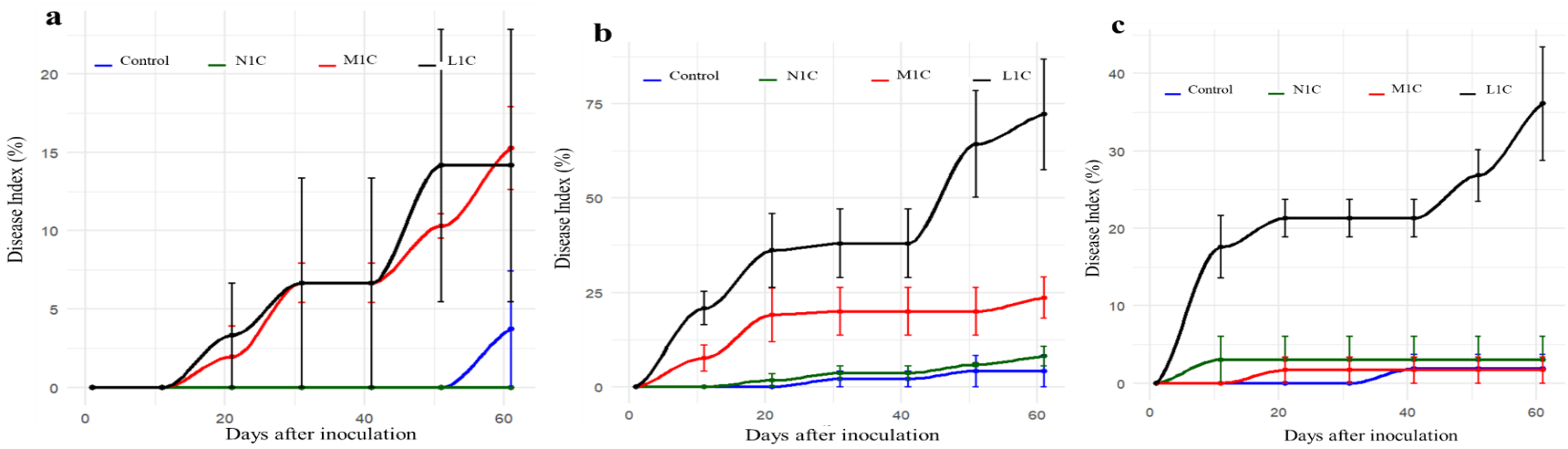

Figure 7: Coffee Red Blister Disease (CRBD) infection progress for 3 isolates L1C, M1C, and N1C (each representing Morphotypes II, III, and IV, respectively) on Robusta coffee varieties KR10 (a), KR6 (b), and KR3 (c), at 65 days post inoculation.

CHAPTER FIVE: DISCUSSION

5.1 Prevalence of Coffee Red Blister Disease (CRBD) in the major Robusta coffee growing regions of Uganda

The survey results revealed that on average, the incidence of Coffee Red Blister Disease (CRBD) was 38% in the three major Robusta coffee growing regions of Uganda. This finding is in agreement with earlier research studies conducted in these regions (Kagezi et al., 2018; Olango et al., 2024), though lower incidences have also been reported (Kyalo et al., 2025). Results of this study confirm that CRBD is still present in the three major Robusta coffee production areas. Historically, CRBD was considered a minor disease in the coffee gardens because of its sporadic nature with low severity ratings recorded (Mwatsiya et al., 2023). However, in recent years, the incidence and severity of the disease have increased dramatically in all the Robusta coffee growing regions of Uganda (Kagezi et al., 2018; Olango et al., 2024). Ayalew et al (2024) explained that increased coffee diseases in a key climate change adversary. In addition, the perennial nature of coffee could be keeping the inoculum in the coffee gardens for a long time (Mwatsiya et al., 2023). Also, the recent expansion of coffee cultivation, mainly in the Robusta coffee growing regions of Uganda could equally be responsible for favoring development of the pathogen.

Results of this study further showed that CRBD incidence varied across the Robusta coffee growing regions, with the highest incidence (13.4%) registered in Central region. Similarly, Olango et al. (2024) reported high incidences of CRBD on Robusta coffee in the districts of Luwero (42%), Mpigi (71%) and Kayunga (81%), which are located in central Uganda. The scholar attributed the heightened

disease incidence majorly to poor agronomic practices, a strong predisposal factor to crop for occurrence of CRBD. More so, this disease has been reported to be severe in low-elevation areas with temperature in ranges of 20–28°C (68–82.4°F) and high relative humidity (Nelson, 2008). These are the characteristic conditions of the districts which were sampled in the central region (Nakaseke, 2016; Nalubwama et al., 2024).

In addition, the disease metric assessments revealed that the lesion incidence, number of lesions per coffee berry, and lesion area per berry differed across the three Robusta coffee growing regions of Uganda. Overall, lesion incidence and lesion area per berry observed in southwestern region were significantly different from those observed in central and eastern regions. Zongo et al. (2023) reported variations between strains of *Cercospora arachidicola* depending on the agroecological zones. The same trend was also observed in other coffee berry diseases. For example, in Ethiopia, the occurrence of Coffee Berry Disease caused by *Colletotrichum kahawae* on Arabica coffee was mostly prevalent in Bonga (40.0%) followed by Yayu (26.3%) and least in Berhane-Kontir (6.0%), suggesting that these berry diseases do vary across locations (Hindorf and Omondi, 2011). The variations in CRBD symptoms observed across different Robusta coffee growing regions of Uganda may be partly attributed to genetic differences among *C. coffeicola* pathotypes or races present in those areas (Nelson, 2008). However, results further showed that the number of lesions per berry did not differ across the three major Robusta coffee growing regions, agreeing with Leucker et al., (2016). Kinanda (2022) also agrees to that significant difference of Cercospora severity between

agroecological zones since a similar situation was reveal in Ntwara region of southern Tanzania.

5.2 Morphological characterization of Coffee Red Blister Disease (CRBD) on berries

Generally, *Cercospora coffeicola* is known to elicit diverse responses on its hosts depending on the genotype, the plant part affected and environmental conditions (Martins et al., 2008; Nelson, 2008; Ramos et al., 2022). On leaves, for example, the pathogen may cause round or irregular dark, brown to black spots (brown eye spot/ dark spots) as well as necrotic lesions surrounded by a "halo" on the leaf surface (Nelson, 2008; Silva et al., 2016; Andrade et al., 2021). On the other hand, on the coffee fruits, *C. coffeicola* may cause berry spots (i.e small, sunken, dark brown to black spots surrounded by a reddish to purplish margin), necrotic lesions on the berry surface as well as berry mummification (Nelson, 2008). Results of this study support this phenomenon, with the diseased coffee berries observed to have diverse kinds and sizes of symptoms including: flat or elevated reddish-tan wet blisters, elevated brownish–yellowish concentric rings, and sunken, dark dry blisters containing or lacking cracks. Six groups (morphotypes) of symptoms with regard to lesion relative size, number of lesions, lesion incidence and visual aspect of lesions caused on the coffee berries by *C. coffeicola* were observed. Similarly, Nelson (2008) reported morphological variation among Cercospora isolates from coffee in Hawaii. Visual aspect of lesions provided the baseline to investigate the symptom variability and this is in agreement with Pavicic et al. (2021). However, Leucker et al. (2016) used lesion size and the visual aspect of lesions to group *Cercospora beticola* leaf spot symptoms on sugar beet into four categories.

Results also showed that the commonest morphotype observed in the current study was “C” that had flat, reddish-tan wet blister of 4 – 20 mm. This happens to be the typical symptom of *C. coffeicola* on Robusta coffee berries in Uganda, and hence the name, Coffee Red blister Disease (CRBD) (Mukasa, 2019). On the other hand, morphotype “D” with elevated, reddish-tan wet blister (4-8 mm) and elevated brownish–yellowish concentric ring (6-10 mm) was the rarest disease symptom.

