DISTRIBUTION, PATHOGEN CHARACTERISATION AND MANAGEMENT OF BRACHIARIA GRASS DISEASES IN RWANDA

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DECLARATION

This thesis is my original work and has not been submitted for award of a degree in any other University.

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DEDICATION

This Ph.D. thesis work is dedicated to my loving Husband Dr. Ndabamenye Telesphore and my precious Children Umuhire Paladi, Kwizera Eloi Anointed, Sibomasimbi Marie Gisele and Mugisha Louis Marie de Montfort whose love, prayers, encouragement, and invaluable support have enriched my soul and inspired me to pursue and complete this research. I also dedicate this work to my parents for their unbelievable and unforgettable support in my education.

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LIST OF ABBREVIATIONS AND ACRONYMS

%	Percentage
°C	Degree Celsius
µg	Microgramme
µM	Micromole
AEZ	Agro-ecological zone
AG	Anastomosis group
ANOVA	Analysis of variance
ATFGRC	Australian Tropical Forage Genetic Resources Centre
AUDPC	Area under the disease progress curve
BecA	Biosciences eastern and central Africa
BLAST	Basic Local Alignment Search Tools
bp	Base pair
BR	Bugesera
BS	*Bipolaris secalis*
BUSCO	Benchmarking Universal Single Copy Ortholog
BWA	Burrows-Wheeler Aligner
Ca	Calcium
CABI	Centre for Agriculture Biosciences International
CENARGEN	Centro Nacional de Recursos Geneticos e Biotechnologia of EMBRAPA
CIAT	International Center for Tropical Agriculture
CO_2	Carbon dioxide
Cv.	Cultivar
DIN	Deoxyribonucleic acid Integrity Number
DNA	Deoxyribonucleic acid
DM	Dry matter
DSMV	*Digitaria striate mosaic virus*
EDTA	Ethylenediaminetetraacetic acid
EAC	East Africa Community
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
g	Gramme

GAPDH	Gglyceraldehyde-3-phosphate dehydrogenase gene
GBK	Genebank of Kenya
GC	Guanine-Cytosine
gDNA	Genomic Deoxyribonucleic acid
GDP	Gross domestic product
GGMV	Guinea grass mosaic virus
GIS	Geographic information system
GPS	Global positioning system
HY	Huye
IFC	Immunofluorescence colony
ILCA	International Livestock Centre for Africa
ILRI	International Livestock Research Institute
IMS	Immunomagnetic separation
IP	Incubation period
ISAR	Rwanda Agricultural Research Institute
ITS	Internal transcribed spacer
JGMV	Johnson grass mosaic virus
KALRO	Kenya Agricultural and Livestock Research Organization
LSD	Least Significant Difference
LSU	Large subunit of nuclear ribosomal RNA
m.a.s.l	Meter above sea level
MB	Megabyte
MDMV	Maize dwarf mosaic virus
MINAGRI	Ministry of Agriculture and Animal Resources
ml	Millilitre
MSV	Maize streak virus
NPS	Nucleotide polymorphism
N	Nitrogen
NA	Nutrient agar
NaOCl	Sodium hypochlorite
NCBI	National centre for biotechnology information
NGS	Next generation sequencing
NJ	Neighbour-joining

ns	Not significant
NY	Nyamagabe
ONT	Oxford Nanopore Technology
PacBio	Pacific Biosciences
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
Ph.D.	Doctor of Philosophy
PSTA	Strategic plan for the transformation of agriculture sector
QC	Quality check
qPCR	Quantity polymerase chain reaction
RAB	Rwanda Agriculture and Animal Resources Development Board
RAPD	Random Amplified Polymorphic DNA
RCBD	Randomised complete blocks design
rep-PCR	Repetitive extragenic palindromic-PCR genomic fingerprinting
RFLP	Restriction Fragment Length Polymorphism
RGI/ARC	Roodeplaat Grassland Institute of the African Research Council
RN	Rwamagana
RPM	Revolutions per minute
SCMV	Sugarcane mosaic virus
SDW	Sterile Distilled Water
Sida	Swedish International Development Cooperation Agency
SNP	Single-nucleotide polymorphism
SSA	Sub-Saharan Africa
T	Tiller
TAE	Tris-acetate-EDTA
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture

