# GENETIC DYNAMICS OF *Fusarium xylarioides*: KEY TO THE MANAGEMENT OF COFFEE WILT DISEASE IN UGANDA

**BY**

**NAMAYANJA CLARE MUSANGO**

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**Declaration**

**Declaration by candidate:**

I, NAMAYANJA CLARE MUSANGO, do solemnly declare to the best of my knowledge that this dissertation is as a result of my own work through research and it has never been submitted in any institution for any award.

…… ……..

Signature Date

**We confirm that the work in this dissertation was done by the candidate under our supervision:**

1. …… ……..

   Signature Date

   Professor Charles Twesigye

   Department of Biological Sciences

   Faculty of Science

   Kyambogo University

2. …… ……

   Signature Date

   Dr. Sammy Olal

   Department of Biological Sciences

   Faculty of Science

   Kyambogo University

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**List of Acronyms**

| | |
|---|---|
| AMOVA | Analysis of Molecular Variance |
| AFLP | Amplified Fragment Length Polymorphisms |
| Bp | Base pair |
| CAR | Central African Republic |
| CLR | Coffee Leaf Rust |
| CORI | Coffee Research Institute |
| CWD | Coffee Wilt Disease |
| DNA | Deoxyribose Nucleic Acid |
| dNTPS | Dinucleotide triphosphates |
| DRC | Democratic Republic of Congo |
| EDTA | Ethylene Diamine Tetra Acetic Acid |
| GPS | Global Positioning System |
| IPM | Integrated Pest Management |
| ISR | Intergenic Space Region |
| ISSR | Inter Simple Sequence Repeats |
| MAAIF | Ministry of Agriculture, Animal Industry and Fisheries |
| mL | millilitre |
| mM | millimolar |
| NaCORI | National Coffee Research Institute |
| NARO | National Research Organization |
| PCR | Polymerase Chain Reaction |
| RAPD | Random Amplified Polymorphic DNA |

| | |
|---|---|
| RFLP | Restriction Fragment Length Polymorphisms |
| SSR | Simple sequence repeats |
| *Taq* | *Thermus aquaticus* polymerase enzyme |
| TBE | Tris Borate EDTA |
| UCDA | Uganda Coffee Development Authority |

**Abstract**

*Fusarium xylarioides* (*F. xylarioides*) is a vascular fungal pathogen that causes Coffee Wilt Disease (tracheomycosis). The pathogen is only endemic to Robusta coffee in Uganda while Arabica coffee is immune to it, yet Robusta coffee contributes 80% of the Uganda traded coffee. Currently, the pathogen is estimated at 3% incidence with economic losses of $17m annually. The pathogen is present in all traditional Robusta coffee growing regions and is spreading to non-traditional areas like Kabale. Despite the importance of *F. xylarioides*, there is limited information on the contribution of environmental factors and its genetic diversity to the spread of the pathogen.

The aim of this study was to determine the genetic dynamics of *F. xylarioides* and the environmental factors that contribute to its spread to new places in Uganda. Environmental data comprising rainfall, relative humidity, speed of wind, and temperature for 2000 to 2005 and 2020 was obtained from the POWER project. Historical pathogen incidence data was obtained from UCDA reports while the primary incidence data was obtained from the coffee fields during specimen collection in 2020. The environmental data was then correlated to the pathogen incidence data in SPSS. Pathogen isolates that had been stored for a period of ten years (2009 to 2019) were used to study the genetic dynamics of the pathogen over a 10-year period by subjecting their DNA to PCR analysis using SSR primers. Twenty-three (23) *F. xylarioides* isolates obtained from 3 coffee growing regions in Uganda were used to study the genetic dynamics of the pathogen as of 2020 using SSR markers. The results revealed that all the environmental factors studied were significant to the spread of the disease. However, it was revealed that rainfall (R= 0.8680, R=1), temperature (R= 0.8680, R=1, R=$^{-}$1) and humidity (R=0.76, R=1, R=-1) were key in the spread of the pathogen. The study observed 0.00% genetic variation in the *F. xylarioides* population in Uganda over a 10-year period. The study also observed 0.00% genetic variation in the pathogen populations from the 3 coffee growing regions of Uganda as of 2020. This implies the spread of the pathogen in Uganda is mainly due to the environmental factors and not the pathogen genetic diversity. However, there is need of consistent annual data collection and storage, and use of a variety of molecular markers in a diverse study.

# CHAPTER ONE: INTRODUCTION

## 1.1 Background of the Problem

Global coffee trade depends on two coffee species, Arabica (*Coffea arabica*) at 60% and Robusta (*Coffea canephora*) at 40 % (Moat *et al*., 2019). Global coffee production provides a livelihood for up to 125 million people (Voora *et al.*, 2019) with world production at 168.68 million 60-kilogram (kg) bags and Africa production at 18.69 million 60-kg bags by 2020 (ICO, 2020). With Arabica production at 96.82 million 60-kg bags, and Robusta at 71.86 million 60-kg bags, globally coffee is one of the most traded agricultural commodities generating a total average value of US$20 billion in the period 2015-2020 (Barometer, 2020; ICO, 2020; Voora *et al.*, 2019). Europe is the world's leading coffee consumer, followed by Asia and Oceania, Latin America and North America, with global consumption at 164.5 million 60-kg bags in 2020 (Barometer, 2020; ICO, 2020). However, in the year 2019/20, coffee demand declined in all regions, apart from Africa, leading to a downward trend in coffee prices (Barometer, 2020; ICO, 2020). This affected the incomes and the livelihoods of households that depend on coffee production and economies of coffee producing countries including Uganda (Development Report, 2019). Global coffee production is further challenged by the expensive highly labor-intensive work, that is aggravated by the effects of climate change, including more unpredictable weather patterns and severe pest and disease outbreaks, for example coffee rust and Coffee Wilt Disease (Barometer, 2020; Voora *et al.*, 2019).

Coffee is Uganda's oldest, most significant cash crop and leading foreign exchange earner and accounts for over an average of 20% of the country's total export earnings and for over a half of the total agricultural exports (Senyonyi, 2018; WORLDBANK, 2011; UCDA, 2019a; UCDA, 2019b). Commercial production of Robusta Coffee in Uganda began in 1920 and by 1960, coffee production had risen to about 2 million 60-kg bags and reached its peak of 3.7 million and 4.2 million 60-kg bags in 1972/73 and 1996/97 respectively (UCDA, 2019b). In the subsequent years, however, volumes fell primarily due to the occurrence and spread of Coffee Wilt Disease (CWD), that was first identified in Uganda in 1993 (UCDA, 2019b;WORLDBANK, 2011). In 2000 to 2010, Uganda's annual coffee exports averaged to just below 2.8 million 60-kg bags, with a high of 3.2 million and a low of 2 million 60-kg bags, increasing in 2016 and 2017 to 4.19 million 60-kg bags and 4.45 million 60-kg bags respectively and 3.3 million 60-kg bags by 2019

(WORLDBANK, 2011; UCDA, 2017; UCDA, 2019b). Uganda coffee production in the year 2019/20 was 7.75 million 60-kg bags amounting to total exports of 5,103,771 60-kg bags worth US$ 496.25 million (UCDA, 2020). With Robusta coffee production at 80%, Uganda remains Africa's and East Africa's largest and the world's fourth largest producer of Robusta coffee (Ngugi & Aluka, 2019; WORLDBANK, 2011; UCDA, 2019b; UCDA, 2019a).

There are two types of coffee, Robusta and Arabica coffee, grown in five regions of Uganda, that is Central, Western, South-Western, Northern and Eastern regions (UCDA, 2019a). Robusta, at 80% production, is the major type of coffee grown in Uganda (UCDA, 2019a; UCDA, 2019b). Robusta coffee, which was initially only cultivated along the Lake Victoria crescent is native to the Nganda regions of Uganda while Arabica coffee has its origins in Ethiopia (UCDA, 2019a). There are two major varieties of Robusta coffee, Nganda and Erecta (UCDA, 2019a). However, since 2009, the National Research Organization (NARO) has released 10 high yielding clonal Robusta Coffee varieties that are resistant to CWD to replace the 6 old clones (1s/2, 1s/3, 1s/6, 223/32, 257/53, 258s/24 (0)) which are now susceptible (UCDA, 2019a; UCDA, 2019b). The 10 coffee varieties for Robusta include: KR1 (NARO-Kituza Robusta1), KR2 (NARO-Kituza Robusta2), KR3 (NARO-Kituza Robusta3), KR4 (NARO-Kituza Robusta4), KR5 (NARO-Kituza Robusta5), KR6 (NARO-Kituza Robusta6), KR7 (NARO-Kituza Robusta7), KR8 (NARO-Kituza Robusta8), KR9 (NARO-Kituza Robusta9) and KR10 (NARO-Kituza Robusta10) (UCDA, 2019b). However, even with the release of the resistant clones, they are not available to all farmers (Hakiza *et al*., 2010), and even so, most farmers tend to prefer the old susceptible clones (Olal, 2021). There are a number of Arabica coffee varieties grown in Uganda and these include SL 28, SL 14, KP 423 and the traditional Nyasaland (UCDA, 2019a; World Coffee Research, 2019).

However, several forms of environmental changes affect species survival, but infectious disease is rarely mentioned as a major cause of species extinction(Smith *et al.*, 2009). Nevertheless, there is substantial evidence that pathogens, due to their interaction with other driving factors, such as climate change, habitat loss, over-exploitation, invasive species, gene erosion, and pollution can cause temporary or permanent declines in species abundance (Moat *et al*., 2019; Smith *et al*., 2009). Currently, resources for coffee conservation and use in breeding are diminishing largely due to climatic inflexibility and susceptibility of coffee to pathogens like *Hemileia vastatrix* and *Fusarium xylarioides* (Moat *et al*., 2019). Coffee pests and diseases are a threat to the coffee

industry and affect farmer livelihoods that depend on coffee in Uganda (Liebig, 2017; Rutherford & Phiri, 2006). Coffee is host to a wide range of arthropod pests, however, there are majorly five significant pests of coffee that include the Coffee Berry Borer, the White Coffee Stem Borer, the Green Scales, the Coffee Root Mealybug and the Nematodes of Coffee (Rutherford & Phiri, 2006; Waller *et al*., 2012; Liebig, 2017). Insects destroy the leaves, berries, green shoots, roots, bark, and twigs of coffee depending on their mode of feeding (Rutherford & Phiri, 2006). They rarely kill the tree, and when they do, they can have a permanent effect on the field, because it is difficult to grow a tree in the gap left by a dead tree (Waller *et al*., 2012). Coffee has also been recorded to host a large number of fungi and bacterial species but a few of these can be regarded as pathogens(Waller *et al.*, 2012). There are five significant pathogens of coffee in Uganda including Coffee Leaf Rust (*Hemileia vastatrix*) on Arabica coffee and Robusta coffee, Coffee Berry Disease (*Colletotrichum kahawae*) on Arabica coffee, CWD (*Fusarium xylarioides*) on Robusta coffee and absent on Arabica coffee in Uganda, Coffee Bark Disease (*Fusarium stilbioides*) on Arabica coffee, and Brown Eye Spot Disease (*Cercospora coffeicola*) on Arabica and Robusta coffee (Rutherford & Phiri, 2006). However, most of the pathogens of coffee have co-evolved with the genus *Coffea* as a result of changes in environmental conditions (Waller *et al.*, 2012). This is evidence that environmental factors influence the incidence and severity of coffee diseases leading to serious implications for the coffee industry in Uganda(Liebig, 2017; Waller *et al.*, 2012).

*F. xylarioides* is a devastating vascular pathogen of Robusta coffee that invades the xylem system of coffee (Olal,*et al.* 2018; Yigletu, 2019; Musoli *et al*., 2013). The pathogen attacks coffee at all stages of growth, from seedlings to mature plants, and leads to 100% mortality of infected plants (Miller *et al*., 2008). In Uganda, it is recorded to have led to the destruction of 44.5% of Robusta coffee by 2002 (Hakiza *et al.*, 2010; Phiri & Baker, 2009; UCDA, 2004; Olal et al., 2018) and it continues to be a major threat to the coffee industry (UCDA, 2002). It is accountable for economic losses of over $100M thus affecting livelihoods of over 1.7 million agricultural households that depend on coffee farming or employed along the value chain (Olal et al., 2018; Phiri & Baker, 2009; UCDA, 2019b). The pathogen was first identified more than 70 years ago on *Coffea excelsa* in the Central African Republic in 1927 (Rutherford, 2006). It then spread to Côte d'Ivoire in the 1940s and 1950s, and the Democratic Republic of Congo (DRC) from about 1949 where it destroyed more than 50% of the coffee-producing areas in both countries (Hakiza *et al*., 2010; Phiri & Baker, 2009; Rutherford, 2006). However, in Central African Republic (CAR) and DRC,

susceptible lines of coffee exhibited resistance to the *F. xylarioides* strain from Côte d'Ivoire, leading to the speculation of the existence variation within the pathogen due to environmental factors (Rutherford, 2006). The pathogen was first reported to Uganda in 1992 by John Schluter, a coffee trader, who warned of a devastating Robusta coffee disease in the DRC and its consequences if it crossed into Uganda (Hakiza *et al.*, 2010; Phiri & Baker, 2009). Measures to control the disease by uprooting and burning of infected plants at the earliest symptoms and other sanitary measures were taken to destroy possible sources of infection but unfortunately, infected plant material carried as firewood led to its spread to Bundibugyo, a district at the border of the DRC with Uganda (Hakiza *et al.*, 2010). In September 1993, it was observed on few Robusta coffee trees at CORI, Kituza, in Mukono district and it continued to rise, and by October 1993 it was reported in Bundibugyo district (Phiri & Baker, 2009).

However, if a disease becomes rampant in an area in which it was previously non-existent, it is important to assess whether; the pathogen populations have changed and/or increased or if the pathogen and its vectors may be newly introduced to the area (Garrett *et al*., 2008). For this reason, the Uganda Coffee Development Authority (UCDA) and the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) conducted the first survey in 1996 that established that only ten districts of Mukono, Mubende, Mpigi, Ntungamo, Kasese, Kabarole, Rukungiri, Bundibugyo, Kiboga and Masaka (Phiri & Baker, 2009) were affected by then. This study paved way for research into the pathogen, and since then a number of molecular markers such as microsatellites, restriction fragment length polymorphisms (RFLPs), Amplified Fragment Length Polymorphisms (AFLPs), random amplified polymorphic DNA markers (RAPD), and inter-simple sequence repeats (ISSRs) have been applied to study intraspecific genetic diversity within the *F. xylarioides* genome (Hakiza *et al.*, 2010). As such, *F. xylarioides* populations responsible for current outbreaks in Africa have been identified, however, studies have revealed very little genetic diversity within the existing pathogen populations (Hakiza *et al*., 2010, Bieysse *et al.*, 2006; Rutherford, 2004; Rutherford, 2006).

However, the pathogen continues to spread and in 2000 it was identified in isolates from the West Nile region indicating that all Robusta coffee districts in Uganda including Kabale had been affected (Hakiza *et al*., 2010, Phiri & Baker, 2009). However, it is unknown whether this spread is due to the pre-existing strain of *F. xylarioides* or a new variant that has evolved from an earlier

existing and more diverse population (Rutherford, 2006). This situation prompted the need of this study to determine the genetic dynamics of *F. xylarioides* and the environmental factors that contribute to its spread to new places in Uganda.