Furthermore, in addition to being the most prevalent, morphotype C equally had a relatively higher number of lesions. The high prevalence of morphotype C may be indicative of the aggressiveness of the pathotype responsible for this disease symptom on the berries (Gashaw et al., 2014). All in all, the existence of diverse morphotypes of *C. coffeicola* on Robusta coffee observed in this study, presents management implications because the different morphotypes can influence the fungus-host-environment interaction to optimize overall pathogen fitness (Francisco et al., 2019). This in turn might influence the way the fungus responds to the different management options.

5.3 Colony characterization

The colony growth characteristics for the isolates obtained from the different symptom morphotypes were in agreement with morphological characteristics manifested by *C. coffeicola* isolates that were reported by Souza et al. (2011). Nearly all the morphotypes except morphotype D isolates produced colonies that were umbonate with light greenish center to dark greenish brown circular periphery but with a dark greenish to light greenish reverse side. As reported by a number of pathologists, plant pathogens display a high extent of phenotypic plasticity when

grown on artificial media (Mwatsiya et al., 2023; Souza, 2012). Pan and May (2009) elaborated that variability in colony morphology for fungal pathogens emanates from genetic variability. In agreement with the latter, the isolates obtained in this study displayed morphological variability to make six groups, which is indicative of the pathogen genetic variability (Martins et al., 2008; Mahmoudi et al., 2018; dos Santos Botelho et al., 2022). However, there was no relationship between pathogen variability and location or origin. This finding agrees with several other researchers (e.g. Souza, 2012; Martins, 2016; Vale et al., 2021; dos Botelho et al., 2022) who noted that the genetic variability of *C. coffeicola* isolates yielded groups associated with most regions and cropping systems where the isolates were sourced.

Furthermore, on average, the fungal isolates grew at a rate of 2.9 to 3.6 mm/day on PDA. According to Silva et al. (2016), mycelia of *C. coffeicola* isolates exhibit differential growth rates depending on the culture conditions (i.e. culture media, incubation temperature and light intensity among others). For instance, on malt agar, *C. coffeicola* isolates grew slowly at high and low light intensity and temperatures, respectively, and vice versa under dark and high temperature conditions (Silva et al., 2016). This study revealed that mycelial growth increased linearly over time, which is in line with what has been observed among other *Cercospora* spp. by Sharma et al. (2025).

Results also showed that the emerged six morphotypes were associated with the major symptom morphotypes. They contained at least 2 (Morphotype III, IV, V, and VI) symptom morphotypes, with some exhibiting 5 (Morphotype II) and 3

(Morphotype I) symptom types. The overlap of colony morphotypes in symptom morphotyping suggests that both host and environmental factors influence the expression of CRBD symptoms as well as the development of *C. coffeicola* colonies. Therefore, symptom and colony morphotyping serve as complementary tools for understanding the diversity of *C. coffeicola*. Lacap et al. (2003) and Gálvez & Palmero (2021) highlighted the importance of fungal morphotyping in disease diagnosis, as it contributes to the formulation of precise disease management strategies.

5.4 Pathogenicity of selected colony morphotypes

Results of pathogenicity assessment revealed that the selected *C. coffeicola* isolates (L1C- Morphotype II, M1C-Morphotype III and N1C -Morphotype VI) caused disease on the inoculated coffee berries, 65 days after inoculation as also reported by Vale et al. (2021) and Ramos et al. (2022). However, the disease incidence and severity elicited by the respective fungal isolates depended on the variety, with the most aggressive fungal morphotypes (L1C and M1C) infecting and producing significantly higher disease index on the berries of susceptible variety KR6 than on tolerant varieties, KR10 and KR3. Similar trends have also been reported by earlier studies of Dell'Acqua et al. (2011), Delima et al. (2018) and Ramos et al. (2022). Although the N1C isolate elicited some infection on coffee berries, it never caused a disease on berries of KR10 variety.

Similarly, a pathogenicity assessment of 10 *C. coffeicola* severity on seedlings of a susceptible coffee variety (Catuaí Vermelho IAC 144) revealed that disease incidence and severity were dependent on the isolate in question (Silva et al., 2016). In

their study, the isolate that produced the highest quantity of cercosporin (>2 μmol.L^{-1}) was the most aggressive with a disease incidence of >45% and severity of ~15%, which more than doubled the disease intensity observed among isolates that produced little cercosporin (<1 μmol.L^{-1}). Such variability in pathogenicity of *C. coffeicola* isolates on coffee may be attributed to the differences in the genetic make – up of the isolates and the coffee varieties as well as gene regulation (Silva et al., 2016). For example, Robusta coffee varieties KR6, KR3, and KR10 express differential susceptibility rates to CRBD because of the differences in their genome. Whereas Robusta coffee variety, KR6 is known to harbor susceptibility genes, KR3 and KR10 coffee varieties are more tolerant to the pathogen (Musoli et al., 2013).

Irrespective of the Robusta coffee variety, the onset of disease expression by the selected *C. coffeicola* isolates on coffee berries started at 5 days post inoculation. However, this was contrary to Ramos et al. (2022) and Silva et al. (2016), who observed that the onset of *C. coffeicola* disease symptom on coffee seedlings was at 22 days post inoculation. This variability may be attributed to several factors including differences in the fungal isolate aggressiveness, plant part inoculated, the coffee variety and inconsistences in management practices of the crop (Wolf and Verreet, 2005).

Furthermore, the disease progress was affected by the fungal morphotype, with the disease infection rate being fastest for the L1C isolate at 1.1% RBD/day followed by M1C and lastly the N1C isolate. This is contrary to Ismailaj et al. (2024), who reported a no clear correlation between the morphotype of the fungus, *Fusarium*

proliferatum, and the intensity of the disease.

Nevertheless, the observed variability in disease progress by these isolates tallied with the morphological attributes observed across the various morphotypes, whereby those with faster mycelial radial growth were more aggressive than the slow ones (Suaza, 2012). A similar research finding was reported by Laurent et al. (2021) in which *Fusarium graminearum* morphotypes with slow radial growth were negatively correlated with aggressiveness while the faster radial growth morphotypes were more aggressive. However, mycelial growth rate may not be indicative of a pathogen's aggressiveness; it is informative but not definitive (Swalarsk-Parry et al., 2024).

All in all, the presence of aggressive morphotypes of *C. coffeicola* on Robusta coffee berries in Uganda creates management challenges because these fungi are more likely to have more widespread damage and severity than the less aggressive ones (Pariauda et al., 2009) and therefore require more intensive management strategies (Doehlemann et al., 2017).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study aimed at: i) identifying *Cercospora coffeicola* morphotypes causing Coffee Red Blister Disease (CRBD) on berries of Robusta coffee, and, ii) determining the virulence of the *C. coffeicola* complex on berries of Robusta coffee.

Results of the survey conducted in the three major Robusta coffee growing regions of Uganda, shows moderate incidence (average of 38%) of Coffee Red Blister Disease (CRBD) which is caused by the fungus, *Cercospora coffeicola* Berk. and Cooke. This implies that since the disease attacks the coffee berries, farmers are losing 38% of their harvests if the disease is not managed. Furthermore, it is observed that that *C. coffeicola* expresses itself on coffee berries in six groups (morphotypes) of symptoms with regard to lesion relative size, number of lesions, lesion incidence and visual aspect of lesions. Morphotype "C" which has flat, reddish-tan wet blister is the most frequently observed type, which happens to be the typical symptom of *C. coffeicola* observed on Robusta coffee berries in Uganda, hence the name, Coffee Red blister Disease (CRBD). This presents management implications because the different morphotypes can influence the fungus-host-environment interaction and therefore, the way the fungus responds to the different management options.

All the selected *C. coffeicola* isolates (L1C- Morphotype II, M1C-Morphotype III and N1C -Morphotype VI) cause disease on the inoculated coffee berries. However, the disease incidence and severity depends on the variety, with the most

aggressive fungal morphotypes (LIC and MIC) infecting and producing significantly higher disease index on the berries of susceptible variety K6 than on tolerant varieties, K10 and K3. This finding has management implications because fungi which are more aggressive are more likely to lead to more widespread damage and severity than the less aggressive ones; and therefore, may require intensive management options and strategies.