ABSTRACT

The genus *Brachiaria* (syn. *Urochroa*) is ranked among the high-quality nutritious forages that originate from Africa. It remains a favourite forage for Sub-Saharan Africa due to different traits including high quantity and quality of its biomass. However, diseases were reported as one of the major constraints of *Brachiaria* production worldwide. The general objective of the study was to increase livestock productivity and improve income of farmers through sustainable management of diseases affecting *Brachiaria* grass in Rwanda. The study analysed the effect of growing seasons on distribution, disease incidence and severity of *Brachiaria* grass diseases in prevailing climatic conditions of Rwanda. Disease surveys were conducted in five districts during the dry season and the wet season in 2018 and 2019. Surveys showed that leaf spot, leaf rust and leaf blight diseases were largely distributed across the country. Incidence and severity of these diseases differed significantly ($p \leq 0.05$) by districts, seasons, and district × season interactions, however, exception was non-significant effect of season and district × season interactions on leaf rust incidence in 2018. Furthermore, isolation and confirmation of causative relationship between *Bipolaris secalis*/ *Phakopsora apoda* and leaf spot/leaf rust were evaluated. *Brachiaria* leaf samples with disease symptoms were taken from farmers' fields during the wet season of the year 2018. Fungi associated with major diseases were isolated and identified based on morphological, molecular characteristics and pathogenicity tests. Molecular identification confirmed the results of morphological identification and revealed *Phakopsora apoda* as the only fungus associated with leaf rust, and predominant association of fungi *Epicoccum* spp. and *Nigrospora* spp. with leaf blight while *Bipolaris secalis* and *Fusarium* spp. were associated with leaf spot symptoms. Morphological, molecular identification and symptoms reproduced on inoculated *Brachiaria* seedlings confirmed *Bipolaris secalis* and *Phakopsora apoda* as causal agents of leaf spot and leaf rust, respectively. Moreover, whole genome sequencing and genomic characterisation of *Bipolaris secalis* isolates from *Brachiaria* grass (Humidicola and Basilisk.) grown in Bugesera, Nyagatare, Rwamagana and Huye districts were performed. Illumina platform to give 151 bp reads in paired – end sequencing was used. The phylogenomic relationships of 12 isolates was also constructed and de novo assembly of one isolate (BS7) was performed. Eleven isolates were re-sequenced based on BS7 and they were mapped to the reference (BS7). Illumina sequencing results of BS7 produced the estimated genome size of 34,813,291 bp with an average GC content of 50.01%, organised into 108 contigs with the longest contig of 2,265,317 bp, the N50 of 1,032,497 bp and the L50 of 12.