## 1.2 Statement of the Problem

Coffee, Arabica produced at 20% and Robusta coffee at 80%, is Uganda's leading foreign exchange earner (UCDA, 2019a; UCDA, 2019b, Senyonyi, 2018). However, the coffee industry is majorly threatened by *F. xylarioides* that currently affects 70% of the Robusta coffee producing areas in Uganda (Nabeta, 2016). Similar studies of pathogen diversity suggest very little genetic diversity within the pathogen and transmission of the pathogen through human activities including pruning, stumping, slashing and hoeing and transporting infected trees and dispersal by rain, wind, insects from diseased fields to healthy fields and humidity and dryness (Rutherford, 2006; Alemu, 2012; Lepoint, 2006). However, there is no up-to-date knowledge if the current spread is due to genetic variability in the pathogen or due to environmental effects on the spread of the pathogen in Uganda. This prompted the need of the current study considering the advent of the pathogen to new places like Kabale district where initially it was not expected to thrive.

## 1.3 General objective

To determine the genetic dynamics of *Fusarium xylarioides* and the environmental factors that contribute to its spread to new places in Uganda.

### 1.3.1 Specific objectives

i. To determine the environmental factors responsible for the spread of *Fusarium xylarioides* to new places in Uganda.
ii. To determine the genetic diversity within the *Fusarium xylarioides* population in Uganda over a 10-year period.
iii. To determine the genetic diversity among the *Fusarium xylarioides* populations from 3 coffee growing regions of Uganda.

## 1.4 Research Questions

The following questions were explored:

1. What environmental factors are responsible for the current increase in the incidence of *Fusarium xylarioides* in Uganda?

2. Is there genetic diversity within the *Fusarium xylarioides* population in Uganda over a 10-year period?
3. Is there genetic diversity among the *Fusarium xylarioides* populations from 3 coffee growing regions of Uganda?

### 1.5 Significance of the study

The study was aimed at providing information on genetic dynamics and environmental factors that contribute to the spread of *F. xylarioides*. This information is fundamental to the design, optimization and successful implementation of sustainable Integrated Pest Management practices of the pathogen thus safeguarding the livelihoods of millions of smallholder coffee farmers across Uganda.

### 1.6 Scope of the study

The study sought to identify environmental factors contributing to the spread of *F. xylarioides* and to determine the genetic diversity within and among the *F. xylarioides* population(s) in Uganda from selected Robusta growing areas of Uganda. Sample analysis, storage and maintenance was conducted at the National Coffee Research Institute (NaCORI) at Kituza, Mukono.

## CHAPTER TWO: LITERATURE REVIEW

### 2.1 Coffee growing regions and Robusta coffee varieties in Uganda

Coffee is grown in five regions of Uganda that is the Central, Western, South-western, Northern and Eastern Regions (UCDA, 2019a). Robusta is the major type of coffee grown in Uganda (Ngugi & Aluka, 2019; UCDA, 2019b), and it grows in low-altitude areas of Uganda including Central, Eastern, Mid North, West Nile, Western and South Western Uganda (UCDA, 2019b). A typical growing area of Robusta coffee is defined by fertile, well aerated, free draining, slightly acidic, deep soil, an altitude range of 900-1500 metres above sea level, warm temperatures ranging from 22°-28° C, rainfall range of 1,200 mm to 1,800 mm, well distributed over a period of 9 months and the appropriate type of cropping system (UCDA, 2019a; UCDA, 2019b). However, a change in these environmental patterns for example prolonged rainfall favours the spread of pathogens including *F. xylarioides* (Hakiza *et al.*, 2010). In this regard, NARO has released 10 varieties of resistant coffee since 2009 to curb the spread of *F. xylarioides* (UCDA, 2019b).

### 2.2 *Fusarium* xylarioides symptoms

*F. xylarioides* is a vascular pathogen that invades the xylem system of the coffee plant therefore symptoms are associated with the impediment of water transport ( Musoli *et al.*, 2008; Olal, *et al*., 2018; Yigletu, 2019, Lepoint, 2006). Typical symptoms proceed with sudden blackening of buds, curling of leaves, followed by wilting and rapid leaf fall (Olal, *et al*., 2014, Lepoint, 2006). The leaves may or may not turn yellow (Figure 1) (Hakiza & Birikunzira, 2000). The leaf fall is followed by die back of stem tips, which spreads down all the primary branches, resulting in death of the stem or the entire plantation (Lewis, *et al*., 2014; Lepoint, 2006; Alemu, 2012).

**Figure 1:** Illustration of yellowing of leaves of a coffee plant infected by *F. xylarioides* (Photo courtesy Olal, 2021).

On fields where coffee is managed on multiple stem system, the stems die one after the other until the whole plant completely dies and dries up, it is difficult to save the plant from the pathogen once infected (Alemu, 2012; Hakiza & Birikunzira, 2000; UCDA, 2019b). Berries ripen prematurely, have brown sunken lesions at the stalk end and remain attached even after the death of the trees (Figure 2) (Rutherford & Phiri, 2006; Alemu, 2012).

**Figure 2:** Illustration of die back on a symptomatic coffee trees killed due to *F. xylarioides* (Photo courtesy Olal, 2021)

Another characteristic of the pathogen is the longitudinal staining of the wood beneath the cracked bark (Lepoint, 2006). Towards the end of the rainy season, when the bark is peeled off at the base of the affected stem or tree, black or blue black bands or streaks are observed in the wood along the xylem vessels (Figure 3) (Rutherford & Phiri, 2006; Hakiza *et al*., 2010; Alemu, 2012).

**Figure 3:** Illustration of blue black lines under the back of a symptomatic coffee plant due to *F. xylarioides* (Photo courtesy Olal, 2021)

### 2.3 Transmission of *Fusarium xylarioides* in Uganda

The spread of *F. xylarioides* in Uganda has been attributed to a number of factors that include:

### 2.3.1 Environmental factors

Changes in environmental factors such as temperature, light, rain, wind, and humidity patterns have impacted negatively on the suitability of coffee-producing areas causing changes in disease and pest patterns and diversity (Lepoint, 2006; Savary *et al*., 2011, Belachew & Teferi, 2015, & Fischersworring *et al.*, 2015). Host-pathogen associations involve a three-dimensional interaction where favourable environmental conditions, in the presence of susceptible germplasm (host) and an aggressive pathogen, lead to rapid spread of disease (Hakiza *et al.*, 2010; Yigletu, 2019). For this reason, the agricultural sector, due to its size and sensitivity, suffers from the impacts of climate change with an anticipated average global loss of 50% by 2050 (Kutywayo *et al*., 2013). A case in point is in Uganda, where there are normally two rainy seasons, one from March to June and another from September to November(Hakiza *et al*., 2010). However, rainfall patterns having become less predictable and more severe (Fischersworring *et al.*, 2015; Haggar & Schepp, 2012), there are high chances of impending new outbreaks and mortality rate due *to F. xylarioides* (Phiri & Baker, 2009). On the other hand, environmental factors such as soil type and health and insects, though refuted by some studies, have been suggested to contribute to the spread of *F. xylarioides* (Lepoint, 2006; Hakiza *et al.*, 2010; Phiri & Baker, 2009). This presents a new challenge to assess whether the increase in pathogen populations is due to existing genetic diversity or environmental factors (Garrett *et al.*, 2008).

### 2.3.2 Susceptibility and genetic modification of existing coffee varieties

Most Robusta coffee strains in Uganda originate from seeds that are susceptible to *F. xylarioides* thus they are vulnerable to the pathogen given this susceptibility can be inherited. (Hakiza *et al.*, 2010, Phiri & Baker, 2009, Musoli *et al.*, 2013). Studies suggest breeding coffee for resistance against *F. xylarioides* through genetic modification, as the most appropriate, cost effective and sustainable method for *F. xylarioides* management (Lepoint, 2006; Yigletu, 2019). However, it has been noted that the Robusta clones being developed have a narrow resistance base (Phiri & Baker, 2009). This presents a challenge that as the pathogen continues to spread, it will breed with a different strain of *F. xylarioides* increasing its genetic diversity and breaking down the already narrow base of the resistant Robusta strains (Phiri & Baker, 2009)

### 2.3.3 Information gaps

Missing information and misinformation on *F. xylarioides* has given time for the pathogen to spread within a very short time making it difficult to control (Hakiza *et al*., 2010). Information on why its spread and severity in Uganda is much higher than in countries like Tanzania and Ethiopia where there is little effort to control it is still missing (Phiri & Baker, 2009). It is also unknown whether the current steady spread is due to the pre-existing strain of *F. xylarioides* or a new variant that has evolved from an earlier existing and more diverse population as a result of selection pressure (Rutherford, 2006). This current lack of knowledge about the diversity of the pathogen has made it difficult to establish its importance as a significant driver of the already diminishing coffee resources(Moat *et al.*, 2019; Smith *et al.*, 2009).

### 2.3.4 Human activities

Human activities such as pruning, stumping, slashing, and hoeing to control weeds, and transporting infected trees have been identified as the main route of transmission of *F. xylarioides* (Alemu, 2012; Rutherford, 2004; Rutherford, 2006). Studies have shown that plant to plant infection plays a key role in *F. xylarioides* spread through wounding that occurs during pruning and cutting dry infected trees for use as firewood (Hakiza & Birikunzira, 2000; Musoli *et al.*, 2008; Hakiza *et al.*, 2010). Other management practices identified as risk factors include; extensive wounding of stems by grazing animals, use of infected planting material like seedlings, seed, soil, and mulching using infected coffee husks (Hakiza *et al.*, 2010; Phiri & Baker, 2009; Lepoint, 2006). However, studies have revealed that *F. xylarioides* is neither seed nor soil transmitted (Hakiza *et al.*, 2004, Lepoint, 2006).

## 2.4 Diversity in the *Fusarium xylarioides* pathogen

Studies have characterized and identified *F. xylarioides* in two basic ways as explained below.

### 2.4.1 Morphological variations

*F. xylarioides* has been initially identified using morphological and phenotypic attributes such as spore morphology, mode of spore production, degree of conidiophore branching, presence/absence of chlamydospores, colony pigmentation, growth rate, and production of secondary metabolites, etc. Morphologically, characterization of the fungus has only been possible between strains growing on Arabica and Robusta, where an orange pigmentation was observed in young *F. xylarioides* cultures from Robusta and is absent in isolates from Arabica (Lepoint, 2006; Phiri &

Baker, 2009). Differences in growth rate and growth response to different temperature levels have also been observed (Hakiza *et al*., 2010). However, no visual characterization is possible beyond this because morphology alone does not allow any reliable differentiation given that the morphological attributes such as spore shape and size, are identical (Phiri & Baker, 2009 & Hakiza *et al.*, 2010).

### 2.4.2 Genetic diversity, Biological species diversity, and mating types

Initially, there was limited information on the genetics of the pathogen, its extent of diversity, or how it relates to other attributes, such as field behaviour (Hakiza *et al.*, 2010). This was attributed to the limited expertise and methodology to investigate genetic diversity, thus the development of a range of molecular markers, such as microsatellites and AFLPs (Hakiza *et al.,* 2010 & Phiri & Baker, 2009). In this regard, studies have identified the existence of limited genetic diversity within the pathogen (Bieysse *et al*., 2006; Hakiza *et al*., 2010; Rutherford, 2004; Phiri & Baker, 2009). This has been attributed to selection pressure due to the widespread cultivation of resistant coffee genotypes or the rapid spread of a single or small number of survivors or variants (Hakiza *et al.*, 2010). As such, biological species variation, based on the ability of two forms with different versions of the mating gene to produce a viable offspring, characterizes the pathogen into three biological species (BS), BS1, BS2, BS3, a residual sterility group (SG), SG4, and two mating types (MAT), *MAT1-1* and *MAT1-2* (Phiri & Baker, 2009, Waller, *et al,* 2012, & Lepoint, 2006). Other forms of genetic characterization of the pathogen include:

- Two genetically distinct populations responsible for current outbreaks; the *Fusarium xylarioides* strain from Robusta in Uganda, DRC, and Tanzania and the *Fusarium xylarioides* strain from Arabica in Ethiopia (Hakiza *et al.*, 2010).
- Four distinct species in Africa that is; *Fusarium abyssiniae* on Arabica in Ethiopia, *Fusarium congoensis* in DRC, Uganda, and Tanzania, *Fusarium guineensis* the asexual form, responsible for the original mid-20$^{th}$ century outbreaks in Guinea, Côte d'Ivoire and CAR and the *Fusarium xylarioides*, in CAR during the first epidemic (Phiri & Baker, 2009) and;
- Three clades that is Clade I a in West African strains in the 1950s to 1960s, Clade I b in CAR in the 1950s to 1960s, and Clade II the Congolese, Ugandan, and Tanzanian strain associated with *C. canephora* /*C. excelsa* from 1960 to 2002 (Bieysse *et al.*, 2006)

However, there is no up-to-date information on the existing genetic diversity of the *F. xylarioides* strain in Uganda.

### 2.5 Management Practices of *Fusarium xylarioides* and Limitations in Uganda

*F. xylarioides* attacks the coffee plant at all stages of growth, from seedlings to mature plants, and once infected plants show 100% mortality and cannot be saved (Miller *et al.*, 2008). Integrated Pest Management practices are therefore based on principles of exclusion, eradication, escape from infection, protection and reduction of the pathogen applied by either biological, cultural, or regulatory methods (Wassie, 2019) as enlisted below.

i. Regular monitoring to identify symptomatic plants accompanied with uprooting and burning of trees in a radius 10 meters away, however, it is expensive for farmers to accept (Phiri & Baker, 2009; Rutherford & Phiri, 2006; Lepoint, 2006).
ii. Sterilization of tools used during hoeing, pruning and marking of infected plants (Phiri & Baker, 2009)
iii. Use of pathogen free planting material including plants and seedlings from reliable sources (Phiri & Baker, 2009; Wassie, 2019).
iv. Site selection by planting Robusta coffee in non-traditional areas free from the pathogen to avoid planting in pathogen high risk areas (Hakiza *et al.*, 2010; Wassie, 2019).
v. Promotion of growth of Arabica in Robusta coffee traditional areas since the pathogen does not thrive on Arabica coffee (Hakiza *et al*., 2010).
vi. Prevention of wounding of plants during pruning, hoeing or grazing (Phiri & Baker, 2009).
vii. Restriction of movement and use of planting materials from infested areas (Phiri & Baker, 2009; Wassie, 2019).
viii. Observation of a fallow period of at least 6 months to 2 years after uprooting and burning before replanting with a susceptible coffee seedling (Phiri & Baker, 2009).
ix. Regular use of fungicides for example Bordeaux mixture, carbolineum mixture, or cupro-insecticides on wounds of coffee plants and on wilted coffee plants before uprooting, and on infected soil after uprooting and burning (Lepoint, 2006).
x. Soil sterilization for eliminating the pathogen in soil, but it is expensive and time consuming (Phiri & Baker, 2009).

xi. Planting of resistant clones in high risk areas although the clones are not available to all farmers (Hakiza *et al*., 2010; Phiri & Baker, 2009; Wassie, 2019).

xii. Farmer support programs involving participation and sensitization of all stakeholders from village level to policy makers through research and training activities by NARO, universities and other private organizations (Hakiza *et al*., 2010; Wassie, 2019).

xiii. Dissemination of information through the mass media in local languages for farmers and through participatory approaches in farmer field schools (Hakiza *et al.*, 2010).