6.2 Recommendations

Based on the study results, it is recommended that:

- Research should be conducted to develop comprehensive management strategies such as breeding Robusta coffee varieties for specific regions and symptoms of the Coffee Red Blister Disease (CRBD) so as to contain it and prevent further spread.
- When managing the disease, farmers should pay closer attention to the susceptible variety KR6 because it is more prone to development of the *C. coffeicola* pathogen which can result into significant yield losses.

For further studies, it is recommended that additional studies be conducted on:

- The symptomatology of *C. coffeicola* caused by CRBD on a wider scope to reveal the disease complex more accurately for Uganda as a whole.
- Delineating the *C. coffeicola* morphotypes and pathotypes using molecular tools for the case of CRBD on Robusta coffee in Uganda.

REFERENCES

Agrios, G. N. (2005). *Plant pathology*. Elsevier.

Ahmed, S., and Stepp, J. R. (2016). Beyond yields: Climate change effects on specialty crop quality and agroecological management. *Elementa*, *4*, 000092.

Ahmed, S., Griffin, T. S., Kraner, D., Schaffner, M. K., Sharma, D., Hazel, M., ... and Cash, S. B. (2019). Environmental factors variably impact tea secondary metabolites in the context of climate change. *Frontiers in plant science*, *10*, 939

Andrade, C. C., de Resende, M. L. V., Moreira, S. I., Mathioni, S. M., Botelho, D. M., Costa, J. R., ... & Alves, E. (2021). Infection process and defense response of two distinct symptoms of Cercospora leaf spot in coffee leaves. *Phytoparasitica*, *49*, 727-737.

Ayalew, B., Hylander, K., Adugna, G., Zewdie, B., Zignol, F., & Tack, A. J. (2024). Impact of climate and management on coffee berry disease and yield in coffee's native range. Basic and Applied Ecology, 76.

Ayesu-Offei, E. N., & Antwi-Boasiako, C. (1996). Production of microconidia by *Cercospora henningsii* Allesch, cause of brown leaf spot of cassava (Manihot esculenta Crantz) and tree cassava (*Manihot glaziovii Muell*.-Arg.). *Annals of Botany*, *78*(5), 653-657.

Bakhshi, M. (2019). A revision on the taxono my of the genus Pseudocercospora. *University of Yasouj Plant Pathology Science*, *8*(1), 1-14.

Bakhshi, M., Arzanlou, M., Babai-Ahari, A., Groenewald, J. Z., & Crous, P. W. (2018). Novel primers improve species delimitation in Cercospora. *IMA fungus*, *9*, 299-332.

Bakhshi, M., Arzanlou, M., Babai-Ahari, A., Groenewald, J. Z., Braun, U., & Crous, P. W. (2015). Application of the consolidated species concept to Cercospora spp. from Iran. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, *34*(1), 65-86.

Battilani, P., Toscano, P., Van der Fels-Klerx, H. J., Moretti, A., Camardo Leggieri, M., Brera, C., Rortais, A., Goumperis, T., & Robinson, T. (2016). Aflatoxin B1 contamination in maize in Europe increases due to climate change. *Scientific Reports*, *6*(1), 24328. https://doi.org/10.1038/srep24328

Bebber, D. P., Ramotowski, M. A., & Gurr, S. J. (2013). Crop pests and pathogens move polewards in a warming world. *Nature climate change*, *3*(11), 985-988.

Bedimo, J. M., Njiayouom, I., Bieysse, D., Nkeng, M. N., Cilas, C., & Notteghem, J. L. (2008). Effect of shade on Arabica coffee berry disease development: Toward an agroforestry system to reduce disease impact. *Phytopathology*, *98*(12), 1320-1325.

Bisikwa, J., Nampala, P., Nsibo, DL., Kwikiriza N., Bukenya C., Otim, MH., Kapting, I., Kabuye, FM, and Felix, J. 2022. Assessment of *Striga hermonthica* Infestation and Effectiveness of Current Management Strategies in Maize-Based Cropping Systems in Eastern Uganda. African Crop Science Journal 30(4):525-538.

Bock CH, Parker PE, Cook AZ, Gottwald TR. 2008. Visual rating and the use of image analysis for assessing different symptoms of citrus canker on grapefruit leaves. Plant Disease 92:530–41.

Bote, A. D. (2016). *Examining growth, yield and bean quality of Ethiopian coffee trees: towards optimizing resources and tree management* (Doctoral

dissertation, Wageningen University and Research).

Botelho, D. M. D. S., Resende, M. L. V. D., Rezende, J. C. D., Ribeiro Júnior, P. M., Andrade, C. C. L. D., Alves, E., & Amaral, D. C. (2019). Difference between isolates from brown eye spot and black spot lesions in coffee plants. *Pesquisa Agropecuária Brasileira*, *54*, 01423.

Braun, U. (1996). Taxonomic notes on some species of the Cercospora complex (IV). *SYDOWIA-HORN-*, *48*, 205-217.

Braun, U. (1999). Taxonomic notes on some species of the Cercospora complex (VI). *Cryptogamie Mycologie*, *20*(3), 155-177.

Bunn, C., Lundy, M., Läderach, P., Fernández Kolb, P., & Castro-Llanos, F. (2019). Climate-smart coffee in Uganda.

Burgess L.W., Knight T.E., Tesoriero L. and Phan H.T. 2008. Diagnostic manual for plant diseases in Vietnam. ACIAR Monograph No. 129, 210 pp. ACIAR: Canberra.

Burnett, J. H. (2003). *Fungal populations and species*. Oxford University Press, USA.

Chand, R., Singh, V., Kumar, P., Pal, C., & Chowdappa, P. (1954). The genus Cercospora: biology and taxonomy. Fungal leaf spot diseases of annual and perennial crops. Westville Publishing House, New Delhi.Chupp C. 1954 – A monograph of the fungus genus Cercospora. Published by the author, Ithaca, New York.

Chupp, C. (1954). A monograph of the fungus genus Cercospola.

Crous, P. W., & Braun, U. (2003). Mycosphaerella and its anamorphs: 1. Names published in Cercospora and Passalora (pp. 571-pp).

Crous, P. W., Braun, U., Hunter, G. C., Wingfield, M. J., Verkley, G. J. M., Shin, H. D., ... & Groenewald, J. Z. (2013). Phylogenetic lineages in Pseudocercospora. Studies in Mycology, 75(1), 37-114.

Crous, P. W., Groenewald, J. Z., Pongpanich, K., Himaman, W., Arzanlou, M., & Wingfield, M. J. (2004). Cryptic speciation and host specificity among Mycosphaerella spp. occurring on Australian Acacia species grown as exotics in the tropics. Studies in Mycology, 50(2), 457-469.

Custódio, A. A. D. P., Pozza, E. A., Guimarães, S. D. S. C., Koshikumo, É. S. M., Hoyos, J. M. A., & Souza, P. E. D. (2011). Comparison and validation of diagrammatic scales for brown eye spots in coffee tree leaves. Ciência e Agrotecnologia, 35, 1067-1076.

DaMatta, F. M., Rahn, E., Läderach, P., Ghini, R., & Ramalho, J. C. (2019). Why could the coffee crop endure climate change and global warming to a greater extent than previously estimated?. Climatic change, 152, 167-178.

Daub, M. E., & Chung, K. R. (2007). Cercosporin: a photoactivated toxin in plant disease. Journal, Saint Paul, USA. Online.

Daub, M. E., Herrero, S., & Chung, K. R. (2013). Reactive oxygen species in plant pathogenesis: the role of perylenequinone photosensitizers. Antioxidants & Redox Signaling, 19(9), 970-989.

Davidson, J. A., Krysinska-Kaczmarek, M., A. McKay, H., & Scott, E. S. (2012). Comparison of cultural growth and in planta quantification of Didymella pinodes, Phoma koolunga and Phoma medicaginis var. pinodella, causal agents of ascochyta blight on field pea (Pisum sativum). Mycologia, 104(1), 93-101.