The self-mapping of BS7 was 97.69%. The results obtained when mapping dataset of 11 isolates to BS7 indicated that the final mapping ratio was in the range of 80 – 95%, consisting of 28,950,637 – 15,611,348 total mapped reads. Finally, field experiments were established to evaluate management options against foliar diseases affecting *Brachiaria* grass in Rwanda. One study evaluated the reaction of nine improved *Brachiaria* cultivars (Marandu, MG 4, Piata, Xaraes, Basilisk, Humidicola, Cayman, Cobra and Mulato II) against leaf rust, leaf spot and leaf blight diseases and agronomic performances in two agro-ecological zones of Rwanda. Experiments were established under natural disease pressure for three distinct consecutive harvests in 2019 and 2020. Analysis of variance (ANOVA) was performed for data on disease and agronomic parameters of *Brachiaria* grass and Pearson correlation analysis was used to determine the relationships between agronomic traits and the extent of the diseases expressed as Area Under Disease Progress Curve (AUDPC). Basilisk, Marandu, MG4 and Xaraes exhibited moderately resistant to resistant response to all three diseases but Cayman and Cobra were the most susceptible to leaf rust. Cultivars differed for biomass production ($p \leq 0.05$), as well as percentage of dry matter content. The highest biomass producers were Marandu, Mulato II and Xaraes but a high percentage of dry matter content was registered for Cayman and Cobra. The interaction of between site, cultivar and harvest was evident ($p \leq 0.05$) for disease development and agronomic performances. Moreover, an experiment was established to evaluate different management options including mineral fertiliser application, fungicide application, manual weeding, no fungicide application, no weeding, and no fertiliser application for leaf rust using the susceptible cultivar (*Brachiaria* hybrid cv. Cayman) for four consecutive harvests. The results showed a significant reduction in the incidence and severity of leaf rust as a result of mancozeb and mineral fertiliser treatments, leading to simultaneous increases in plant growth, number of tillers and biomass production. The findings of the study provide baseline information on diseases affecting *Brachiaria* grass in Rwanda. Farmers can use resistant cultivars identified in this study and they can be explored for further use in breeding programmes. The information generated in the study is therefore, useful for sustainable management of diseases affecting *Brachiaria* grass and other crops in Rwanda and in the Sub-Saharan Africa region. Likewise, this is the first study to provide a whole genome sequence of *Bipolaris secalis* hence, the generated genome data under this study will contribute to the database improvement of *Bipolaris secalis* for future investigation and it will contribute to identification of novel sources of genetic resistance for improving disease management in *Brachiaria* grass and other new strategies for the control of this pathogen.

CHAPTER ONE: INTRODUCTION

1.1 Background

Rwanda is one of the seven countries of East African Community (EAC). It is a tropical landlocked country and its geographical location lies between 1° to 3° South and 28° to 31° East (Karamage *et al.*, 2017). The country borders with Burundi to the South, Uganda to the North, Tanzania to the East and the Democratic Republic of Congo to the West. With the total surface area of 26,338 km^2 and around 11,262,564 population, about 83% of the later live in rural areas. It has the highest population density in the region with an average of 467 inhabitants per km^2 (NISR, 2017). The economy of Rwanda depends on three main sectors, namely agriculture, industry and services. Most people in Rwanda rely on agriculture sector for food, income and employment. About 90 percent of food demand in nation is produced by agriculture sector and it generates 90 percent of employment, especially for women (Rwirahira, 2009). According to the 2017 Agriculture household survey, Rwanda has an estimated 2.1 million agricultural households, constituting about 80.2 percent of all households in the country. This means that 9.7 million Rwandans reside in agricultural households, with a working population of 5.4 million people (NISR, 2017).

In Rwanda, the agriculture sector remains a significant source of comparative advantages for national economy and it contributes about a third of the Gross Domestic Product (GDP) whereby the contribution of animal resources sub-sector is 3.5 % of agricultural GDP (MINAGRI, 2015; NISR, 2017). In addition to income generation, livestock is a source of nutritious food such as meat, milk and eggs, manure and power for plant cultivation (Bazarusanga, 2008). About 70 percent of the population of Rwanda own livestock with the average of one to three cows per family. Despite the importance of the agriculture sector in the economy of Rwanda, the agriculture situation analysis indicated the low productivity and volatility of the agriculture sector. The indicators of agricultural poor performance include low use of agricultural inputs, vulnerability to weather related shocks, subsistence production system, lack of value addition and export barriers (Rwirahira, 2009). Both crop and livestock were shown to be below production potential due to several challenges including limited lands and adverse effects of climate change.