The existing management practices, however, lack information on the management of the spread of the pathogen due to environmental changes in Uganda.

## 2.6 Conceptual framework

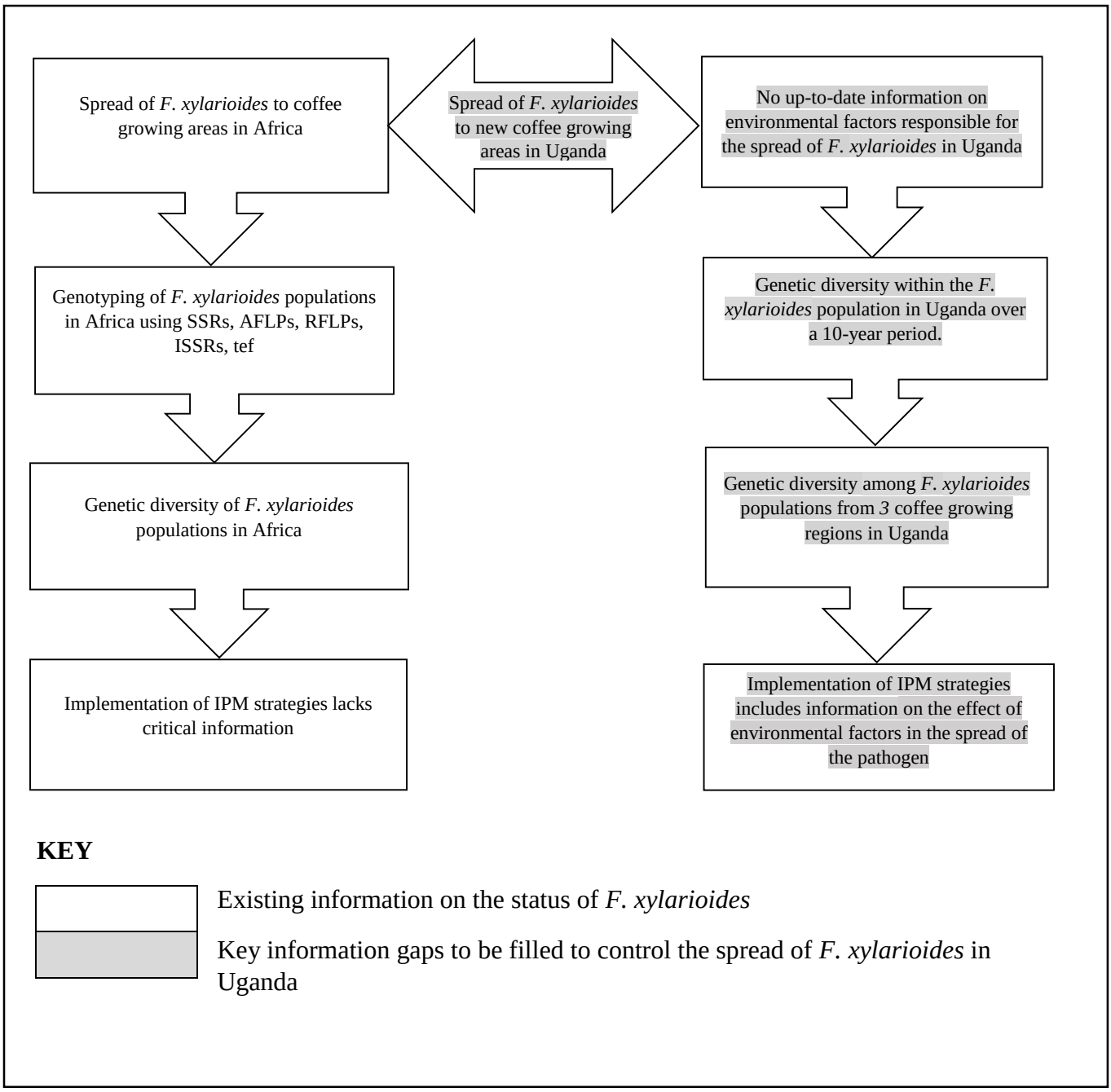


**Figure 4:** Conceptual framework illustrating the existing information on the status of *F. xylarioides* and key information gaps to be filled to curb the spread of the pathogen in Uganda

## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Study design

A snapshot randomized sampling was conducted using, a handheld GPS, paper envelopes, and pangas. Ten (10) samples were collected from fields in 12 Robusta coffee growing districts of Uganda with high risk and incidence of the pathogen, in the year 2020. Incidence data from the sampling was used to study the relationship between the incidence of *F. xylarioides* and environmental data obtained from the POWER project (http://power.larc.nasa.gov) using SPSSv22 software. Historical incidence data of *F. xylarioides* was obtained from UCDA Annual Reports, and likewise historical environmental data was collected form the POWER project. Location and incident maps were then generated from the data using Arcmapv10.8. Simple sequence repeats (SSR) markers were designed from the pathogen sequence data at NaCORI (unpublished data) and were applied to study the genetic diversity of *F. xylarioides.*

### 3.2 Study area

Symptomatic stem tissues were collected from infected Robusta coffee trees from 12 districts of Bundibugyo, Kibaale, Rukiga, Buikwe, Mukono, Ntungamo, Masaka, Butambala, Gomba, Kassanda, Luweero, and Kayunga to represent the major coffee growing regions of Uganda (Figure 5). The choice of these districts was based on the inclusion and exclusion criteria (Setia, 2016) where areas with a high incidence of *F. xylarioides* were sampled (UCDA, 2004 & UCDA, 2005). The locations of the study sites were captured in form of coordinates using a handheld GPS receiver (etrex 10-Garmin, Olathe, Kansas USA).

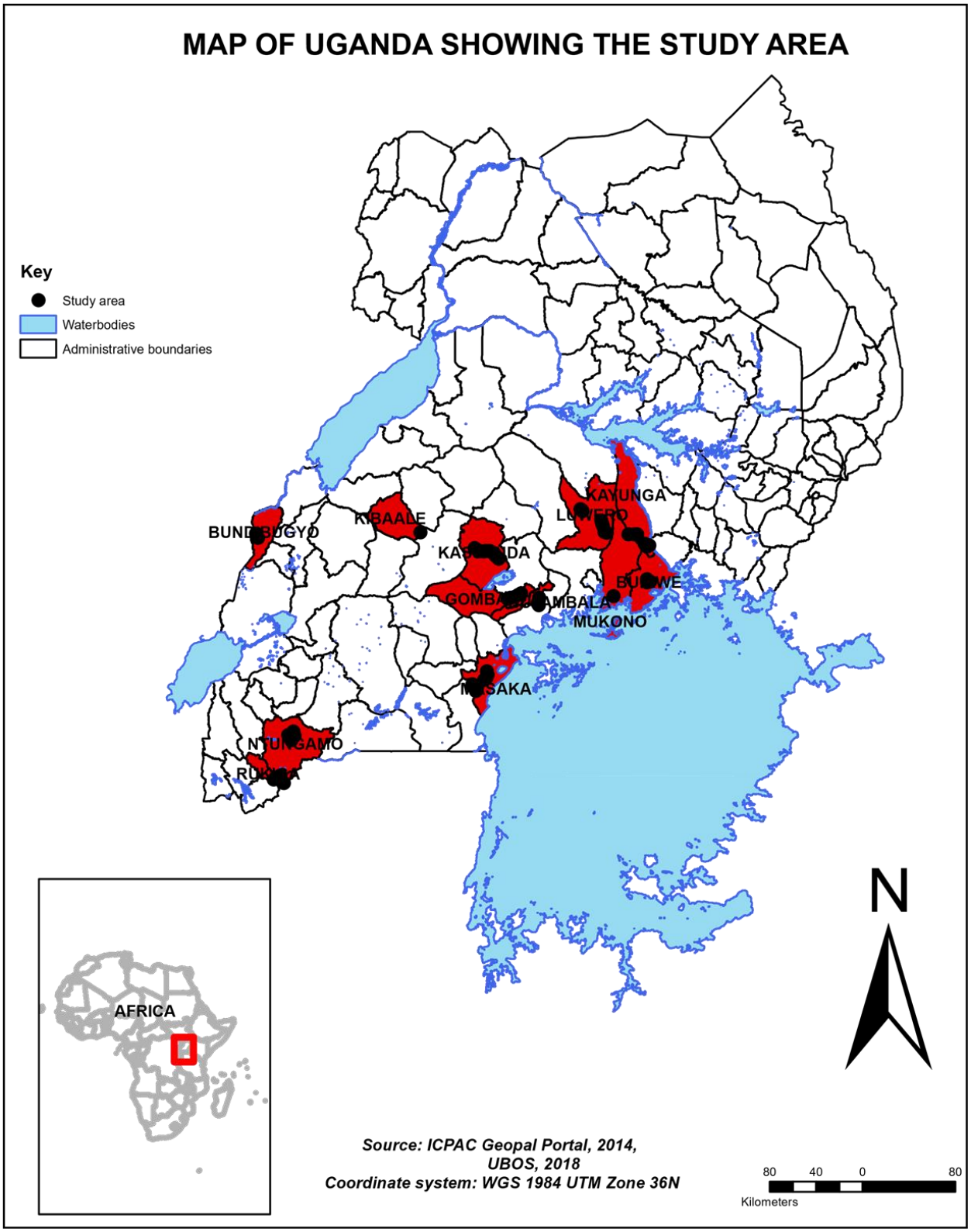


**Figure 5:** Map of Uganda showing the 12 selected coffee growing districts from which symptomatic specimen were collected in the year 2020.

### 3.3 Data Collection Instrument(s)

A handheld GPS (etrex 10-Garmin, Olathe, Kansas USA) was used to capture the locations of the fields in form of coordinates. Pangas were used to harvest coffee specimens from the field while paper envelopes were used to transport specimens to the laboratory.

### 3.4 Data Collection Procedure

Data collected included primary and historical data. Historical data including annual average data of rainfall, relative humidity, temperature and speed of wind was collected from the Prediction of Worldwide Energy Resources (POWER) website for both time frames, equally, historical *F. xylarioides* incidence data was collected from UCDA Reports as enlisted below. Secondary allelic data was collected from pathogen cultures collected and maintained from 2009 to 2019 at NaCORI, likewise primary allelic data was obtained from specimens collected from the selected Robusta coffee growing districts for the year 2020 as enlisted below. Primary *F. xylarioides* incidence and location data was collected alongside the specimen from selected 12 Robusta coffee growing districts as enlisted below.

#### 3.4.1 Environmental factors responsible for the spread of *Fusarium xylarioides* to new places in Uganda

Datasets explaining variation in the disease incidence were captured from fields from the 12 Robusta coffee growing districts of Uganda with high risk and incidence of the pathogen in the year 2020. These datasets, including information on the field latitude, and longitude and *F. xylarioides* incidence, were recorded on individual sample labels. Historical *F. xylarioides* incidence data for correlation of the disease incidence with environmental data was obtained from the UCDA Annual Reports. Data from 2000 to 2005 was used, because there was no record of *F. xylarioides* incidence data from 2006 to 2019. Historical environmental data including average rainfall, average temperature, average speed of wind and average humidity was obtained from the POWER project website. The data obtained was then used in SPSS to study the factors responsible for the spread of the pathogen in Uganda.

### 3.4.2 Genetic diversity within the *Fusarium xylarioides* population in Uganda over a 10-year period

Specimens from naturally *F. xylarioides* infected Robusta coffee plants from the field were collected and maintained on a yearly basis for the period 2009 to 2019 in a refrigerator at 4°C with reliable power supply at NaCORI, Kituza, Mukono, Uganda. Fungal spores, stored on Synthetic Nutrient Agar (SNA) a low nutrition media that reduces the degeneration of isolates morphologically, and thereby preserves other traits such as metabolite production and pathogenicity, were isolated as described by Olal, *et al.*, 2014. The cultures were removed from the fridge and allowed to attain room temperature and were sub-cultured on fresh Tap Water Agar to determine their survival and purity. The cultures were transferred to Potato Dextrose Agar incubated at 25° C under 12 hour alternating fluorescence light and dark cycles for 4 days before DNA extraction.

### 3.4.3 Genetic diversity among the *Fusarium xylarioides* populations from 3 Robusta coffee growing regions of Uganda

Ten (10) symptomatic stem tissues were collected in duplicates (2 samples) from Robusta coffee trees from the districts of Bundibugyo, Kibaale, Rukiga, Buikwe, Mukono, Ntungamo, Masaka, Butambala, Gomba, Kassanda, Luwero, and Kayunga in 2020. The expected number of fields to be visited per district was 10, however, the pathogen is not uniformly distributed and thus only fields with symptomatic trees were sampled. The specimens were transported to the laboratory wrapped and labelled in individual envelopes to avoid condensation from specimens and reduce contamination of the specimens.

Fungal isolation and identification were performed as described by Olal, *et al.*, 2014. The bark of the stem specimen was carefully removed from plant tissues exhibiting clear symptoms. The tissues exhibiting symptoms along with adjacent small unaffected tissue were cut into small pieces (05 mm squares) for laboratory analysis. These sections were transferred to sterile petri dishes containing 1% v/v sodium hypochlorite solution for 1 to 2 min using flame-sterilized forceps, and then rinsed twice with sterile distilled water. The sterilized sections were then pressed dry using sterile paper towels. The edges were trimmed to expose fresh tissue, placed on Tap Water Agar (TWA) comprising 2% agar, and incubated at 25° C for three days. The emerging hyphae from the inoculated pieces were sub-cultured on fresh Synthetic Nutrient Agar (SNA) and four pieces of

sterile filter paper of 1 x 1 cm were placed on the periphery of the set agar to induce sporulation (Rutherford, 2004). The hyphae were then transferred to Potato Dextrose Agar (PDA) to encourage development of pigmentation. The cultures were incubated at 25° C under 12 hours alternating fluorescence light and dark cycles for 10 days. Identification was then done basing on typical cultural and morphological characteristics of *F. xylarioides* on culture media.

#### 3.4.3.1 Preparation of mycelial homogenates

The mycelial homogenates were prepared as described by Olal, *et al.*, 2014. The isolates were then grown separately by transferring four (4 x 4 mm) plugs taken from the periphery of 5 days old cultures on PDA into 100ml conical flasks containing 20 ml of potato dextrose broth. The cultures were then incubated stationery in the dark for 6 days at 20°C. The broth containing fungal hyphae was poured into sterile steel tea strainer to harvest the mycelium. The mycelial mats harvested were rinsed thoroughly with sterile distilled water, blotted dry ready for DNA extraction (Olal, *et al.*, 2018). *F. xylarioides* isolate obtained from a wilted coffee plant at NaCORI, Kituza, Mukono, Uganda was used as a reference.

#### 3.4.3.2 DNA extraction and quantification

DNA was extracted from the mycelia using ZR Plant/Seed DNA MiniPrep (ZYMO RESEARCH, USA) kit according to the manufacturer's instructions. The purity and quantity of the extracted DNA was verified using the Synergy 2 multi-mode microplate reader (BioTek Instruments, Inc., USA). DNA samples having absorbent readings at wavelength (λ) 260/280 ratio ranging from 1.8-2.0 were considered pure and of substantial quality.

#### 3.4.3.3 Primer Design and optimization

Ten (10) SSR primers were developed from the genomic sequence of the pathogen at NaCORI (unpublished data). The designed primers were then synthesized from Inqaba Biotec East Africa Ltd. The synthesized primers were then tested for polymorphism and reproducibility using 3 isolates of *F. xylarioides*. Two (2) primers, described in the table below, were found with good polymorphism and reproducibility were selected for use in the study.