Davis, A. P., & Kiwuka, C. (2023). A review of the indigenous coffee resources of Uganda and their potential for coffee sector sustainability and development. Frontiers in Plant Science, 13, 1057317.

De Carvalho, A. M., De Abreu Cardoso, D., Carvalho, G. R., De Carvalho, V. L., Pereira, A. A., Ferreira, A. D., & Carneiro, L. F. (2017). Behavior of coffee cultivars under the incidence of diseases of rust and gray leaf spot in two cultivation environments. Coffee Science, 12(1), 100–107. https://doi.org/10.25186/cs.v12i1.1248

De Lima, AE., Sampaio Jr., HG., de Melo Castro, E., de Carvalho, SP., da Silva, FF., de Lima Jr. S. and de Carvalho, AM. 2018. Incidence and Severity of Coffee Leaf Rust, Cercosporiosis and Coffee Leaf Miner in Coffee Progenies. Coffee Science 13(3) :349-355.

De Lima, L. M., Pozza, E. A., & Da Silva Santos, F. (2012). Relationship between incidence of brown eye spot of coffee cherries and the chemical composition of coffee beans. Journal of Phytopathology, 160(4), 209-211.

Deighton FC. (1971) Studies on Cercospora and allied genera. III. Centrospora. Kew: Commonwealth Mycological Institute. Mycological Papers; vol 124.

Deighton FC. (1979) Studies on Cercospora and allied genera. VII. New species and redispositions. Kew: Commonwealth Mycological Institute. Mycological Papers; vol 144.

Del Peloso, M. J., Cardoso, A. A., Vieira, C., Saraiva, L. S., & Zimmermann, M. D. O. (1989). Genetic system for the reaction of Phaseolus vulgaris to the BA-2 (Alpha) race of *Colletotrichum lindemuthianum*.

Dell' Acqua, R., Mantovani, E. S., Braghini, M. T., Oliveira, C. M. G., Harakava,

R., Robaina, A. S., ... & Patricio, F. R. (2011). Variabilidade in vitro, in vivo e molecular de isolados de *Cercospora coffeicola*. Tropical Plant Pathology, 36, 313-326.

Doehlemann G, Ökmen B, Zhu W, Sharon A. 2017. Plant pathogenic fungi. Microbiol Spectrum 5(1):FUNK-0023-2016.

dos S. Botelho, D. M., de Resende, M. L. V, Andrade, V. T., Pereira, A. A., Patricio, F. R. A., Junior, P. M. R., Ogoshi, C., & de Rezende, J. C. (2017). Cercosporiosis resistance in coffee germplasm collection. Euphytica, 213(6), 117. https://doi.org/10.1007/s10681-017-1901-9

dos Santos Botelho, D.M., de Resende, M.L.V., Andrade, V.T., de Castro Guerra Guimarães, L., Andrade, AC. and de Rezende Abrahão, JC. 2022. Heritability of aggressiveness components in *Cercospora coffeicola* isolates. Eur J Plant Pathol 164, 495–505.

Donzelli, B. G. G., & Churchill, A. C. (2007). A quantitative assay using mycelial fragments to assess virulence of *Mycosphaerella fijiensis*. Phytopathology, 97(8), 916-929

Durand-Bessart, C., Tixier, P., Quinteros, A., Andreotti, F., Rapidel, B., Tauvel, C., & Allinne, C. (2020). Analysis of interactions amongst shade trees, coffee foliar diseases and coffee yield in multistrata agroforestry systems. Crop Protection, 133, 105137.

Echandi, E. (1959). Coffee leaf spot caused by the fungus *Cercospora coffeicola* Berk. & Cooke. Turrialba , 9 (2), 54-67.

Egonyu, J. P., Kucel, P., Kangire, A., Sewaya, F., & Nkugwa, C. (2009). Impact of the black twig borer on Robusta coffee in Mukono and Kayunga districts,

central Uganda.

Ellis, M. B. (1971). Dematiaceous hyphomycetes. 608 pp. Commonwealth Mycological Institute, Kew.

Fazuoli, L.C., H.P. Medina-Filho, W. Gonçalves, O. Guerreiro Filho, and M.B. Silvarolla. 2002. Melhoramento do cafeeiro: Variedades do tipo arábica obtidas no Instituto Agronômico de Campinas. In: Zambolim L (eds) O estado da arte de tecnologias na produção de café. Viçosa, Brazil. 163–251.

Francisco, C. S., Ma, X., Zwyssig, M. M., Mc- Donald, B. A., & Palma-Guerrero, J. (2019). Morphological changes in response to environmental stresses in the fungal plant pathogen *Zymoseptoria tritici*. Scientific Reports, 9(1), 1–18.

Gálvez, L., & Palmero, D. (2021). Incidence and etiology of postharvest fungal diseases associated with bulb rot in garlic (*Alllium sativum*) in Spain. Foods, 10(5), 1063.

Gashaw, G., Alemu, T. and Tesfaye, K, 2014. Evaluation of disease incidence and severity and yield loss of finger millet varieties and mycelial growth inhibition of *Pyricularia grisea* isolates using biological antagonists and fungicides in vitro condition. Journal of Applied Biosciences 73:5883– 5901.

Geiser, D. M., Lewis Ivey, M. L., Hakiza, G., Juba, J. H., & Miller, S. A. (2005). *Gibberella xylarioides* (anamorph: *Fusarium xylarioides*), a causative agent of coffee wilt disease in Africa, is a previously unrecognized member of the G. fujikuroi species complex. Mycologia, 97(1), 191-201.

Godoy, CV, Bergamin Filho, A., & Salgado, CL (1997). Coffee diseases. Manual of phytopathology: diseases of cultivated plants.

Goulart, M., Silva, D., Pozza, E. A., Vitor, C., Vaz De Lima, R., & Fernandes, T.

J. (2016). Temperature and light intensity interaction on *Cercospora coffeicola* sporulation and conidia germination. Ciência e Agrotecnologia, 40(2), 198–204. http://dx.doi.org/10.1590/1413-70542016402025915

Groenewald, J. Z., Nakashima, C., Nishikawa, J., Shin, H. D., Park, J. H., Jama, A. N., ... & Crous, P. W. (2013). Species concepts in Cercospora: spotting the weeds among the roses. Studies in Mycology, 75(1), 115-170.

Groenewald, M., Groenewald, J. Z., & Crous, P. W. (2005). Distinct species exist within the *Cercospora apii* morphotype. Phytopathology, 95(8), 951-959.

Groenewald, M., Groenewald, J. Z., Braun, U., & Crous, P. W. (2006). Host range of *Cercospora apii* and *C. beticola* and description of *C. apiicola*, a novel species from celery. Mycologia, 98(2), 275-285.

Gu, X., Yang, S., Yang, X., Yao, L., Gao, X., Zhang, M., Liu, W, Zhao, H., Wang, Q., Li, Z., Li, Z. and Ding, J. 2020. Comparative transcriptome analysis of two *Cercospora sojina* strains reveals differences in virulence under nitrogen starvation stress. BMC Microbiology, 20(1).:doi:10.1186/s12866-020-01853-0.

Guo, L. D., Hyde, K. D., & Liew, E. C. Y. (2000). Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. The New Phytologist, 147(3), 617-630.

Guo, Y., Olsen, R. T., Kramer, M., & Pooler, M. (2016). Use of mycelium and detached leaves in bioassays for assessing resistance to boxwood blight. Plant Disease, 100(8), 1622-1626.

Gupta, V. P., Tewari, S. K., Bajpai, A. K., & Datta, R. K. (1995). Observations on the surface ultrastructure of conidial stage of *Cercospora moricola* and its

infection process in mulberry. Sericologia, 35(1), 123-131.

Hartati, S., Meliansyah, R., & Mayanti, T. (2024). Potency of Yeast's Volatile Compounds to Control *Cercospora coffeicola*. Jurnal Fitopatologi Indonesia, 20(1), 1-16.