Challenges associated with livestock production such as poor quality and quantity of animal feeds, small land holding and pest and diseases are common in East African countries including Rwanda, Kenya and Uganda (Lukuyu, 2009; MINAGRI, 2018). The consumption of animal source food in Rwanda is reported to be below the Food and Agriculture Organization of the United Nations (FAO) recommendations of 50 kg meat and 200 L milk per capita per year, respectively. Consistent deficits of almost all livestock products were reported in Burundi and Rwanda. In 2004, only 40% and 41% demand for meat and milk, respectively, was met by domestic productions. The remaining amount was met through imports. Despite the significant contribution of livestock to food and nutrition security, South Asia and Sub-Saharan Africa have high numbers of poor livestock keepers with a global increase of 1.4 percent per year. The number of livestock keepers in Eastern Europe and Central Asia was at 3.7% per year (FAO, 2012). The Government of Rwanda has been implementing different programmes to support livestock sub-sector including the home-grown solution so-called "One Cow Per Poor Family" (Girinka) Programme and the Intensification of Livestock (MINAGRI, 2015). The Girinka programme (translated as "may you have a cow") was initiated in Rwanda in 2006 to reduce child malnutrition rates and to increase income of vulnerable poor families. Prerequisites to benefit from Girinka programme include not having any cow, availability of a cowshed or suitable building, having 0.25 to 0.75 ha of land for forage production, being a trusted and poor person in the community and lack of other sources of income (Beyi, 2016). Rwanda's Strategic Plan for the Transformation of Agriculture sector (PSTA phase 4) highlights the need for both public and private sector supports and financial investments in both crop and animal resources production during the period from 2018 to 2024. On the livestock side, apart from genetic improvement and animal health, ensuring the availability of enough animal feed while analysing animal feed production systems is of high priority. The emphasis is put on the improvement of animal feeding and increasing on-farm crop-livestock integrated outputs (MINAGRI, 2018). In order to increase food conversion efficiency and reduce input costs, it is important to promote the production and processing of improved fodder crops at farm level. The above strategy ensures that the local community shall have access to improved fodder seeds and planting materials, and this improves animal feeding through commercial improved fodder production. The niches for improved grass cultivation include farm hedge backyard, fodder banks, live fences at the farm and fallows. Achievement of above mission requires diversification of forage production, forage improvement and management of forages pests and diseases.

Limited availability and low-quality animal feed, especially in dry seasons is one of the main challenges of livestock production in Kenya (Njarui *et al.*, 2016) and it holds true for Rwanda (MINAGRI, 2018). The use of non-conventional feeds such as banana leaves and kitchen leftovers were reported in Rwanda (Mutimura *et al.*, 2013).

1.2 Problem statement

Livestock productivity is far below the genetic potential in Rwanda. For instance, milk production is estimated at 300 litres per cow per lactation compared to 2−4 litres per cow per day (610 – 1,220 liters per lactation) for cows provided under Girinka Programme (Klapwijk *et al.*, 2014). As a result, the domestic production meets about 40 percent of meat and milk demand, and the rest has been met through import. Limiting factors of low livestock productivity include use of crop residues that are of low nutritive values and shortage of feed, which mostly occurs during the dry growing period. Even though a lot of efforts were put in place for improvement of forage productivity and quality in Rwanda, feed shortage in wet and dry seasons is still a challenge for farmers (Mutimura *et al.*, 2013).

Some studies reported Napier grass as the most frequently used animal feed in the region but it is seriously affected by Napier stunt disease. This becomes a big problem and negatively affects livestock production (Lukuyu *et al.*, 2009; Umunezero *et al.*, 2016). Considering the susceptibility of Napier grass to stunt disease and prolonged dry spells in East Africa, *Brachiaria* grass has become potential forage to overcome the challenge of lack of quantity and quality feed for livestock sub-sector (Machogu, 2003). However, diseases affecting *Brachiaria grass* in Rwanda have not studied so far. Elsewhere, several diseases of *Brachiaria* were reported and they may cause substantial yield loss (Nzuoki *et al.*, 2016). Foliar blight caused by *Rhizoctonia* spp was reported to reduce the production of about 50% in the tropics (Alvarez *et al.*, 2013). Yield losses caused by leaf rust are very high and can reach up to 100 percent and the reduction of crude proteins of *Brachiaria humidicola* leaves was reported to be between 49 – 43%. Furthermore, the availability of other nutrients was shown to be highly affected even at infection of 5% of leaf area, resulting to negative impact on livestock production (Lenné and Trutman, 1994). Other reported diseases include ergot, bacterial blight, leaf spot, smut and viral diseases and their presence in Africa is documented. They can reduce the production of *Brachiaria* grass (Miles *et al.*, 1996; Ramirez *et al.*, 2015).