**Table 1:** SSR primers used and their relevant characteristics to study genetic diversity of *Fusarium xylarioides*

| Primer Name | Primer sequence | Length | GC Content (%) | Molecular weight | Melting temperature (°C) |
|---|---|---|---|---|---|
| SSR 33F | ATTGAAGAGAGACCTGAAAGG | 21 | 42 | 6633.04 | 50.45 |
| SSR 33R | AGTTTGTGGTTTGCTCACTT | 20 | 40 | 6200.63 | 47.68 |
| SSR 41F | CCTCTTCTTCATAGCCTCTTT | 21 | 42 | 6329.72 | 50.45 |
| SSR 41R | AAACTACAACTGCATCACCTG | 21 | 42 | 6423.87 | 50.45 |

#### 3.4.3.4 PCR amplification and electrophoresis

The PCR (Polymerase Chain Reaction) mixtures containing 3.0 µl (25 ng) genomic DNA, 0.125 µl One Taq Hot Start DNA Polymerase (New England BioLabs, Inc., USA), SSR forward primer 0.5 µl (10 µM), SSR reverse primer 0.5 µl (10 µM), 0.5 µl (10mM) dNTPs, 5 µl (1X) One Taq Standard Reaction Buffer in a total volume of 25 µl were prepared for each of the 23 isolates. PCR was performed in the eppendorf vapo.protect thermal cycler (Hamburg, Germany) using thermal cycling conditions; 30 seconds at 94° C for initial denaturation, 30 cycles of 30 seconds at 94° C for denaturation, 60 seconds at 55° C for annealing, 1 minute at 68° C for extension, 5 minutes at 68° C for the final extension, and an additional hold at 10° C for 10 minutes. The PCR products were separated by electrophoresis on 2% agarose at 100 Volts for 1.5 hours (90 minutes) in 1X TBE buffer (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA). The gel was stained with ethidium bromide at 0.5µg/ml final concentration and then the bands were visualized using ultraviolet light in the G: BOX gel documentation machine (Syngene Inc, USA).

### 3.5 Data Analysis and Presentation

The data collected recorded was subjected to various statistical analyses using different software packages. Regression analyses were performed in SPSS to establish the relationship between the environmental data and *F. xylarioides*, while allelic data was analysed in GenAlEx 6.5 (Peakall & Smouse, 2012) as described below.

### 3.5.1 Environmental factors responsible for the spread of *Fusarium xylarioides* to new places in Uganda

Time series regression analyses were performed in SPSSv 22 to establish the relationship between *F. xylarioides* incidence and environmental factors. The Box-Jenkins (ARIMA) autoregressive model fitted was:

$$y_t = c + \phi_1 y_{t-1} + \phi_2 y_{t-2} \ldots + \phi_p y_{t-p} + \varepsilon_t$$

where $y_t$ is the dependent variable (*F. xylarioides* incidence), c is the constant, $y_{t-1}$, $y_{t-2}$, $y_{t-p}$ are the time series values and ε is the error (Box, *et al.*, 1976).

### 3.5.2 Genetic diversity of *Fusarium xylarioides* in Uganda over a 10-year period and from 3 Robusta coffee growing regions of Uganda

The banding patterns obtained by gel electrophoresis were compared for polymorphism by visual observation. The visible bands of isolates at the same migration distance were considered the same. These data were numerically coded for and entered in a spreadsheet. Each band was considered as polymorphic and was thus scored one (1) for presence or zero (0) for absence on the spreadsheet. Only reproducible bands in repeated PCR amplification for the different primers were considered for analysis. The numerically coded data was grouped into years, districts and regions and was used to determine the pairwise genetic distance of *F. xylarioides* populations, and analysis of molecular variance (AMOVA), as described below.

#### 3.5.2.1 Genetic diversity, Analysis of molecular variance and Genetic distance

The numerically coded allelic data were transferred to GenAlEx 6.5 to determine the existing genetic structure and genetic diversity within and among *F. xylarioides* populations. The data was analysed for genetic diversity indices including observed number of alleles, effective number of alleles, percentage polymorphic loci, gene diversity, and Shannon information index. The data was also analysed for AMOVA where the variations of the dataset were subdivided into two constituents namely:

1. within the *F. xylarioides* population
2. among the *F. xylarioides* populations.

Pairwise comparisons of the genetic distance between populations were also conducted to determine the existing genetic structure and genetic diversity within and among the *F. xylarioides* populations in Uganda.

## CHAPTER FOUR: RESULTS

### 4.1 Description of environmental factors of the coffee growing areas

The average relative humidity ranged from 61.9% to 79.4% for both 2000-2005 and 2020 (Appendix II and Appendix V). The average humidity for the years 2000 to 2005, was generally highest in the Central region, followed by the Eastern region, then the Western region, and was lowest in the South-western region. The general pattern of relative humidity for 2000 to 2005 was such that relative humidity increased from 2000 to 2001, decreased in 2002 and continued to decrease in 2003, it then increased in 2004 and finally decreased in 2005 (Figure 6).

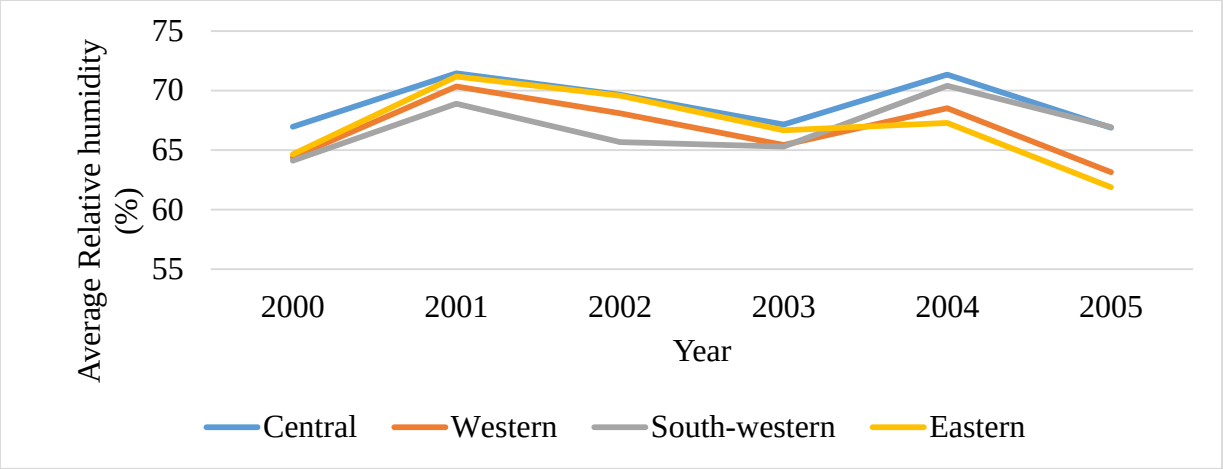


**Figure 6:** Trend graph of the annual average relative humidity of the different coffee growing regions for the years 2000 to 2005

The average relative humidity in the year 2020 was highest in the South-western region, followed by the Western region and lowest in the Central region (Figure 7).

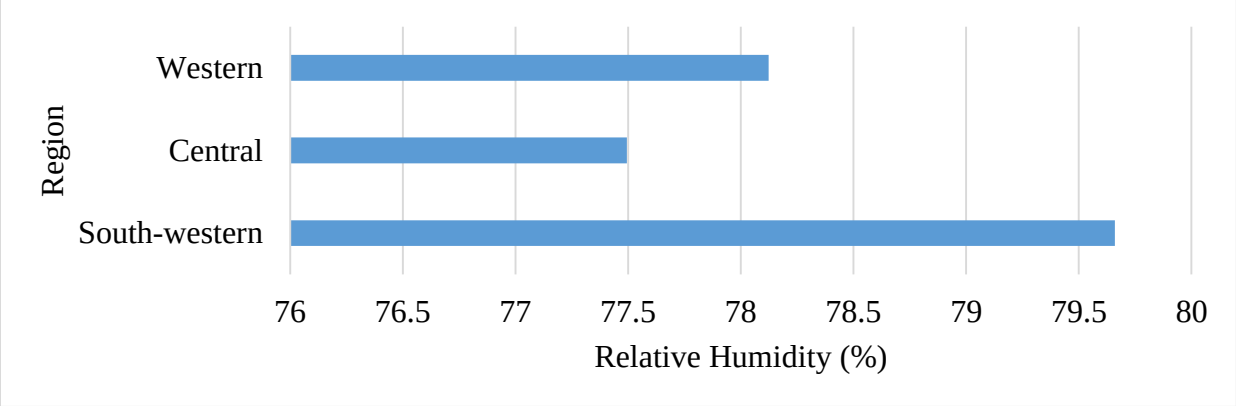


**Figure 7:** Illustration of the cumulative average relative humidity of the selected areas in the 3 coffee growing regions of Uganda for the year 2020

The average rainfall ranged from 910 mm/day to 1743 mm/day (Appendix I and Appendix V). The average rainfall for the years 2000 to 2005, was highest in the Eastern region, followed by the Central region, then the Western region, and was lowest in the South-western region. The general pattern of rainfall for 2000 to 2005 was such that the rainfall increased from 2000 to 2001 then decreased in 2002, it flattened off between 2002 and 2003, then increased in 2004 to finally reduce in 2005 (Figure 8).

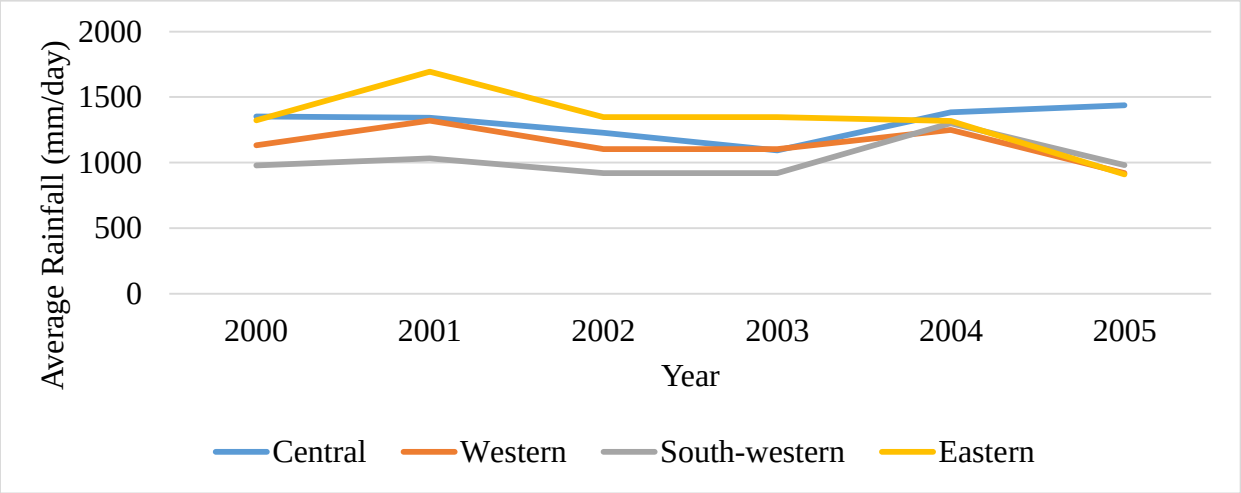


**Figure 8:** Trend graph of the annual average rainfall of the different coffee growing regions for the years 2000 to 2005

The average rainfall was highest in the South-western region, followed by the Central region and lowest in the Western region for the year 2020 (Figure 9).

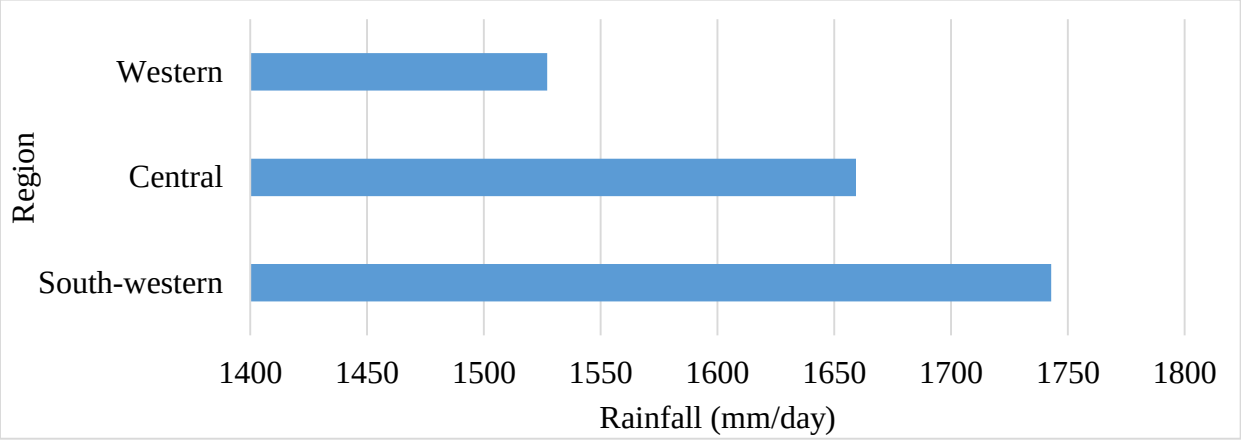


**Figure 9:** Illustration of the cumulative average rainfall of the selected areas in the 3 coffee growing regions of Uganda for the year 2020

The temperature ranged from 19.3° C to around 24.5° C (Appendix III and Appendix V). The average temperature for the years 2000 to 2005 was highest in the Eastern region, followed by the Western region, then the Central region, and was least in the South-western region. The general pattern of temperature for 2000 to 2005 was such that the temperature decreased from 2000 to 2001 then increased in 2002, it continued to increase in 2003, then decreased in 2004 to finally increase in 2005 (Figure 10).

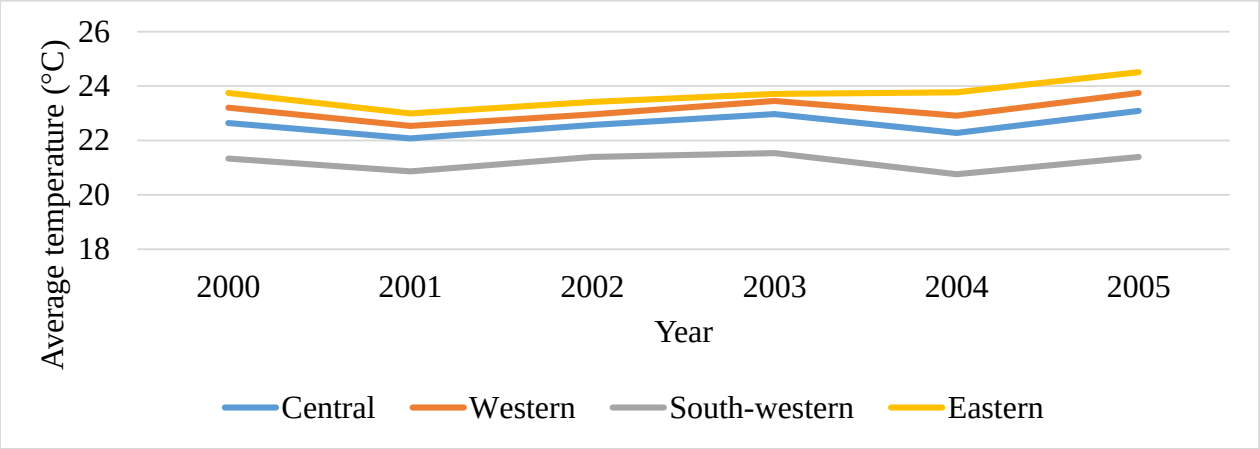


**Figure 10:** Trend graph of the annual average temperature of the different coffee growing regions for the years 2000 to 2005

The average temperature for the year 2020 was highest in the Central region, followed by the Western region and lowest in the South-western region (Figure 11).