Hindorf, H., & Omondi, C. O. (2011). A review of three major fungal diseases of *Coffea arabica* L. in the rainforests of Ethiopia and progress in breeding for resistance in Kenya. Journal of advanced research, 2(2), 109-120.

Hoseini, M., Cocco, S., Casucci, C., Cardelli, V., & Corti, G. (2021). Coffee by-products derived resources. A review. *Biomass and Bioenergy*, *148*, 106009.

IITA, 2024. Enhancing Resilient and Adaptive Agricultural Livelihoods in Uganda - Scaling of Successful Technologies - 2024 Annual Outcome Survey Dataset. International Institute of Tropical Agriculture (IITA). https://data.iita.org/dataset/enhancing-resilient-and-adaptive-agricultural-livelihoods-in-uganda-annual-outcome-survey-2024.

Intergovernmental panel on climate change (ICPP). Climate Change 2014. Fifth Assessment Synthesis Report. In The Core Writing Team, Pachauri RK, Meyer L, editors; 2014. p. 132. Available from: URL: http://www.ipcc.ch/pdf/ assessment-report/ar5/syr/SYR_AR5_LONGERREPORT_ Corr2.pdf

INTERNATIONAL COFFE ORGANIZATION (ICO). (2021). A Flagship Report of the International Coffee Organization. 1–94.

Isingiro District, 2016. District Multi-hazard, Risk and Vulnerability Profile for Isingiro District. Republic of Uganda. 84 pp.

Ismailaj, E., Huqi, A., Palmieri, D., Ianiri, G. and Varaku, S. (2024).

Morphological and Molecular Identification of *Fusarium Oxysporum* as the Main Cause of Wilt Disease of Tomatoes Grown in Greenhouses in Albania. Evergreen. 11. 1655-1665.

Jassogne, L., van Asten, P. J., Wanyama, I., & Baret, P. V. (2013). Perceptions and outlook on intercropping coffee with banana as an opportunity for smallholder coffee farmers in Uganda. International Journal of Agricultural Sustainability, 11(2), 144-158.

Kagezi GH., Kucel P., Kobusinge J., Nakibuule J., Akwatulira F., Ivette P. and Wagoire WW. 2018. Characterising the coffee-banana agroforestry systems: An entry point for promoting coffee and banana growing in mid-northern Uganda. Uganda Journal of Agricultural Sciences 18(2):111-121.

Kagezi GH., Kucel, P., Olango, N., Tumuhaise, V., Nakibuule L., Kobusinge, J., Kabole, C. and Wagoire WW. 2016. Current distribution and impact of the Black Coffee Twig Borer (BCTB) and Coffee Wilt Disease (CWD) in Uganda. Study report submitted to the Uganda Coffee Development Authority (UCDA). June 2016.

Kagezi, GH., Kyalo, G., Twesigye, V., Helerimana, C., Anyijuka, M., Musasizi, J. K., ... & Niyibigira, E. I. (2021). Coffee Berry Moth, *Prophantis smaragdina* (Butler)(Lepidoptera: Pyralidae): Another threat to Robusta coffee, *Coffea canephora* production in Uganda. Uganda Journal of Agricultural Sciences, 21(2), 15-30.

Kagezi, GH., Patrick, K., Judith, K., Nicholas, O. D., Lilian, N., & Wagoire, W. (2018). Predicting the response of insect pests and diseases of Arabica coffee to climate change along an altitudinal gradient in Mt. Elgon region. Uganda.

Journal of Agriculture and Environmental Sciences, 7(1), 134-140.

Kinanda, R. I. (2022). Distribution and management of groundnuts rust (*Puccinia arachidis*) and leaf spot (*Cercospora arachidicola* and *Cercosporidium personatum*) diseases in Mtwara region.

Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. (2008). Dictionary of the Fungi. 10º.

Knight, N. L., Vaghefi, N., Kikkert, J. R., & Pethybridge, S. J. (2019). Alternative hosts of *Cercospora beticola* in field surveys and inoculation trials. Plant disease, 103(8), 1983-1990.

Kobusinge J., Kagezi GH., Kasoma A., Kucel P., Nakibuule L., Perfecto I. and Wagoire WW. 2018. Farmers' knowledge of pests and diseases in the coffee-banana agroforestry systems of mid-eastern Uganda. Journal of Agriculture and Environmental Sciences 7(2)109-119.

Krinner, G., Germany, F., Shongwe, M., Africa, S., France, S. B., Uk, B. B. B. B., ... & Lucas, C. (2013). Long-term climate change: Projections, commitments and irreversibility. Climate change, 1029-1136.

Kyalo, G., Kagezi, G. H., Olango, N. D., Anyijuka, M., Twesigye, V., Musasizi, J. K., ... & Niyibigira, E. I. (2024). Incidence, Damage and Management of the Major Pests and Diseases of Robusta Coffee,[*Coffea canephora* (Pierre Ex A. Froehner)] in Uganda. Journal of Agricultural Science, 17(1), 1-70.

Lacap, D. C., Hyde, K. D., & Liew, E. C. Y. (2003). An evaluation of the fungal 'morphotype' concept based on ribosomal DNA sequences. Fungal Diversity.

Laurent, B., Moinard, M., Spataro, C., Chéreau, S., Zehraoui, E., Blanc, R., ... & Foulongne-Oriol, M. (2021). QTL mapping in *Fusarium graminearum*

identified an allele of FgVe1 involved in reduced aggressiveness. Fungal genetics and biology, 153, 103566.

LePage, B. A., Currah, R. S., & Stockey, R. A. (1994). The fossil fungi of the Princeton chert. International Journal of Plant Sciences, 155(6), 828-836.

Leucker, M., Mahlein, A. K., Steiner, U., & Oerke, E. C. (2016). Improvement of lesion phenotyping in *Cercospora beticola* sugar beet interaction by hyperspectral imaging. Phytopathology, 106(2), 177-184.

Liebig, T. I. (2017). Abundance of pests and diseases in Arabica coffee production systems in Uganda-ecological mechanisms and spatial analysis in the face of climate change (Doctoral dissertation).

Lorenzetti, E. R., Pozza, E. A., Souza, P. E. D., Santos, L. A., Alves, E., Silva, A. C. D., ... & Carvalho, R. R. D. C. (2015). Effect of temperature and leaf wetness on Phoma tarda and phoma leaf spot in coffee seedlings.

Lucas, P., & Sarniguet, A. (1998). Biological control of soil-borne pathogens with resident versus introduced antagonists: Should diverging approaches become strategic convergence?. In Conservation biological control (pp. 351-370). Academic Press.

Luuka District, 2023. Luuka District Wash Master Plan 2021 – 2030. 65 pp.

Luweero District, 2016. Luweero District Hazard, Risk and Vulnerability Profile. 66 pp.

Madaraiga BR, Scharen AL (1986) Interactions of *Puccinia striiformis* and *Mycosphaerella graminicola* on wheat *Triticum aestivum*. Plant Disease, 70:651–654

Madrid-Casaca, H., Salazar-Sepúlveda, G., Contreras-Barraza, N., Gil-Marín, M.,

& Vega-Muñoz, A. (2021). Global trends in coffee agronomy research. Agronomy, 11(8), 1471.

Mahmoudi, SB., Abbaszadeh, MS., Abbasi, S. and Farrokhinejad, R. 2018. Genetic diversity and pathogenic variability among *Cercospora beticola* Sacc. isolates causing leaf spot of sugar beet in Iran. J. Crop Prot. 7 (2): 207-217.

Martinez, H. E. P., Andrade, S. A. L. D., Santos, R. H. S., Baptistella, J. L. C., & Mazzafera, P. (2023). Agronomic practices toward coffee sustainability. A review. Scientia Agricola, 81, e20220277.