The *Brachiaria* cultivars grown in Africa were selected and improved in other continents without their native diseases and pests. Thus, they may be highly vulnerable to the native pests and pathogens in Africa. Within a few years of introduction, these *Brachiaria* cultivars have suffered from different diseases often at high levels. Moreover, there is a very little understanding of diseases that affect *Brachiaria* grass in Africa. As there is an increase in *Brachiaria* grass cultivation over years, an increase in the disease incidence and severity can also be anticipated. It warrants enriching the understanding on diseases of *Brachiaria* grass and development of effective management strategies.

1.3 Justification

A large number of livestock farmers in the East African region are relying on traditional natural pastures and mostly use Napier grass as the main source of fodder for their dairy production (Klapwijk *et al.*, 2014). Role of cultivated pasture is very important for sustainable livestock production and intensification of the livestock sector. Over the years, Governmental and non-governmental organizations across East Africa have been popularising and promoting Napier grass as an important fodder source. Unfortunately, Napier grass has been attacked by two major diseases including Napier Stunt and Smut diseases and the incidence and severity of both diseases are high in Rwanda (Nyiransengimana *et al.*, 2015). These diseases reduce forage quality and biomass production (Lenné and Trutmann, 1994). Therefore, it is very important to develop effective disease management strategies for sustainable productivity of pastures (Lenné and Trutmann, 1994).

Introduction of forages has been a major effort towards pasture improvement for many decades in Africa. Many national programmes have given high priority to introduce improved forages and diversifying the forage species cultivation. It has been quite important to mitigate the negative effect of Napier diseases on livestock feed availability in the region. Rwanda Agricultural Research Institute (ISAR) initiated research on *Brachiaria* grass in partnership with the International Center for Tropical Agriculture (CIAT) in 2006. Since then, productivity trials of improved *Brachiaria* cultivars (*Brachiaria decumbens* cv. Basilisk, *Brachiaria brizantha* cv. Toledo, *Brachiaria brizantha* cv. Marandu, *Brachiaria* hybrid cv. Mulato, *Brachiaria* hybrid Bro2/0465, *Brachiaria* hybrid Bro2/1452 and *Brachiaria* hybrid Bro2/1485) were initiated (CIAT, 2010). Through International Livestock Research Institute (ILRI)'s climate smart *Brachiaria* grass programmes, new *Brachiaria* cultivars were introduced to Rwanda and evaluated in different agro-ecological zones for adaptation.

The importance and gains of *Brachiaria* grass on dairy productivity were documented, and *Brachiaria* grass was successfully integrated and promoted into mixed crop-livestock systems (Ghimire *et al.*, 2015). *Brachiaria* grass is known as a forage with good attribute to produce high amount of above ground biomass with superior forage quality. About 70 percent of the population of Rwanda own livestock, which is the result of national and strategic livestock programmes including Girinka (One Cow per poor family) and the intensification of livestock activities. These programmes require promotion and sustainable production of forage with good quality for livestock feeding. Previous studies on *Brachiaria* grass adaptability have demonstrated that different *Brachiaria* cultivars perform well in distinct agro-ecologies of Rwanda with differences in rainfall, altitude and temperature and contribute significantly to the availability of livestock feed all year round for dairy farmers including the dry growing season (Mutimura and Everson, 2012).