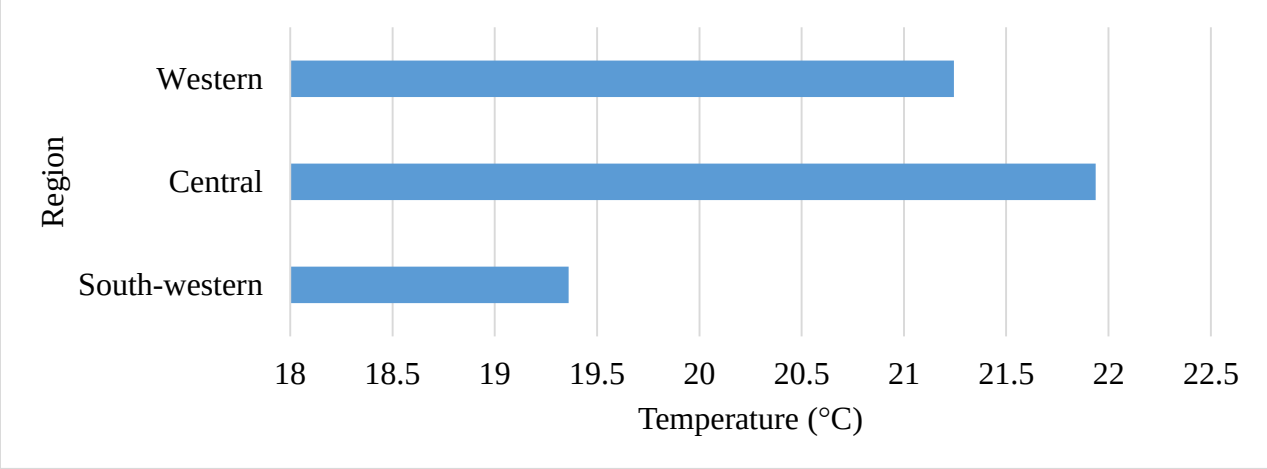


**Figure 11:** Illustration of the cumulative average temperature of the selected areas in the 3 coffee growing regions of Uganda for the year 2020

The speed of wind ranged from 0.877 m/s to 2 m/s (Appendix IV and Appendix V). The average speed of wind for the years 2000 to 2005 was generally highest in the Central region, followed by the Eastern region, then the Western region and was lowest in the South-western region. The general pattern of the speed of wind for 2000 to 2005 was such that the speed of wind decreased slightly from 2000 to 2001 then increased slightly in 2002, it continued to increase slowly in 2003, then decreased slightly in 2004 and finally increased slightly in 2005 (Figure 12).

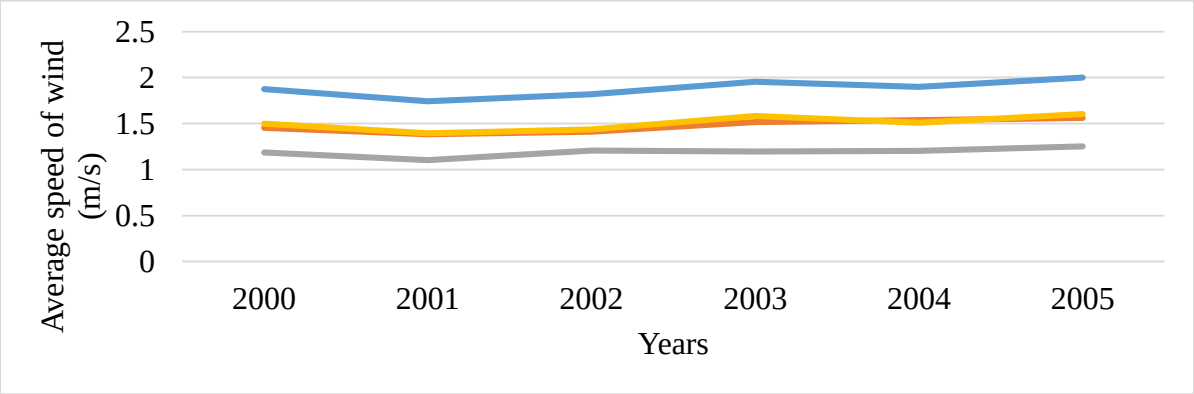


**Figure 12:** Trend graph of the annual average speed of wind of the different coffee growing regions for the years 2000 to 2005

The average speed of wind was highest in the Central region, followed by the Western region and lowest in the South-western region in 2020 (Figure 13).

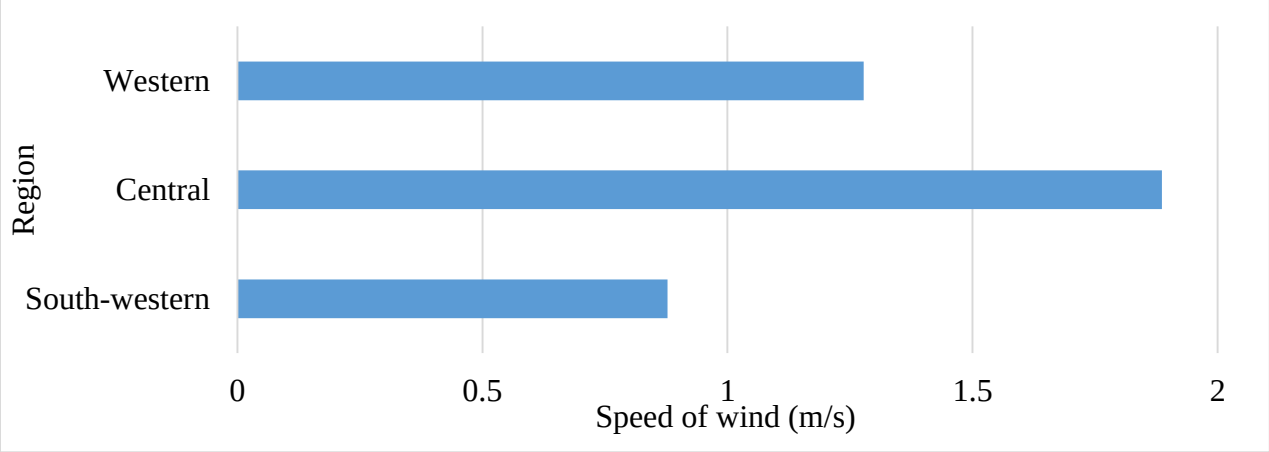


**Figure 13:** Illustration of the cumulative average speed of wind of the selected areas in the 3 coffee growing regions of Uganda for the year 2020

### 4.2 *Fusarium xylarioides* incidence and variability characteristics

The general pathogen incidence was highest in the Western region, then South-western region, then the Central region and lowest in the Eastern region for 2000-2005. In the Western, South-

western and Central regions, there is a sharp increase in the pathogen incidence increases from 2000 to 2001. From 2001 to 2005, these regions experience a gradual increase of the pathogen incidence. However, in the Eastern region the increase in the pathogen incidence is gradual from 2000 to 2005 (Figure 14).

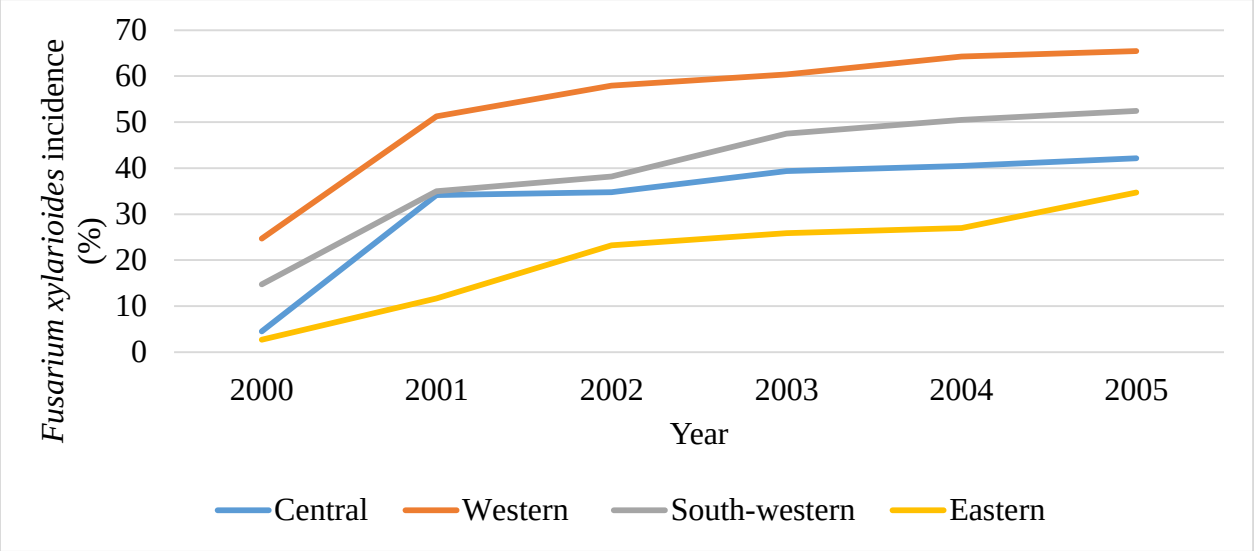


**Figure 14:** Illustration of the annual average *Fusarium xylarioides* incidence for the years 2000 to 2005

In the year 2020 for the 3 coffee growing regions studied, the pathogen incidence was highest in the Western region, then South-western region and lowest in the Central region (Figure 15).

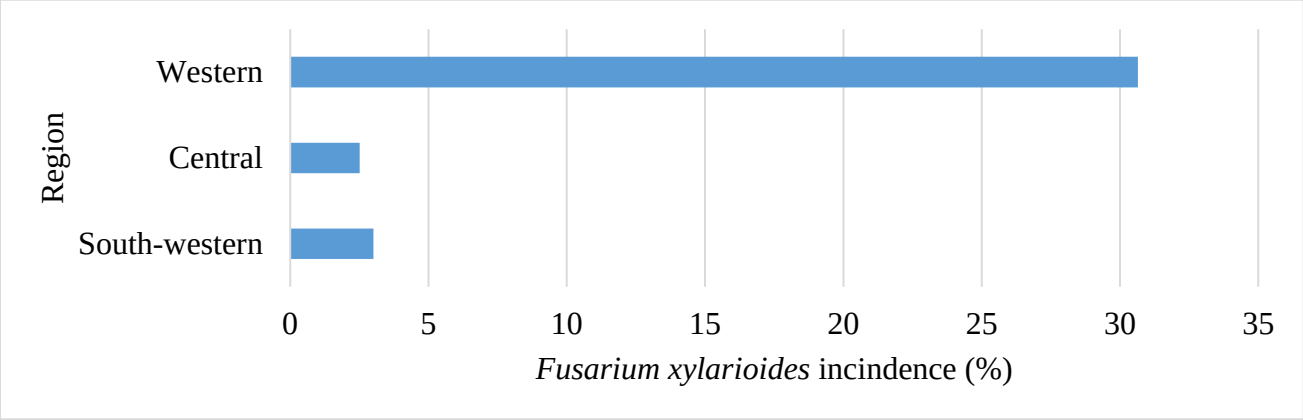


**Figure 15:** Illustration of the cumulative average *Fusarium xylarioides* incidence of the selected areas in the 3 coffee growing regions of Uganda for the year 2020

The pattern of the spread of the pathogen in 2020 was such that the *F. xylarioides* incidence was highest in the Western region, then South-western region and lowest in the Central region (Figure 16).

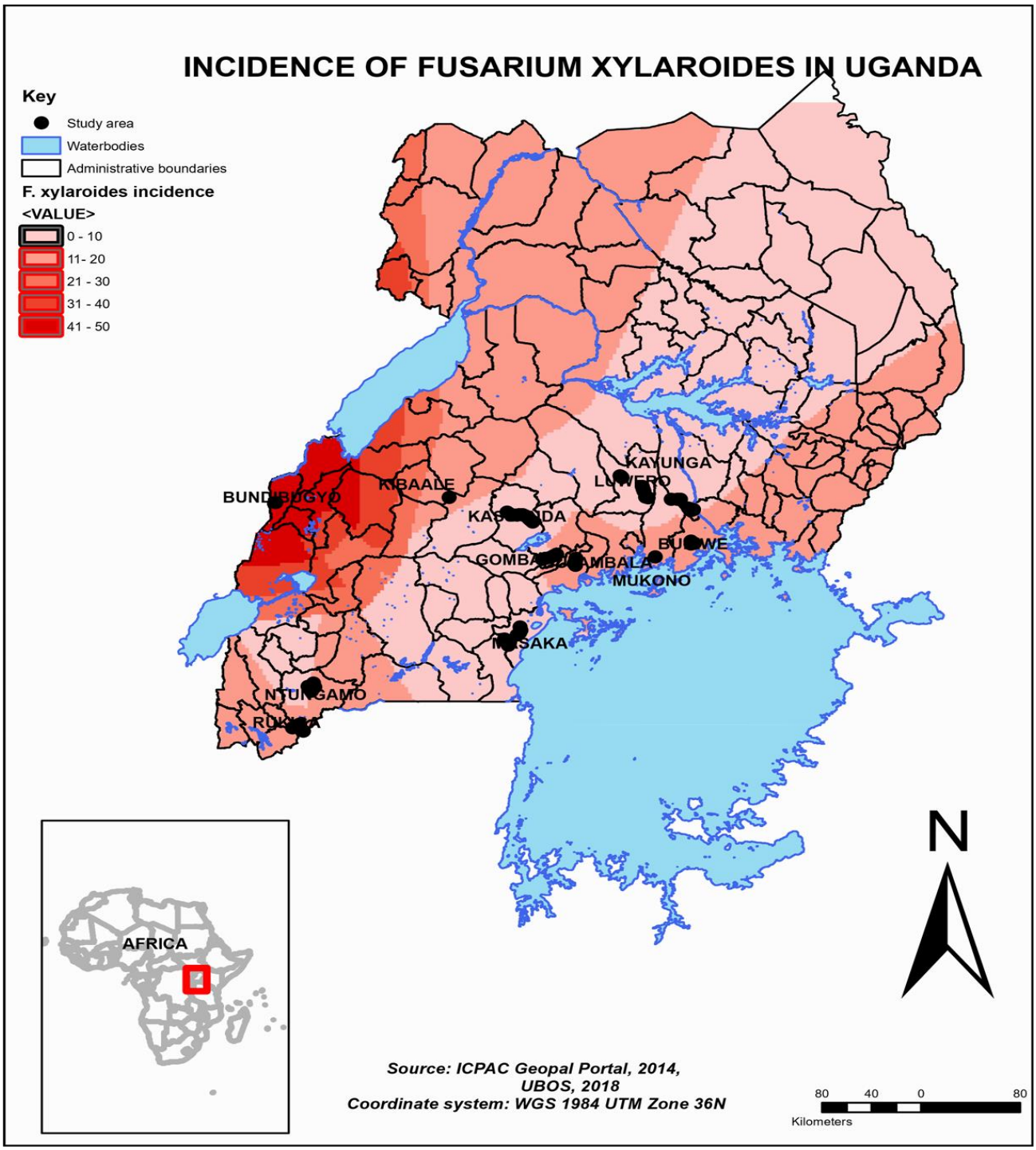


**Figure 16:** Illustration of the incidence of *F. xylarioides* in the selected districts from the 3 coffee growing regions of Uganda in the year 2020.