Martins, R. B., Maffia, L. A., & Mizubuti, E. S. (2008). Genetic variability of *Cercospora coffeicola* from organic and conventional coffee plantings, characterized by vegetative compatibility. Phytopathology, 98(11), 1205–1211. https://doi.org/10.1094/PHYTO-98-11-1205

Matovu, R. J., Kangire, A., Phiri, N. A., Hakiza, G. J., Kagezi, G. H., & Musoli, P. C. (2013). Ecological factors influencing incidence and severity of coffee leaf rust and coffee berry disease in major Arabica coffee growing districts of Uganda. Uganda Journal of Agricultural Sciences, 14(1), 87-100.

Mbolanyi, B., Egeru, A., & Mfitumukiza, D. (2017). Choice Options to Meet Household Food Security in the Cattle Corridor of Uganda. Environment and Natural Resources Journal, 15(1), 19–29. https://doi.org/10.14456/ennrj.2017.2.

Mizubuti, E. S., Yamada, J. K., Santiago, T. R., & Lopes, C. A. (2022). On the assessment of the sources of inoculum of bacterial wilt in Brazil. Tropical Plant Pathology, 47(5), 685-692.

Monteiro, J., Alves, M. G., Oliveira, P. F., & Silva, B. M. (2019). Pharmacological potential of methylxanthines: Retrospective analysis and future expectations. Critical reviews in food science and nutrition, 59(16), 2597-2625.

Mukasa, NZ. 2019. Cercospora leaf spot on coffee-Uganda; *Cercospora coffeicola*: Brown eye spot, Berry blotch. PlantwisePlus Knowledge Bank. Pest Management Decision Guides. https://doi.org/10.1079/pwkb.20207800.

Mukono DDP (District Development Plan) (2015/2016-2019/2020). Retrieved from http://npa.go.ug/wp-content/uploads/2017/05/Mukono-DISTRICT-Development-Plan-Final.pdf

Muller, R. A., Berry, D., Avelino, J., & Bieysse, D. (2009). Coffee diseases.

Musoli, P. C., Cilas, C., Pot, D., Nabaggala, A., Nakendo, S., Pande, J., ... & Bieysse, D. (2013). Inheritance of resistance to coffee wilt disease (Fusarium xylarioides Steyaert) in Robusta coffee (Coffea canephora Pierre) and breeding perspectives. Tree Genetics & Genomes, 9, 351-360.

Mwatsiya, N., Mahoya, C., Chidoko, P., Kutywayo, D. and Makuvara Z. 2023. Culture Media Options for Growth and Morphological Characterisation of *Cercospora coffeicola* Affecting Coffee in Zimbabwe. NASS Journal of Agricultural Sciences 5(1):19-27.

NaCORI/ MAAIF/ UCDA 2022. A rapid assessment report on the status of pests and diseases in southwestern, eastern, greater Masaka, western and Rwenzori regions - A joint survey undertaken by the National Coffee Research Institute (NaCORI), Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) and Uganda Coffee Development Authority (UCDA). 37 pp.

Naik, B. J., Kim, S. C., Seenaiah, R., Basha, P. A., & Song, E. Y. (2021). Coffee

cultivation techniques, impact of climate change on coffee production, role of nanoparticles and molecular markers in coffee crop improvement, and challenges. Journal of Plant Biotechnology, 48(4), 207-222.

Nakaseke District, 2016. Nakaseke District Hazard, Risk and Vulnerability Profile. 84 pp.

Nalubwama, S., Bagamba, F., Kabi, F., Nampijja, Z., Kiggundu, M., Kamatara, K., Kwikiriza, N., Nampanzira, D., Nalunga, A., Lutwama, V. and Nasaka, J.. (2024). Knowledge and perceptions towards organic farming: a case of pineapple farmers in central Uganda. Discover Agriculture. 2. 10.1007/s44279-024-00100-8.

Nega, A., Lemessa, F. and Berecha, G. 2020. Virulence of four *Cercospora zeae-maydis* isolates on tolerant and susceptible maize varieties under greenhouse condition. International Journal of Research in Agricultural Sciences 7:2348-3997.

Nelson, S. C. (2008). Cercospora leaf spot and berry blotch of coffee. University of Hawai‘i at Manoa, College of Tropical Agriculture and Human Resources, Cooperative Extension Service.

Neto, C. B., Silveira, E. D., Mello, S. D., & Fontes, E. M. G. (1998). Scanning electron microscopy of the infection process of *Cercospora caricis* on purple nutsedge.

Ngugi, H. K., King, S. B., Holt, J., & Julian, A. M. (2001). Simultaneous temporal progress of sorghum anthracnose and leaf blight in crop mixtures with disparate patterns. Phytopathology, 91(8), 720-729.

Ntungamo District, 2016. Ntungamo District Hazard, Risk and Vulnerability

(HRV) profile. 76 pp.

Oerke, E. C., Leucker, M., & Steiner, U. (2019). Sensory assessment of *Cercospora beticola* sporulation for phenotyping the partial disease resistance of sugar beet genotypes. Plant Methods, 15, 1-12.

Okech, S. H., Gold, C. S., Bagamba, F., Masanza, M., Tushemereirwe, W. K. & Ssenyonga, J. (2005). Cultural control of banana weevils in Ntungamo, southwestern Uganda. In: G. Blomme, C. Gold and E. B. Karamura (Editors), Farmer participatory testing of integrated pest management options for sustainable banana production in Eastern Africa, Proceedings of workshop on Farmer-participatory testing of IPM options for sustainable banana production in Eastern Africa, Seeta, Uganda, December 8-9, 2003, pp. 116-128.

Olango N D, Olal S, Kagezi G, Kucel P, Ekwaru R, Kobusinge J, Arinaitwe G. 2023. Occurrence and damage/severity of coffee pests and diseases in central Uganda. Proceeding. Third NARO-MAK Joint Scientific Conference, Kampala, 2023, pp. 62.

Olango, N. D. K., Kagezi, G., Olal, S., Kucel, P., Ekwaru, R., Judith, K., & Arinaitwe, G. (2024). Distribution and Severity of Coffee Pests and Diseases in Central Uganda. Uganda Journal of Agricultural Sciences, 22(2), 1-9.

Oligo, S., Nanteza, A., Nsubuga, J., Musoba, A., Kazibwe, A., Lubega, G.W. 2023. East Coast Fever Carrier Status and Theileria parva Breakthrough Strains in Recently ITM Vaccinated and Non-Vaccinated Cattle in Iganga District, Eastern Uganda. Pathogens 12, 295.

Otieno, H. M., Alwenge, B. A., & Okumu, O. O. (2019). Coffee production

challenges and opportunities in Tanzania: the case study of coffee farmers in Iwindi, Msia and Lwati Villages in Mbeya Region.

Pan, J. J., & May, G. (2009). Fungal-fungal associations affect the assembly of endophyte communities in maize (*Zea mays*). Microbial Ecology, 58, 668-678.

Pariauda, B., Ravignéb, V., Halkettc, F., Goyeaua, H., Carlierb, J. and Lannoua, C. 2009. Aggressiveness and its role in the adaptation of plant Pathogens. Plant Pathology 58, 409–424.

Paula, P. V. A. A. D., Pozza, E. A., Alves, E., Moreira, S. I., Paula, J. C. A., & Santos, L. A. (2019). Infection process of *Cercospora coffeicola* in immature coffee fruits. Coffee Science, 14, 127-130.

Pavicic, M., Overmyer, K., Rehman, A. U., Jones, P., Jacobson, D., & Himanen, K. (2021). Image-based methods to score fungal pathogen symptom progression and severity in excised Arabidopsis leaves. Plants, 10(1), 158.

Peck, L. D., & Boa, E. (2024). Coffee wilt disease: The forgotten threat to coffee. Plant Pathology, 73(3), 506-521.

Pollack, F. G. (1987). An annotated compilation of Cercospora names (pp. 212-pp).

Pozza, EA, Carvalho, VD, & Chalfoun, SM (2010). Symptoms of injuries caused by diseases in coffee plants. Semiology of coffee plants: symptoms of nutritional, phytosanitary and physiological disorders, 68-106.