Feeding animals on *Brachiaria* grasses showed increase in milk and meat production by 35% and 44%, respectively (Ghimire *et al.*, 2015; Mutimura *et al.*, 2018). Mutimura and Everson (2012) revealed that farmers in Nyamagabe and Bugesera districts appreciated *Brachiaria* grass as forage with good quality. *Brachiaria* grass is now a preferred forage option to thousands of farmers in Rwandan agro-ecologies as well as in Sub-Saharan Africa region (SSA). High biomass production, drought tolerance, high palatability and nutritive values, improvement in soil fertility, significant increase in livestock productivity and positive impact on environmental are major attributes that make *Brachiaria* grass a preferred forage in SSA. This grass is also being used in push pull system for controlling pests and parasitic weed striga (ICIPE, 2017).

The susceptibility of improved *Brachiaria* grass bred in South America to pest and diseases present in East Africa was reported (Ghimire *et al.*, 2015). Therefore, the expansion of acreage in SSA, the native home of *Brachiaria* grass, makes this grass vulnerable to diseases and insect pests. Since 2013, when improved *Brachiaria* varieties were introduced by the International Livestock Research Institute, various *Brachiaria* diseases including leaf rust, leaf spot, leaf blight and smut have been reported (Nzioki *et al.*, 2016). As of now, there is a very little understanding on *Brachiaria* diseases in Africa. As the acreage under *Brachiaria* pasture is increasing over years, an increase in the diseases and pest incidence and their possible outbreak is anticipated. Understanding diseases affecting *Brachiaria* grass and associated pathogens is very important for sustainable production of qualitative and quantitative biomass.

1.4 Objectives of the study

1.4.1 Overall objective

The overall objective of the study was to increase livestock productivity and improve income of farmers through sustainable management of diseases affecting *Brachiaria* grass in Rwanda.

1.4.2 Specific objectives

The specific objectives were:

i. To determine the distribution, incidence and severity of *Brachiaria* grass diseases in Rwanda.
ii. To isolate and confirm causative relationship between two major fungal pathogens (*Bipolaris secalis* and *Phakopsora apoda*) and leaf spot and leaf rust.
iii. To determine the whole genome sequence and genomic characterisation of one pathogen (*Bipolaris secalis*).
iv. To evaluate the reaction to foliar diseases and agronomic performances of improved *Brachiaria* (*Urochloa*) grass cultivars.
v. To evaluate management options of one major disease (Leaf rust) affecting *Brachiaria* grass in Rwanda.

1.5 Hypotheses

i. In Rwanda, *Brachiaria* grass diseases are widespread with varying incidence and severity.
ii. *Brachiaria* grass diseases are associated with different organisms with high diversity.
iii. Diseases significantly reduce *Brachiaria* grass forage quality and biomass yield.
iv. Integrated disease management techniques including pesticide application reduce significantly the incidence and severity of *Brachiaria* leaf rust.

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin and classification of *Brachiaria* grass

The genus *Brachiaria* consists of a large number of species (Over 100 species are documented), that are found in most of the tropical regions of Africa. Even though Africa is reported as the centre of both origin and diversity of *Brachiaria* grass, improvement of this genus was done outside Africa especially in America and Australia (Maass *et al.*, 2015). It belongs to the *Poaceae* family. It is mostly under extensive cultivation in the tropics of Central and Southern America regions. Seven *Brachiaria* species of African origin including *Brachiaria decumbens, Brachiaria humidicola, Brachiaria ruziziensis, Brachiaria brizantha, Brachiaria mutica, Brachiaria arrecta* and *Brachiaria dictioneura* are used as forages. *Brachiaria* species were classified in nine groups based on morphology of inflorescence and panicle (Renvoize *et al.*, 1996). However, 14 species were reported ungrouped where nine species are from Africa, two species from America, one species from India and Southeast Asia and two species from Australia (Miles *et al.*, 1996). The figure 2.1 indicates photos of different cultivars of *Brachiaria* grass (*Brachiaria brizantha*).

Figure 2.1: Photos of different cultivars of *Brachiaria brizantha*. A: Piata, B: Xaraes (Toledo), C: MG4 and D: Marandu. Photos were taken in Rubona Research Station, Rwanda Agriculture and Animal Resources Development Board, November 2019.