### 4.3 Environmental factors responsible for the spread of *Fusarium xylarioides* to new places in Uganda

At R= 0.8680, rainfall and temperature had the highest correlation with the pathogen incidence for 2000-2005. The correlation coefficients of the average relative humidity with *F. xylarioides* incidence ranged from -0.311 in the Eastern region to 0.760 in the Western region. The correlation coefficients of the average rainfall with *F. xylarioides* incidence ranged from -0.619 in the Eastern region to 0.868 in the Central region. The correlation coefficients of the average temperature with *F. xylarioides* incidence ranged from -0.072 in the South-western region to 0.868 in the Central region. The correlation coefficients of the average speed of wind with *F. xylarioides* incidence ranged from 0.241 in the Central region to 0.586 in the Eastern region for the period 2000 to 2005 (Table 2).

**Table 2:** Correlation coefficients of different environmental factors with *F. xylarioides* for the different coffee growing regions of Uganda for the years 2000 to 2005

| **Environmental factors** | **Correlation coefficients of environmental factors with *F. xylarioides*** | **P-Value(p**)** | **Coefficient of determination (%)** |
|---|---|---|---|
| | **Central** | | |
| Humidity | 0.313 | 0.005** | 9.7969 |
| Rainfall | 0.8680 | 0.008** | 75.3424 |
| Temperature | 0.8680 | 0.000** | 75.3424 |
| Speed of wind | 0.241 | 0.006** | 5.8081 |
| | **Western** | | |
| Humidity | 0.760 | 0.016** | 57.76 |
| Rainfall | -0.195 | 0.007** | 3.8025 |
| Temperature | 0.745 | 0.001** | 55.5025 |
| Speed of wind | 0.450 | 0.003** | 20.25 |
| | **South-western** | | |
| Humidity | 0.525 | 0.002** | 27.5625 |
| Rainfall | 0.287 | 0.000** | 8.2369 |
| Temperature | -0.072 | 0.008** | 0.5184 |
| Speed of wind | 0.427 | 0.003** | 18.2329 |
| | **Eastern** | | |
| Humidity | -0.311 | 0.004** | 9.6721 |
| Rainfall | -0.619 | 0.019** | 38.3161 |
| Temperature | 0.576 | 0.002** | 33.1776 |
| Speed of wind | 0.586 | 0.001** | 34.3396 |

At R=1 and R=-1, the correlation coefficients of all the environmental factors with the pathogen indicate that all the environmental factors had a high correlation with *F. xylarioides* incidence for 2020 (Table 3). The correlation coefficients of the average relative humidity with *F. xylarioides* incidence ranged from -1 the Western region to 1 in the South-western. The correlation coefficients of the average rainfall with *F. xylarioides* incidence ranged from 0.528 in the Central region to 1 in the Western and South-western regions. The correlation coefficients of the average temperature with *F. xylarioides* incidence ranged from -1 in the South-western region to 1 in the Western region. The correlation coefficients of the average speed of wind with *F. xylarioides* incidence ranged from -1 in the South-western region to 1 in the Western region in the year 2020 (Table 3).

**Table 3:** Correlation coefficients of different environmental factors with *F. xylarioides* for the 3 coffee growing regions of Uganda for the year 2020.

| **Environmental factors** | **Correlation coefficients of environmental factors with *F. xylarioides*** | **P-Value(p**)** | **Coefficient of determination (%)** |
|---|---|---|---|
| | **Central** | | |
| Humidity | -0.442 | 0.027** | 19.5364 |
| Temperature | 0.402 | 0.003** | 16.1604 |
| Rainfall | 0.523 | 0.018** | 27.3529 |
| Speed of wind | 0.201 | 0.006** | 4.0401 |
| | **Western** | | |
| Humidity | -1.000 | 0.000** | 100 |
| Temperature | 1.000 | 0.000** | 100 |
| Rainfall | 1.000 | 0.000** | 100 |
| Speed of wind | 1.000 | 0.000** | 100 |
| | **South Western** | | |
| Humidity | 1.000 | 0.000** | 100 |
| Temperature | -1.000 | 0.000** | 100 |
| Rainfall | 1.000 | 0.000** | 100 |
| Speed of wind | -1.000 | 0.000** | 100 |

### 4.4 Genetic diversity within the *Fusarium xylarioides* population in Uganda over a 10-year period

The average number of alleles (Na) and the average effective number of alleles (Ne) were constant at 0.500 and 1.0 respectively for the 10-year period. The average Shannon's Information Index (I) at 0.000, average expected heterozygosity (He) at 0.000, average unbiased expected heterozygosity

(uHe) at 0.000, and average percentage of polymorphic loci (PPL) at 0.00% indicated 0.00% genetic variation in the *F. xylarioides* populations for the 10-year period, 2009 to 2019 (Table 4).

**Table 4:** Gene diversity indices of *F. xylarioides* populations for the 10-year period based on SSR markers analysis

| Year | Na | Ne | I | He | uHe | PPL |
|---|---|---|---|---|---|---|
| **2010** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **2012** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **2014** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **2017** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **2019** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Grand mean** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |

Na is the Number of alleles, Ne is the Effective number of alleles, I is the Shannon's Information Index, He is the Expected heterozygosity, uHe is the unbiased Expected heterozygosity, PPL is the percentage of polymorphic loci

### 4.4.1 Genetic variation within and among *Fusarium xylarioides* populations for the 10-year period

Analysis of molecular variance exhibited a non-significant (p=1.000) genetic variation within and among pathogen populations. Total variance was 0% within and among populations suggesting no observed genetic variation among and within *F. xylarioides* populations for the years 2009 to 2019 (Table 5).

**Table 5:** Analysis of molecular variance of *F. xylarioides* populations for the 10-year period

| Source of variation | Df | Sum of squares | Mean Square value | Est. Variance | Total variance | P (rand >= data) |
|---|---|---|---|---|---|---|
| **Among Populations** | 4 | 0.000 | 0.000 | 0.000 | 0% | 1.000 |
| **Within Populations** | 5 | 0.000 | 0.000 | 0.000 | 0% | 1.000 |
| **Total** | **9** | **0.000** | | **0.000** | **0%** | |

Df. degree of freedom, PhiPT is genetic divergence Probability, P (rand >= data), for PhiPT is based on standard permutation across the full data set

### 4.4.2 Genetic distance of Fusarium xylarioides populations for the 10-year period

Pairwise comparison of the *F. xylarioides* populations studied for the years 2010 to 2019 indicated 0.000 genetic distance (Table 6).

**Table 6:** Pairwise genetic distance matrix for the *F. xylarioides* populations for the 10-year period

| 2010 | 2012 | 2014 | 2017 | 2019 | |
|---|---|---|---|---|---|
| 0.000 | | | | | 2010 |
| 0.000 | 0.000 | | | | 2012 |
| 0.000 | 0.000 | 0.000 | | | 2014 |
| 0.000 | 0.000 | 0.000 | 0.000 | | 2017 |
| 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 2019 |

## 4.5 Genetic diversity among the *Fusarium xylarioides* populations from 3 coffee growing regions of Uganda

The average number of observed alleles (Na) within the populations varied from 0.5 for the 11 districts to 1.00 for Kassanda, while the effective number of alleles (Ne) within the populations varied from 1.000 for the 11 districts to 1.3554 for Kassanda. The expected heterozygosity varied from He 0.000 in the rest of the populations from other districts to He 0.207 in Kassanda making an average expected heterozygosity of He 0.017. The unbiased expected heterozygosity varied from uHe 0.000 to uHe 0.276 while the Shannon's information index (I) within the populations varied from 0.000 to 0.302 for the 11 districts and Kassanda respectively. The PPL within the populations varied from 0.00% for the 11 districts to 50.00% in Kassanda, with total average PPL of 4.17% for the 12 populations (Table 7).

**Table 7:** Gene diversity indices among *Fusarium xylarioides* populations from 12 coffee growing districts in Uganda based on SSR markers analysis in the year 2020

| District | Na | Ne | I | He | uHe | PPL |
|---|---|---|---|---|---|---|
| **Buikwe** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Mukono** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Luwero** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Kassanda** | 1.000 | 1.354 | 0.302 | 0.207 | 0.276 | 50.00% |
| **Masaka** | 0.500 | 1.000 | 0.000 | 0.00 | 0.000 | 0.00% |
| **Gomba** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Butambala** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Kayunga** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Ntungamo** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Rukiga** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Bundibugyo** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Kibaale** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Grand mean** | 0.542 | 1.029 | 0.025 | 0.017 | 0.023 | 4.17%% |

Na is the Number of alleles, Ne is the Effective number of alleles, I is the Shannon's Information Index, He is the Expected heterozygosity, uHe is the unbiased Expected heterozygosity, PPL is the percentage of polymorphic loci

The average number of observed alleles (Na) varied from 0.5 in the South-western and Western regions to 2.00 in the Central region, while the effective number of alleles (Ne) varied from 1.000 South-western and Western regions to 1.607 in the Central region. The expected heterozygosity varied from He 0.000 in the South-western and Western regions to He 0.342 Central region making an average observed gene diversity of He 0.114. The unbiased expected heterozygosity varied from uHe 0.000 in the South-western and Western regions to uHe 0.360 in the Central region while the Shannon's information index (I) varied from 0.00 in the Central to 0.513 in the South-western and Western. The PPL varied from 0.00% in the in the South-western and Western regions to 100.00% in the Central region, with an average PPL of 33.33% among the 3 regions in 2020 (Table 8).

**Table 8:** Gene diversity indices of *Fusarium xylarioides* populations from 3 coffee growing regions of Uganda based on SSR markers analysis for the year 2020

| Region | Na | Ne | I | He | uHe | PPL |
|---|---|---|---|---|---|---|
| **Central** | 2.000 | 1.607 | 0.513 | 0.342 | 0.360 | 100.00% |
| **South-western** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Western** | 0.500 | 1.000 | 0.000 | 0.000 | 0.000 | 0.00% |
| **Grand mean** | 1.000 | 1.202 | 0.171 | 0.114 | 0.120 | 33.33% |

Na is the Number of alleles, Ne is the Effective number of alleles, I is the Shannon's Information Index, He is the Expected heterozygosity, uHe is the unbiased Expected heterozygosity, PPL is the percentage of polymorphic loci

### 4.5.1 Genetic variation within and among *Fusarium xylarioides* populations from the 3 coffee growing regions of Uganda

Analysis of molecular variance exhibited a non-significant (p=1.000) genetic variation within and among pathogen population. The total variance observed was 0% among *F. xylarioides* populations suggesting no observed genetic variance among *F. xylarioides* populations from 3 coffee growing regions of Uganda for the year 2020. There was 100% observed total variance within *F. xylarioides* populations suggesting genetic variance among *F. xylarioides* populations from 3 coffee growing regions of Uganda for the year 2020 (Table 9).

**Table 9:** Analysis of molecular variance of *Fusarium xylarioides* populations from 3 coffee growing regions of Uganda for the year 2020

| Source of variation | Df | Sum of squares | Mean Square value | Est. Variance | Total variance | P (rand >= data) |
|---|---|---|---|---|---|---|
| **Among Populations** | 2 | 0.371 | 0.186 | 0.000 | 0% | 1.000 |
| **Within Populations** | 11 | 3.700 | 0.336 | 0.336 | 100% | 1.000 |
| **Total** | 13 | 4.071 | | 0.336 | 100% | |

Df. degree of freedom, Probability, P (rand >= data), for PhiPT is based on standard permutation across the full data set

### 4.5.2 Genetic distance of *Fusarium xylarioides* populations from the 3 coffee growing regions of Uganda

Pair-wise comparison of the *F. xylarioides* populations from the 12 coffee growing districts showed that the genetic distance varied in populations from Kassanda with populations from other

districts with observed genetic distances from 0.000 to 0.924. Pair-wise comparison of the genetic distance between populations revealed 0.000 genetic distance, with the exception of Kassanda (Table 10).

**Table 10:** Pairwise genetic distance matrix for the *Fusarium xylarioides* populations from 12 coffee growing districts in Uganda for the year 2020

| Buikwe | Mukono | Luwero | Kassanda | Masaka | Gomba | Butambala | Kayunga | Ntungamo | Rukiga | Bundibugyo | Buikwe |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | | | | | | | | | | | **Mukono** |
| 0.000 | 0.000 | | | | | | | | | | **Luwero** |
| 0.000 | 0.000 | 0.000 | | | | | | | | | **Kassanda** |
| 0.924 | 0.320 | 0.320 | 0.000 | | | | | | | | **Masaka** |
| 0.000 | 0.000 | 0.000 | 0.320 | 0.000 | | | | | | | **Gomba** |
| 0.000 | 0.000 | 0.000 | 0.924 | 0.000 | 0.000 | | | | | | **Butambala** |
| 0.000 | 0.000 | 0.000 | 0.320 | 0.000 | 0.000 | 0.000 | | | | | **Kayunga** |
| 0.000 | 0.000 | 0.000 | 0.320 | 0.000 | 0.000 | 0.000 | 0.000 | | | | **Ntungamo** |
| 0.000 | 0.000 | 0.000 | 0.320 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | **Rukiga** |
| 0.000 | 0.000 | 0.000 | 0.320 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | **Bundibugyo** |
| 0.000 | 0.000 | 0.000 | 0.320 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | **Kibaale** |

Likewise, for the 3 regions, pairwise comparison of the *F. xylarioides* populations showed that the pairwise genetic distance varied from 0.000 to 0.172. There was 0.000 pairwise genetic distance between other regions with the exception of the Central region population (Table 11).

**Table 11:** Pairwise genetic distance matrix for *Fusarium xylarioides* populations from 3 coffee growing regions in Uganda

| Central | South-western | Western | |
|---|---|---|---|
| 0.000 | | | Central |
| 0.172 | 0.000 | | South-western |
| 0.172 | 0.000 | 0.000 | Western |

## CHAPTER FIVE: SUMMARY, CONCLUSION AND RECOMMENDATIONS

### 5.1 Introduction

The aim of the study was to determine the effects of environmental factors and genetic diversity on the spread of *F. xylarioides*. The findings of the study revealed that the environmental factors were significant to the spread of the pathogen and no genetic diversity was observed for the isolates of the 10-year period and from the 3 coffee growing regions. There is therefore need of consistent annual data collection and storage, and use of a variety of molecular markers in a diverse study.