Priwiratama, H., Wiyono, S., Tondok, E. T., Hidayat, S. H., Wening, S., Wijayanti, E. & Rozziansha, T. A. P. 2024. Genus Curvularia and Pestalotiopsis as the primary pathogen of leaf spot disease on oil palm

seedlings throughout Indonesia. IOP Conference Series: Earth and Environmental Science, 1308, 012018. 14-16 March, 2023, Bali, Indonesia.

Radhakrishnan, GV., Cook, NM., Bueno-Sancho, V., Lewis, CM., Persoons, A., Mitiku, AD., Heaton, M., Davey, PE., Abeyo, B., Alemayehu Y., Ayele Badebo, A., Barnett, M., Bryant, R., Chatelain, J., Chen, X., Suomeng Dong, S., Henriksson, T., Holdgate, S., Justesen, AF., Kalous, J., Kang, Z., Laczny, S., Legoff, J-P., Lesch, D., Richards, T., Randhawa, HS., Thach, T., Wang, M., Hovmøller, MS., David P. Hodson, DP. and Saunders, GO. 2019. MARPLE, a point-of-care, strain-level disease diagnostics and surveillance tool for complex fungal pathogens. BMC Biology, 17(1). doi:10.1186/s12915-019-0684.

Ramos, J. B., Resende, M. L. V. D., Botelho, D. M. D. S., Pereira, R. C. M., Reichel, T., Balieiro, A. A. F., Botega, G. P., & Abrahão, J. C. D. R. (2022). Screening coffee genotypes for brown eye spot resistance in Brazil. PloS one, 17(1), e0258822.

Riggins, J. J., Fraedrich, S. W., & Harrington, T. C. (2011). First report of laurel wilt caused by *Raffaelea lauricola* on Sassafras in Mississippi. Plant Disease, 95(11), 1479-1479.

Sacristán, S. and García-Arenal, F. (2008). The evolution of virulence and pathogenicity in plant pathogen populations. Molecular plant pathology. 9. 369-84. 10.1111/j.1364-3703.2007.00460.x.

Sarma, M. S., & Subramanian, S. (1967). Studies on the Brown-eye-spot Disease (*Cercospora coffeicola* Berk, et Cke.) of Coffee (*Coffea arabica* L.) V. Nutritional requirements of the pathogen. Journal of Phytopathology, 60(3),

247-258.

Schumann, G. L., & D'Arcy, C. J. (2012). CHAPTER 3: Coffee and Rubber: Monocultures and Quarantines. In Hungry Planet: Stories of Plant Diseases (pp. 39-54). The American Phytopathological Society.

Sharma, P., Murphy, S., Kikkert, J. R., & Pethybridge, S. J. (2025). Spatiotemporal Spread of Cercospora Leaf Spot and Cercospora beticola Genotypes in Table Beet in New York. Plant Pathology.

Siddiqi, MA. 1970. Incidence, development and symptoms of Cercospora disease of coffee in Malawi. Transactions of the British Mycological Society 54(3):415-421.

Silva, M. G. D., Pozza, E. A., Monteiro, F. P., & Lima, C. V. R. V. D. (2016). Effect of light and temperature on *Cercospora coffeicola* and *Coffea arabica* pathosystem.

Silva, M., Barreto, R. W., Pereira, O. L., Freitas, N. M., Groenewald, J. Z., & Crous, P. W. (2016). Exploring fungal mega-diversity: Pseudocercospora from Brazil. Persoonia-Molecular Phylogeny and Evolution of Fungi, 37(1), 142-172.

Silva, P. (2018). Universidad Nacional Agraria De La Selva Facultad. La Satisfacción De Las Necesidades De Los Clientes En Los Centros Veterinarios De La Ciudad De Tingo María". http://repositorio.unas.edu.pe/bitstream/handle/UNAS/1680/TS_MAR_2019.pdf?sequence=1&isAllowed=y.

Simon-Gruita, A., Pojoga, M. D., Constantin, N., Duta-Cornescu, G., Grumezescu, A. M., & Holban, A. M. (2019). Caffeinated and Cocoa Based Beverages.

Genetic Engineering in Coffee.

Smith, D. H., Pauer, G. D. C., & Shokes, F. M. (1992). Cercosporidium and Cercospora leaf spots of peanut (groundnut).

Smith, S. J., Edmonds, J., Hartin, C. A., Mundra, A., & Calvin, K. (2015). Near-term acceleration in the rate of temperature change. Nature Climate Change, 5(4), 333-336

Soliman, A., Mettleq, A., & Abu-Naser, S. S. (2019). A Rule Based System for the Diagnosis of Coffee Diseases. International Journal of Academic Information Systems Research, 3(3), 1–8. https://ssrn.com/abstract=3369019

Solymosi, K., & Techel, G. (2019). Brewing up climate resilience in the coffee sector: Adaptation strategies for farmers, plantations, and producers.

Sontirat, P., Phitakpraiwan, P., Choobamroong, W., & Giatgong, P. (1980). Plant pathogenic Cercosporae in Thailand.

Souza, A. G. C., Herrero, S., & Daub, M. E. (2019). The toxin cercosporin is a virulence factor for infection of coffee by *Cercospora coffeicola*. BioRxiv, 818328.

Souza, A. G. C., Herrero, S., Maffia, L. A., & Daub, M. E. (2014). Methods for *Cercospora coffeicola* protoplast isolation and genetic transformation with the green fluorescent protein. European journal of plant pathology, 139, 241-244.

Souza, A. G. C., Rodrigues, F. Á., Maffia, L. A., & Mizubuti, E. S. G. (2011). Infection process of *Cercospora coffeicola* on coffee leaf. Journal of Phytopathology, 159(1), 6-11.

Souza, A. G., Maffia, L. A., & Mizubuti, E. S. (2012). Cultural and aggressiveness

variability of *Cercospora coffeicola*. Journal of Phytopathology, 160(10), 540-546.

Souza, A.G.C.; Maffia, L.A.; Silva, F.F.; Mizubuti, E.S.G.; Teixeira, H. 2015. A time series analysis of brown eye spot progress in conventional and organic coffee production systems. Plant Pathology, v.64, p.157-166, 2015.

Sseremba, G., Kagezi, GH., Kobusinge, J., Musoli, P., Akodi, D., Olango, N., Kucel, P., Chemutai, J., Mulindwa, J., Arinaitwe, G. 2021. High Robusta coffee plant density is associated with better yield potential at mixed responses for growth robustness, pests and diseases: which way for a farmer? Australian Journal of Crop Science 15(4):494-503.

Stewart, E. L., Liu, Z., Crous, P. W., & Szabo, L. J. (1999). Phylogenetic relationships among some cercosporoid anamorphs of Mycosphaerella based on rDNA sequence analysis. Mycological Research, 103(11), 1491-1499.

To-Anun, C., Hidayat, I., & Meeboon, J. (2011). Genus Cercospora in Thailand: taxonomy and phylogeny (with a dichotomous key to species). Plant Pathology & Quarantine, 1(1), 11-87.

Tondok, E. T., & Sa'adah, R. N. H. (2023). Isolation and Morphological Characterisation of *Cercospora janseana* Infecting Rice Leaves. Perhimpunan Fitopatologi Indonesia.

Tuggle, J. C., Smith, O. D., & Starr, J. L. (1999). Variation in Aggressiveness and Virulence Among Isolates of *Cercospora arachidicola*. Peanut Science, 26(2), 95–98.

Tumwesigye, W., Osiru, D., Tefera, TL., Bedai, B. and Majaliwa, M.J-G. (2022). Effect of Intercropping Maize and Beans on the Maize Yields in Isingiro

Town Council, Isingiro District, South Western Uganda. Universal Journal of Food Security, 1(1), 65–75.

UCDA, 2024. Uganda Coffee Development Authority (UCDA). Monthly Report, November 2024. Report CY 2024/25 Issue 2.