### 5.2 Summary

Environmental factors especially temperature, rainfall and speed of wind directly affect the migratory patterns of pathogen populations specifically fungal pathogens (Hunjan *et al*., 2020; Prakash *et al.*, 2015). In agreement to this, all the environmental factors studied including rainfall, relative humidity, temperature, and speed of wind, were found significant to the spread of *F. xylarioides* at 95% confidence interval ($p \leq 0.05$). Rainfall, at R (correlation coefficient) = 0.8680, p = 0.008 in the Central region (2000 to 2005), and R=1.000, p=0.000 in the Western and South-western regions (2020), was observed key in the spread of *F. xylarioides* for both time frames. Likewise, temperature at R= 0.8680, p = 0.000 in the Central region (2000 to 2005), R=1.000, p=0.000 in the Western region (2020), and R=-1.00, p=0.000 in the South-western region (2020) was observed key in the spread of the pathogen for both time frames. Relative humidity, at R= 0.760, p= 0.016 in the Western region (2000 to 2005), R=-1.000, p=0.000 in the Western region (2020), and R=1.000, p=0.000 in the South-western region (2020), was also found key in the spread of the pathogen for both time frames. This is in agreement to Rutherford, 2004 and Hakiza *et al.*, 2010 who noted increased pathogen incidence in the rainy season and attributed it to increased planting during the wet periods and the fact that the symptoms of the disease are not very recognizable so it is easy to transmit the disease when planting. Furthermore, it has been noted that increased rainfall and relative humidity cause an increase in fungal infection and colonisation of the plants, leading to increase in expression of symptoms (Lepoint, 2006; Prakash *et al.*, 2015; Rutherford, 2004). However, previous studies have noted no apparent effect of temperature on the spread of the pathogen (Phiri & Baker, 2009; Rutherford, 2004).

While the effect of speed of wind on the spread of the pathogen was significant for both time frames, the relationship of speed of wind with the pathogen varied for both time frames. Speed of

wind was therefore not considered a key factor. However, the speed of wind, at R=1.000, and p=0.000 for the South-western and the Western in 2020, was noted key in the spread of the pathogen in these regions for the year 2020. This is in agreement with Lepoint, 2006 who suggested that the ascospores and conidia of the pathogen are dispersed by wind.

Genetic analysis of the pathogen populations using SSR markers showed 0.00% genetic variability among *F. xylarioides* populations for both periods (2009 to 2019 and 2020) and 100% variations observed within populations in 2020. Genetic diversity indices demonstrated no genetic diversity within *F. xylarioides* populations with percentage polymorphism (PPL) at 0.000% and expected heterozygosity (He) at 0.000 for the 10-year period. Further analysis by AMOVA, at p=1.000, exhibited 0.000% genetic variation within and among populations suggesting no variability in the *F. xylarioides* population in Uganda for the 10-year period.

Genetic diversity indices of the 3 coffee growing regions demonstrated no genetic diversity within *F. xylarioides* populations in South-western and Western regions (0.000% PPL and 0.000 He) and 100% genetic diversity in the Central region. Results demonstrated 100.00% percentage polymorphism (PPL) and expected heterozygosity (He) at 0.342 in the Central region with genetic diversity in *F. xylarioides* populations from Kassanda (PPL 50.00% and He 0.207). Analysis by AMOVA, at p=1.000, exhibited 100.000% genetic variation within and 0.000% among populations. The genetic variation observed within the Central region, at p=1.000, is not significant. It was hypothesized that it was due to the fact that the isolates from that region specifically Kassanda were in advanced stages whereby as the infected plants die off the pathogen changes from the asexual to the sexual form thus the variation observed (Lepoint, 2006). These findings are in agreement with Rutherford, 2004 and Hakiza *et al.*, 2010 who found very limited and absence of genetic diversity within the existing populations of *F. xylarioides* from of East and Central Africa using AFLP and RAPD markers respectively.

### 5.3 Conclusion

In this study, environmental factors were found significant in the spread of *F. xylarioides* in Uganda, therefore the current spread is attributed to environmental changes in the coffee growing regions.

The study did not observe genetic diversity in the *F. xylarioides* population in Uganda over the 10-year period.

The study did not observe genetic diversity among the *F. xylarioides* populations from the 3 coffee growing regions of Uganda as of 2020.

### 5.4 Recommendations

In this study, environmental factors were found significant to the spread of the pathogen, however, studies of the effects of environmental factors on the pathogen incidence between 2009 to 2019 were not possible due to unavailability of data. Furthermore, the study area in 2020 was not sufficient to draw conclusions on the effects of environmental factors to the spread of the pathogen. Efforts should therefore be taken to ensure consistent 10-year pathogen incidence data collection and avail sufficient data for climate change studies on the pathogen. Studies should also be conducted to cover all the Robusta growing regions including areas where the pathogen has not been previously known to thrive.

Similarly, in this study, no genetic diversity of the *F. xylarioides* population was observed for both periods, in agreement to previous studies. However, it is highly unlikely that the two sets of SSR markers used were sufficient for the study to accurately or reliably reflect the existing genetic diversity. Therefore, there is need to conduct a similar study using a variety of molecular markers to better study the genetic diversity of the pathogen in Uganda.

### 5.5 Suggestions for Further Research

Forecasting models should be developed to study the effect of climate change on the spread of *F. xylarioides*.

Studies should be conducted to develop digitalised disease monitoring and forecasting applications of *F. xylarioides*.

Studies should be conducted using a whole genome comparison of the isolates from different years and regions in a diverse study of the diversity of the *F. xylarioides* populations in Uganda.

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## APPENDICES

| District | Region | Annual Average Rainfall (mm/day) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 |
| Mukono | Central | 1421 | 1743.82 | 1344.4 | 1344.36 | 1404.96 | 1007.9 |
| Luwero | Central | 1238.3 | 1507.25 | 1239.6 | 1239.63 | 1270.87 | 875.28 |
| Mpigi | Central | 1330.4 | 1531.92 | 1186.1 | 1186.09 | 1448.36 | 995.03 |
| Rakai | Central | 1114.27 | 1043.43 | 830.43 | 830.433 | 1350.89 | 944.51 |
| Sembabule | Central | 1195.37 | 1310.44 | 1010 | 1009.97 | 1432.13 | 942.42 |
| Masaka | Central | 1215.78 | 1205.81 | 944.93 | 944.927 | 1403.08 | 981.31 |
| **Average Rainfall** | **Central** | **1252.52** | **203.887** | **1092.6** | **1092.57** | **1385.05** | **957.73** |
| Mubende | Western | 1182.32 | 1400.7 | 1110.3 | 1110.26 | 1383.7 | 914.39 |
| Kiboga | Western | 1190.32 | 1385.63 | 1166.1 | 1166.13 | 1306.52 | 895.98 |
| Hoima | Western | 1022.43 | 1097.65 | 977.16 | 977.158 | 1093.87 | 838.78 |
| Masindi | Western | 963.936 | 1054.61 | 938.64 | 938.639 | 1024.68 | 783.58 |
| Kabarole | Western | 1188.49 | 1469.71 | 1187.3 | 1187.25 | 1313.57 | 1028.8 |
| Bundibugyo | Western | 1280.93 | 1512.61 | 1279.5 | 1279.47 | 1361.35 | 1103.5 |
| Kibaale | Western | 1101.71 | 1338.7 | 1068.3 | 1068.31 | 1261.63 | 878.42 |
| **Average Rainfall** | **Western** | **1132.88** | **1320.15** | **1103.9** | **1103.89** | **1249.33** | **920.5** |
| Mbarara | South-western | 977.232 | 1045.68 | 869.15 | 869.146 | 1251.42 | 935.51 |
| Bushenyi | South-western | 965.323 | 1042.27 | 900.08 | 900.077 | 1257.25 | 960.93 |
| Ntungamo | South-western | 960.53 | 998.526 | 917.09 | 917.094 | 1323.09 | 982.68 |
| Rukungiri | South-western | 1014.28 | 1040.9 | 995.34 | 995.342 | 1370.73 | 1047.9 |
| **Average Rainfall** | **South-western** | **979.341** | **1031.84** | **920.41** | **920.415** | **1300.62** | **981.75** |
| Iganga | Eastern | 1342.97 | 1747.49 | 1389.5 | 1389.47 | 1345.37 | 924.23 |
| Jinja | Eastern | 1398.6 | 1803.02 | 1395.8 | 1395.8 | 1376.26 | 951.62 |
| Kamuli | Eastern | 1223.37 | 1527.95 | 1258.2 | 1258.17 | 1230.38 | 854.45 |
| **Average Rainfall** | **Eastern** | **1321.65** | **1692.82** | **1347.8** | **1347.82** | **1317.34** | **910.1** |

**Appendix I:** Table showing the annual average rainfall data for the years 2000 to 2005 Source*:* The POWER project *(POWER | Data Access Viewer)*

| District | Region | Annual average relative humidity (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 |
| Mukono | Central | 66.642 | 70.809 | 69.904 | 67.825 | 69.044 | 65.317 |
| Luwero | Central | 65.784 | 71.903 | 69.879 | 66.908 | 68.85 | 63.157 |
| Mpigi | Central | 67.866 | 72.247 | 71.003 | 68.493 | 72.057 | 67.841 |
| Rakai | Central | 66.406 | 70.281 | 67.726 | 65.514 | 72.793 | 68.727 |
| Sembabule | Central | 68.091 | 74.092 | 71.824 | 68.377 | 74.83 | 69.107 |
| Masaka | Central | 66.046 | 69.301 | 67.556 | 65.75 | 70.412 | 67.089 |
| **Average Relative humidity** | **Central** | **66.958** | **71.439** | **69.648** | **67.145** | **71.331** | **66.873** |
| Mubende | Western | 67.535 | 74.504 | 72.711 | 68.676 | 74.085 | 67.131 |
| Kiboga | Western | 66.061 | 72.376 | 70.101 | 66.889 | 70.88 | 64.322 |
| Hoima | Western | 58.473 | 63.233 | 60.906 | 59.033 | 62.031 | 57.599 |
| Masindi | Western | 58.061 | 63.341 | 60.12 | 57.982 | 61.536 | 56.569 |
| Kabarole | Western | 66.811 | 72.94 | 70.951 | 68.797 | 70.087 | 65.66 |
| Bundibugyo | Western | 69.144 | 74.548 | 72.227 | 70.825 | 71.208 | 67.396 |
| Kibaale | Western | 64.438 | 71.348 | 69.476 | 65.805 | 69.713 | 63.327 |
| **Average Relative humidity** | **Western** | **64.361** | **70.327** | **68.071** | **65.429** | **68.506** | **63.144** |
| Mbarara | South-western | 64.049 | 69.294 | 66.315 | 64.91 | 70.802 | 66.989 |
| Bushenyi | South-western | 63.142 | 68.11 | 64.66 | 64.23 | 68.921 | 65.277 |
| Ntungamo | South-western | 64.713 | 69.397 | 66.508 | 66.205 | 71.734 | 68.461 |
| Rukungiri | South-western | 64.536 | 68.795 | 65.156 | 65.835 | 70.158 | 66.929 |
| **Average Relative humidity** | **South-western** | **64.11** | **68.899** | **65.66** | **65.295** | **70.404** | **66.914** |
| Iganga | Eastern | 63.826 | 70.91 | 69.285 | 66.324 | 66.555 | 60.787 |
| Jinja | Eastern | 66.209 | 72.283 | 71.129 | 68.25 | 68.746 | 63.735 |
| Kamuli | Eastern | 63.902 | 70.332 | 68.273 | 65.415 | 66.518 | 61.082 |
| **Average Relative humidity** | **Eastern** | **64.645** | **71.175** | **69.562** | **66.663** | **67.273** | **61.868** |

**Appendix II:** Table showing the annual average relative humidity data for the years 2000 to 2005

Source: The POWER project (*POWER | Data Access Viewer*)

| District | Region | Annual average temperature (°C) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 |
| Mukono | Central | 23.41 | 22.94 | 23.26 | 23.53 | 23.38 | 24.02 |
| Luwero | Central | 23.28 | 22.59 | 23.03 | 23.44 | 23.22 | 24.05 |
| Mpigi | Central | 22.73 | 22.27 | 22.6 | 23.02 | 22.45 | 23.2 |
| Rakai | Central | 21.76 | 21.46 | 21.92 | 22.3 | 21.24 | 21.92 |
| Sembabule | Central | 21.7 | 21.09 | 21.5 | 22.07 | 21.09 | 21.99 |
| Masaka | Central | 22.99 | 22.72 | 23.1 | 23.45 | 22.68 | 23.33 |
| **Average Temperature** | **Central** | **22.64** | **22.07** | **22.57** | **22.97** | **22.27** | **23.09** |
| Mubende | Western | 21.89 | 21.14 | 21.47 | 22.15 | 21.31 | 22.34 |
| Kiboga | Western | 22.82 | 22.14 | 22.57 | 23.09 | 22.5 | 23.46 |
| Hoima | Western | 25.24 | 24.71 | 25.19 | 25.6 | 25.05 | 25.75 |
| Masindi | Western | 25.06 | 24.48 | 25.09 | 25.52 | 24.91 | 25.64 |
| Kabarole | Western | 22.34 | 21.64 | 21.98 | 22.4 | 22.1 | 22.86 |
| Bundibugyo | Western | 22.14 | 21.54 | 21.93 | 22.24 | 22.05 | 22.73 |
| Kibaale | Western | 22.92 | 22.11 | 22.46 | 23.11 | 22.46 | 23.44 |
| **Average Temperature** | **Western** | **23.2** | **22.54** | **22.96** | **23.45** | **22.91** | **23.74** |
| Mbarara | South-western | 21.44 | 20.91 | 21.41 | 21.7 | 20.84 | 21.5 |
| Bushenyi | South-western | 22 | 21.47 | 22.04 | 22.18 | 21.45 | 22.12 |
| Ntungamo | South-western | 20.56 | 20.16 | 20.63 | 20.76 | 19.96 | 20.55 |
| Rukungiri | South-western | 21.32 | 20.91 | 21.5 | 21.49 | 20.8 | 21.41 |
| **Average Temperature** | **South-western** | **21.33** | **20.86** | **21.39** | **21.54** | **20.76** | **21.39** |
| Iganga | Eastern | 23.98 | 23.16 | 23.6 | 23.85 | 24 | 24.77 |
| Jinja | Eastern | 23.43 | 22.72 | 23.1 | 23.38 | 23.46 | 24.17 |
| Kamuli | Eastern | 23.83 | 23.1 | 23.57 | 23.89 | 23.86 | 24.6 |
| **Average Temperature** | **Eastern** | **23.75** | **22.99** | **23.42** | **23.71** | **23.77** | **24.51** |