UCDA. (2019) Annual report. Retrieved from http://www.ugandacoffee.org

UCDA. (2021) Annual report. Retrieved from http://www.ugandacoffee.org

UCDA. (2023). Annual report. Retrieved from http://www.ugandacoffee.org

Vaghefi N, Kikkert JR, Hay FS, Carver GD, Koenick LB, Bolton MD, Hanson LE, Secor GS, Pethybridge SJ (2018) Cryptic diversity, pathogenicity, and evolutionary species boundaries in Cercospora populations associated with Cercospora leaf spot of Beta vulgaris. Fungal Biol 122:264–282

Vaghefi, N., Kikkert, J. R., Bolton, M. D., Hanson, L. E., Secor, G. A., Nelson, S. C., & Pethybridge, S. J. (2017). Global genotype flow in *Cercospora beticola* populations confirmed through genotyping-by-sequencing. PLoS One, 12(10), e0186488.

Vale, P. A. S., de Resende, M. L. V., dos Santos Botelho, D. M., de Andrade, C. C. L., Alves, E., Ogoshi, C., ... & Pfenning, L. H. (2021). Epitypification of *Cercospora coffeicola* and its involvement with two different symptoms on coffee leaves in Brazil. European Journal of Plant Pathology, 159(2), 399-408.

Velásquez, A.C., D.M. Christian, Castroverde and Y.H. Sheng. 2018. Plant Pathogen warfare under changing climate conditions. Curr. Biol., 28: 619-634.

Veloso, T. G. R., Da Silva, M. D. C. S., Moreira, T. R., Da Luz, J. M. R., Moreli,

A. P., Kasuya, M. C. M., & Pereira, L. L. (2023). Microbiomes associated with *Coffea arabica* and *Coffea canephora* in four different floristic domains of Brazil. Scientific Reports, 13(1), 18477.

Wang, N., Jassogne, L., Asten, P. J. A. Van, Mukasa, D., Wanyama, I., Kagezi, G., & Giller, K. E. (2015). Evaluating coffee yield gaps and important biotic, abiotic , and management factors limiting coffee production in Uganda. European Journal of Agronomy, 63, 1–11. https://doi.org/10.1016/j.eja.2014.11.003

Wolf, P. F. J., and Verreet, J. A. 2005. Factors affecting the onset of Cercospora leaf spot epidemics in sugar beet and establishment of disease-monitoring thresholds. Phytopathology 95:269-274.

Zamani-Noor, N., Brand, S. and Söchting, H-P. 2008. Effect of Pathogen Virulence on Pathogenicity, Host Range, and Reproduction of *Plasmodiophora brassicae*, the Causal Agent of Clubroot Disease. Plant disease. 106. 10.1094/PDIS-02-21-0410-RE.

Zambolim, L., Vale, F. D., Pereira, A. A., & Chaves, G. M. (1997). Café (Coffea arabica L.), controle de doenças. *Controle de doenças de plantas: grandes culturas*, *1*, 83-40.

Zhou, D., & Hyde, K. D. (2001). Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. *Mycological Research*, *105*(12), 1449-1457.

Zongo, A., Kassankogno, AI., Nana, AT., Zombre, PJW. and Ouedraogo, I. 2023. Morphological diversity, pathogenicity and biofungicides efficacity on *Cercospora arachidicola* strains causal agent of early leaf spot disease of groundnut. International Journal of Agronomy and Agricultural Research, 23(6):1-9.

APPENDICES

Appendix 1: Geographic description of sampled districts

In Mukono district, which is in the Lake Victoria crescent agro-ecological zone, the National Coffee Resources Research Institute (NaCORI) was selected as one of the experimental site. NaCORI is located at 0.2575° N, 32.7911° E north of the equator and an altitude of 800–1160 meters above sea level (m.a.s.l) Temperatures at NaCORI range between 16 and 28°C throughout the year. The area has a tropical climate with a bi-modal rainfall pattern. Mean annual rainfall is 1,100mm distributed over 106 rain days, with peaks in March – May and September – November (Mukono DDP, 2020). The topography of the area consists of sloping land with many undulations, and is dominated by black forest sandy loam soils. Generally, the vegetation cover is of the forest/savannah characterized by patches of dense forest in the area (Mukono DDP, 2020). Luweero district on the other hand is located at 00 50°N, 32 30°E (Latitude: 0.8333; Longitude: 32.500), and an altitude of 1000 – 1500 m.a.s.l (Luweero District, 2016). Rainfall is well distributed throughout the year, with an average annual precipitation of 1300 mm and a mean annual temperature range of 15 to 30 °C (Nalubwama et al., 2024). The landscape is generally made up of elevated and dissected plateau consisting of a series of flat topped hills and intervening valleys. The soil is generally red sandy loam; however, the Southern part is relatively fertile and can support all kinds of crops (Luweero District, 2016). Nakaseke district is situated between coordinates: 0° 44′ 0″ N and 32° 25′ 0″ E, at altitude ranges of 1000-1250 m.a.s.l (average of 1150 m). Its climate can be described as modified equatorial climate with a mean annual

maximum temperature of between 27.5and 30^{o}C and a minimum of 15and 17.5^{o}C (Nakaseke, 2016). The district receives an average rainfall of 1300mm, with two rainfall seasons. The main season runs from March to May, and the second one from mid-August to early December (Mbolanyi et al., 2017). The soils in the district are generally red sandy loamy. Although the north of the district have a relatively low nutrient/ fertility content, soils in the south are clay-loamy and are therefore relatively fertile, which support growth/cultivation of a variety of crops (Nakaseke District, 2016).

Ntungamo district lies at latitudes 0^{o}35' and 1^{o}15'south and longitudes 30^{o}05' East (Ntungamo District, 2016) at an elevation of 1300-1560 m.a.s.l (Okech et al., 2005). The district has a bimodal distribution of rainfall (800-1500 mm) falling between March to mid-May, and September to December. It also experiences a mean annual temperature of 26^{o}C and mean annual minimum of 14.5^{o}C. High temperatures are recorded in the months of January – February and June- August, which correspond to dry spells (Okech et al., 2005; Ntungamo District, 2016). Isingiro district is located at 00 50'S, 300 50'E and an altitude of 1200 - 1810 m.a.s.l (Isingo District, 2016). The district has a tropical wet and dry or savanna climate with the annual rainfall of 973 – 1200 mm and the average temperature of 15.17 to 27.18^{o}C for the last 30 years. The area has a tropical climate and Acrisol soils with rich clay content, limited soil nutrients thus limited potential for crop productivity (Tumwesigye et al., 2022).

Iganga district is located at 0°36'33.01" N and 33°28'7.00" E (Oligo et al., 2023) at an average altitude of 1,138 m.a.s.l (Bisikwa et al., 2022). The mean annual

rainfall of the district ranges from 900 to 1,200 mm; while the mean temperatures range from 25 to 35^{o}C (Oligo et al., 2023). The soils are predominantly ferralitic, with reddish brown sandy loams associated with the gneisses and granites as parent rocks (Bisikwa et al., 2022). On the other hand, Luuka district is located at 00° 42'00" N and 33° 18'59" E and is generally flat at an average elevation of 1132 m.a.s.l with a gentle slope towards the northern part of the district, where elevation is about 1100 m a.s.l. The district lies in a tropical climate zone, with a limited seasonal variation. It experiences a bimodal rainfall distribution pattern with two peak seasons: i.e. April to June and August to November, but with a mean annual rainfall of 1,200 mm. The annual temperatures range from 18-27.5°C, with a daily mean temperature of 21.5^{o}C. The hottest season is from January to March and the coolest period is from April to October (Luuka District, 2023).

Appendix 2: Interactions between varieties and isolates used.

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
VARIETY	2	3.4316872	1.7158436	210.87	<.0001
ISOLATE	3	9.68551855	3.22850618	396.78	<.0001
VARIETY*ISOLATE	6	4.16478298	0.6941305	85.31	<.0001

ANOVA table

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Control	2	0.01263918	0.00631959	6.85	0.0013
N1C	2	0.06313963	0.03156981	32.04	<.0001
M1C	2	1.21945509	0.60972754	118.77	<.0001
L1C	2	6.30123628	3.15061814	123.53	<.0001