**Appendix III:** Table showing the annual average temperature data for the years 2000 to 2005 Source: The POWER project (*POWER | Data Access Viewer*)

| District | Region | Annual average speed of wind (m/s) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 |
| Mukono | Central | 1.96262 | 1.80147 | 1.87379 | 2.04522 | 2.00272 | 2.13598 |
| Luwero | Central | 1.43962 | 1.35951 | 1.40507 | 1.5387 | 1.49815 | 1.57441 |
| Mpigi | Central | 2.0144 | 1.85085 | 1.92337 | 2.09291 | 2.03651 | 2.18026 |
| Rakai | Central | 1.7905 | 1.68388 | 1.7649 | 1.84529 | 1.77456 | 1.83001 |
| Sembabule | Central | 1.72356 | 1.57809 | 1.64728 | 1.75894 | 1.70998 | 1.79598 |
| Masaka | Central | 2.32382 | 2.18853 | 2.29004 | 2.43888 | 2.37914 | 2.48945 |
| **Average speed of wind** | **Central** | **1.87576** | **1.74373** | **1.81741** | **1.95332** | **1.90018** | **2.00102** |
| Mubende | Western | 1.58385 | 1.4713 | 1.51223 | 1.62562 | 1.61731 | 1.68763 |
| Kiboga | Western | 1.49989 | 1.43754 | 1.47231 | 1.59882 | 1.63324 | 1.63324 |
| Hoima | Western | 1.66225 | 1.63313 | 1.66244 | 1.80046 | 1.79137 | 1.82045 |
| Masindi | Western | 1.62229 | 1.55228 | 1.59345 | 1.78293 | 1.72139 | 1.79757 |
| Kabarole | Western | 1.25064 | 1.16447 | 1.18756 | 1.22208 | 1.30449 | 1.28629 |
| Bundibugyo | Western | 0.9576 | 0.90029 | 0.9243 | 0.93721 | 1.0034 | 0.98957 |
| Kibaale | Western | 1.60664 | 1.51617 | 1.52455 | 1.63758 | 1.68535 | 1.70689 |
| **Average speed of wind** | **Western** | **1.45474** | **1.38217** | **1.41097** | **1.51496** | **1.53665** | **1.56024** |
| Mbarara | South-western | 1.46204 | 1.35022 | 1.45769 | 1.48026 | 1.46811 | 1.48476 |
| Bushenyi | South-western | 1.26497 | 1.17402 | 1.29547 | 1.27789 | 1.29821 | 1.29766 |
| Ntungamo | South-western | 0.96799 | 0.88640 | 0.96838 | 0.96079 | 0.96293 | 0.97127 |
| Rukungiri | South-western | 1.04816 | 0.99394 | 1.10249 | 1.06319 | 1.08842 | 1.07514 |
| **Average speed of wind** | **South-western** | **1.18579** | **1.10114** | **1.20599** | **1.19553** | **1.20442** | **1.25123** |
| Iganga | Eastern | 1.47299 | 1.37126 | 1.41275 | 1.54887 | 1.4598 | 1.56254 |
| Jinja | Eastern | 1.54335 | 1.41737 | 1.45923 | 1.62098 | 1.55307 | 1.64731 |
| Kamuli | Eastern | 1.47555 | 1.39717 | 1.43327 | 1.57907 | 1.51154 | 1.60231 |
| **Average speed of wind** | **Eastern** | **1.4973** | **1.39527** | **1.43507** | **1.58297** | **1.50814** | **1.60405** |

**Appendix IV:** Table showing the annual average speed of wind data for the years 2000 to 2005

Source: The POWER project (*POWER | Data Access Viewer*)

| District | Region | Rainfall (mm/day) 2020 | Relative Humidity (%) 2020 | Temperature (°C) 2020 | Speed of wind (m/s) 2020 |
|---|---|---|---|---|---|
| Ntungamo | South-western | 1691.614548 | 79.34964466 | 19.70266128 | 0.920658499 |
| Rukiga | South-western | 1794.197981 | 79.97076743 | 19.01886259 | 0.834401037 |
| **Cumulative Averages** | | **1742.906264** | **79.66020605** | **19.36076193** | **0.877529768** |
| Buikwe | Central | 1940.111654 | 75.46341705 | 22.58088938 | 1.934224129 |
| Mukono | Central | 1848.122864 | 74.54613113 | 22.6685276 | 2.186873913 |
| Masaka | Central | 1514.59259 | 76.83500748 | 21.84814559 | 2.327067852 |
| Butambala | Central | 1663.575146 | 77.42263947 | 21.80103111 | 2.097458458 |
| Gomba | Central | 1559.016553 | 78.83914185 | 21.40181503 | 1.944720149 |
| Kassanda | Central | 1396.106885 | 79.85420303 | 21.098769 | 1.643209302 |
| Luwero | Central | 1552.759741 | 78.78479843 | 21.98556709 | 1.469326866 |
| Kayunga | Central | 1799.991199 | 78.20377808 | 22.11540298 | 1.485372019 |
| **Cumulative Averages** | | **1659.284579** | **77.49363956** | **21.93751847** | **1.886031586** |
| Bundibugyo | Western | 1681.808594 | 77.80485535 | 21.34820938 | 0.907231212 |
| Kibaale | Western | 1372.30542 | 78.44071198 | 21.14038467 | 1.648015022 |
| **Cumulative Averages** | | **1527.057007** | **78.12278366** | **21.24429703** | **1.277623117** |

**Appendix V:** Table showing the cumulative average of environmental factors of 3 coffee growing regions of Uganda for the year 2020. Source: The POWER project (*POWER | Data Access Viewer*)

| District | Region | *Fusarium xylarioides* Incidence (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 |
| Mukono | Central | 4 | 63 | 65 | 66 | 67 | 71 |
| Luwero | Central | 12 | 60 | 56.8 | 63 | 65 | 68.2 |
| Mpigi | Central | 5 | 62 | 57 | 65 | 66 | 68.5 |
| Rakai | Central | 1 | 5 | 6.5 | 12 | 13 | 12 |
| Sembabule | Central | 3 | 10 | 12.5 | 15 | 16 | 18 |
| Masaka | Central | 2 | 5 | 11.2 | 15 | 16 | 15 |
| **Cumulative incidence** | **Central** | **4.5** | **34.17** | **34.78** | **39.33** | **40.5** | **42.12** |
| Mubende | Western | 6 | 55 | 58 | 60 | 65 | 67.3 |
| Kiboga | Western | 8 | 50 | 54 | 56 | 60 | 68.2 |
| Hoima | Western | 9 | 40 | 50.3 | 56 | 57 | 52.5 |
| Masindi | Western | 7 | 35 | 45 | 46.2 | 47 | 47.5 |
| Kabarole | Western | 55 | 57 | 58.5 | 59 | 70 | 67.2 |
| Bundibugyo | Western | 75 | 82 | 85 | 85.5 | 90 | 90 |
| Kibaale | Western | 13 | 40 | 55 | 60 | 61 | 65.5 |
| **Cumulative incidence** | **Western** | **24.71** | **51.29** | **57.97** | **60.39** | **64.29** | **65.46** |
| Mbarara | South-western | 4 | 30 | 33.2 | 42 | 43 | 47.5 |
| Bushenyi | South-western | 5 | 25 | 30.4 | 40 | 44 | 48.2 |
| Ntungamo | South-western | 5 | 30 | 32.3 | 43 | 45 | 45.5 |
| Rukungiri | South-western | 45 | 55 | 56.9 | 65 | 70 | 68.5 |
| **Cumulative incidence** | **South-western** | **14.75** | **35** | **38.2** | **47.5** | **50.5** | **52.43** |
| Jinja | Eastern | 2 | 5 | 22.9 | 25 | 26 | 33 |
| Iganga | Eastern | 3 | 20 | 23.9 | 28 | 30 | 45 |
| Kamuli | Eastern | 3 | 10 | 22.9 | 24.5 | 25 | 26 |
| **Cumulative incidence** | **Eastern** | **2.67** | **11.67** | **23.23** | **25.83** | **27** | **34.67** |

**Appendix VI:** Table showing the cumulative average *Fusarium xylarioides* incidence for the years 2000 to 2005 Source: (UCDA, 2004 & UCDA, 2005)

| District | Region | Collecting locality | Latitude (N) | Longitude (E) | No of trees | No of symptomatic trees | *F. xylarioides* incidence |
|---|---|---|---|---|---|---|---|
| Ntungamo | South-western | Central division | -0.8687778 | 30.25441667 | 880 | 6 | 0.7 |
| Ntungamo | South-western | Central division | -0.8716111 | 30.24658333 | 220 | 1 | 0.5 |
| Ntungamo | South-western | Central division | -0.8706111 | 30.25400556 | 1320 | 15 | 1.1 |
| Ntungamo | South-western | Central division | -0.8836111 | 30.25680556 | 880 | 20 | 2.3 |
| Ntungamo | South-western | Eastern division | -0.9099167 | 30.26866667 | 90 | 7 | 7.8 |
| Ntungamo | South-western | Eastern division | -0.8983056 | 30.27255556 | 420 | 4 | 1.0 |
| Ntungamo | South-western | Eastern division | -0.877 | 30.29469444 | 110 | 0 | 0.0 |
| Ntungamo | South-western | Eastern division | -0.8423889 | 30.28361111 | 1100 | 2 | 0.2 |
| Rukiga | South-western | Kamwesi | -1.1840278 | 30.18402778 | 410 | 0 | 0.0 |
| Rukiga | South-western | Kamwesi | -1.2421667 | 30.21133333 | 8 | 1 | 12.5 |
| Rukiga | South-western | Kamwesi | -0.8423889 | 30.28361111 | 240 | 0 | 0.0 |
| Rukiga | South-western | Kamwesi | -1.2420278 | 30.20033333 | 7 | 1 | 14.3 |
| Rukiga | South-western | Kamwesi | -1.241 | 30.20205556 | 206 | 0 | 0.0 |
| Rukiga | South-western | Kamwesi | -1.239222 | 30.20386111 | 30 | 1 | 3.3 |
| Rukiga | South-western | Kamwesi | -1.2159722 | 30.13333333 | 400 | 1 | 0.3 |
| Buikwe | Central | Buikwe TC | 0.3335555 | 33.02258333 | 2200 | 506 | 23.0 |
| Buikwe | Central | Buikwe | 0.3159722 | 33.04777778 | 820 | 30 | 3.7 |
| Buikwe | Central | Buikwe | 0.3103888 | 33.02311111 | 220 | 1 | 0.5 |
| Mukono | Central | Ntenjeru | 0.2068611 | 32.77166667 | 780 | 7 | 0.9 |
| Mukono | Central | Ntenjeru | 0.20369444 | 32.76461111 | 390 | 8 | 2.1 |
| Masaka | Central | Kyanamukaka | -0.478 | 31.66933333 | 200 | 0 | 0.0 |
| Masaka | Central | Kyanamukaka | -0.4899444 | 31.67175 | 400 | 5 | 1.25 |
| Masaka | Central | Kyanamukaka | -0.498722 | 31.68055556 | 810 | 1 | 0.1 |
| Masaka | Central | Kyanamukaka | -0.5060556 | 31.70633333 | 830 | 1 | 0.1 |
| Masaka | Central | Lwankoni | -0.5295278 | 31.69961111 | 430 | 4 | 0.9 |
| Masaka | Central | Buwunga | -0.4451389 | 31.76361111 | 450 | 2 | 0.4 |
| Masaka | Central | Buwunga | -0.4265833 | 31.78191667 | 1700 | 40 | 2.4 |
| Masaka | Central | Buwunga | -0.4043889 | 31.78952778 | 610 | 0 | 0.0 |
| Masaka | Central | Buwunga | -0.3775278 | 31.7825 | 400 | 0 | 0.0 |
| Masaka | Central | Buwunga | -0.378833 | 31.78241667 | 810 | 2 | 0.2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Butambala | Central | Budde | 0.13475 | 32.18527778 | 210 | 0 | 0.0 |
| Butambala | Central | Budde | 0.1502778 | 32.17655556 | 200 | 0 | 0.0 |
| Butambala | Central | Budde | 0.17075 | 32.18661111 | 3010 | 753 | 25.0 |
| Butambala | Central | Budde | 0.1835556 | 32.18733333 | 220 | 3 | 1.4 |
| Butambala | Central | Budde | 0.193 | 32.18844444 | 230 | 10 | 4.3 |
| Gomba | Central | Ntenja | 0.2254444 | 32.04927778 | 315 | 1 | 0.3 |
| Gomba | Central | Mpenja | 0.21227778 | 32.02772222 | 530 | 0 | 0.0 |
| Gomba | Central | Mpenja | 0.19930556 | 32.00063889 | 410 | 0 | 0.0 |
| Gomba | Central | Kigonza | 0.1927778 | 31.96725 | 200 | 0 | 0.0 |
| Gomba | Central | Kyegonza | 0.18180556 | 31.94613889 | 415 | 0 | 0.0 |
| Kassanda | Central | Mwanzi | 0.49322222 | 31.87422222 | 820 | 33 | 4.0 |
| Kassanda | Central | Nalutuntu | 0.50991667 | 31.85986111 | 420 | 0 | 0.0 |
| Kassanda | Central | Nalutuntu | 0.52038889 | 31.84747222 | 2975 | 5 | 0.2 |
| Kassanda | Central | Town council | 0.54619444 | 31.82094444 | 430 | 1 | 0.2 |
| Kassanda | Central | Town council | 0.55386111 | 31.80166667 | 100 | 5 | 5.0 |
| Kassanda | Central | Kassanda | 0.55294444 | 31.77730556 | 410 | 2 | 0.5 |
| Kassanda | Central | Kassanda | 0.55075 | 31.75841667 | 500 | 16 | 3.2 |
| Kassanda | Central | Kalwana | 0.55261111 | 31.72877778 | 110 | 0 | 0.0 |
| Kassanda | Central | Kalwana | 0.56158333 | 31.70975 | 420 | 0 | 0.0 |
| Kassanda | Central | Kalwana | 0.57697222 | 31.69055556 | 200 | 1 | 0.5 |
| Luwero | Central | Butuntumula | 0.86266667 | 32.51927778 | 168 | 1 | 0.6 |
| Luwero | Central | Butuntumula | 0.86558333 | 32.51091667 | 66 | 3 | 4.5 |
| Luwero | Central | Butuntumula | 0.87702778 | 32.50916667 | 200 | 5 | 2.5 |
| Luwero | Central | Butuntumula | 0.87175 | 32.51833333 | 210 | 6 | 2.9 |
| Luwero | Central | Butuntumula | 0.86322222 | 32.52433333 | 2500 | 0 | 0.0 |
| Luwero | Central | Zirobwe | 0.78805556 | 32.66894444 | 180 | 2 | 1.1 |
| Luwero | Central | Zirobwe | 0.76616667 | 32.67586111 | 98 | 2 | 2.0 |
| Luwero | Central | Zirobwe | 0.7479444 | 32.681 | 300 | 0 | 0.0 |
| Luwero | Central | Zirobwe | 0.6991111 | 32.69508333 | 380 | 0 | 0.0 |
| Luwero | Central | Zirobwe | 0.6944444 | 32.71377778 | 400 | 2 | 0.5 |
| Kayunga | Central | Kangulumira | 0.59686111 | 33.04755556 | 620 | 0 | 0.0 |
| Kayunga | Central | Kangulumira | 0.59638889 | 33.04052778 | 440 | 1 | 0.2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Kayunga | Central | Kangulumira | 0.5893333 | 33.03080556 | 210 | 0 | 0.0 |
| Kayunga | Central | Town council | 0.59680556 | 33.01341667 | 390 | 0 | 0.0 |
| Kayunga | Central | Kangulumira | 0.60825 | 33.00480556 | 200 | 0 | 0.0 |
| Kayunga | Central | Kayunga | 0.68130556 | 32.88116667 | 405 | 0 | 0.0 |
| Kayunga | Central | Kayunga | 0.68227778 | 32.94233333 | 600 | 0 | 0.0 |
| Kayunga | Central | Kayunga | 0.68188889 | 32.94811111 | 760 | 0 | 0.0 |
| Kayunga | Central | Kayunga | 0.68355556 | 32.93205556 | 616 | 1 | 0.2 |
| Kayunga | Central | Kayunga | 0.68127778 | 32.92463889 | 208 | 0 | 0.0 |
| Bundibugyo | Western | Bundibugyo | 0.6545 | 30.0114444 | 13 | 6 | 46.153846 |
| Kibaale | Western | Kibaale | 1.3653611 | 31.2686944 | 33 | 5 | 15.151515 |
| **Average CWD Incidence** | | | | | | | **2.8** |

**Appendix VI:** Table showing sites sampled for *Fusarium xylarioides* populations in Uganda and number of symptomatic trees collected for the year 2020