2.2 Distribution and production of *Brachiaria* grass

Brachiaria grass species are distributed in all tropics and its intercontinental distribution was found related to its adaptability to several types of soils including poor and acidic soils (Renvoize *et al.*, 1996). *Brachiaria* grass is originated from Eastern and Central Africa and it is widely cultivated in South America, Australia, and East Asia (Ghimire *et al.*, 2015). Collecting *Brachiaria* germplasm started in the 1950s and more than 987 accessions with 33 known species have been collected (Miles *et al.*, 1996). International Center for Tropical Agriculture (CIAT) has one of the major collections that comprises about 700 accessions with 27 known species. *Brachiaria brizantha* is the predominant species representing 50 percent of all CIAT accessions, followed by *Brachiaria humidicola, Brachiaria jubata* and *Brachiaria nigropedata* represented at 11%, 8% and 5%, respectively.

Other *Brachiaria* germplasm collection centres include International Livestock Research Institute (ILRI), Centro Nacional de Recursos Geneticos e Biotechnologia of EMBRAPA (CENARGEN), Australian Tropical Forage Genetic Resources Centre of CSIRO, Australia (ATFGRC), United States Department of Agriculture (USDA), Genebank of Kenya (GBK), and Roodeplaat Grassland Institute of the African Research Council, South Africa (RGI/ARC) with 520, 420, 174, 90, 51, 39 accessions, respectively (Miles *et al.*, 1996).

The annual herbage yield of *Brachiaria* grass is between 5 and 36 tons of dry matter per hectare and depends on soil fertility, fertiliser application and moisture. *Brachiaria brizantha, B. ruziziensis, Brachiaria decumbens* and *Brachiaria mutica* are the main *Brachiaria* species used in pastures production and they can multiply through seeds and root splits. Its use in improvement of pasture started around 1950 in several African countries including Uganda, Democratic Republic of Congo, Kenya, Tanzania, and Nigeria (Ndikumana and de Leeuw, 1996). The land occupied by *Brachiaria* grass is more than 70 million hectares in Brazil with the seed production of 100,000 tonnes per year. Characteristics of commonly used *Brachiaria* species are presented in the Table 2.1.

Table 2.1: Major characteristics of common *Brachiaria* grass species used in livestock production system

Species	Characteristics and growing conditions	Negative characteristics	Forage quality	Examples of cultivars	References
Brachiaria brizantha	Spittle bug tolerance respond positively to fertiliser application, ability for resistance to drought; ability to spread and produce under shade.	Low adaptation to poor soils and flooding, susceptible to foliar blight	Good	Marandu, Xaraes, BRS Piata, MG4	Collins, 2010; Miles *et al.*, 1996; Rao *et al.*, 1996
Brachiaria decumbens	Increased production when used intensively, low fertility tolerance, performs well in shaded environment	The adaptation is low in not drained soils, susceptible to spittle bug and foliar blight	Good	Basilisk	Miles *et al.*, 1996; Rao *et al.*, 1996
Brachiaria humidicola	Strong root system at stolon nodes, ability to adapt in low fertility soils, rapid ground cover and competition with weeds, adaptation to poorly drained soils, low P and Ca requirements, some level of spittlebug tolerance	The seed production is low in low altitudes, susceptibility to rust disease	Low digestibility and low dry matter yield. Low concentration of Nitrogen (N) and Calcium (Ca) content	Humidicola	Collins, 2010; Rao *et al.*, 1996
Brachiaria dictyoneura	Adaptation to infertile and acidic soils, spittlebug tolerance	High seed dormancy	Moderate, higher than *Brachiaria humidicola*	Llanero	Miles *et al.*, 1996
Brachiaria ruziziensis	Grow very fast in wet seasons, high seed production potential, ability to establish easily	No tolerance to water logging and poor soils, susceptible to spittlebug and foliar blight, low ability to compete with weeds	Good	Kennedy	Collins, 2010; Rao *et al.*, 1996
Brachiaria hybrid		Susceptible to foliar blight	Good	Mulato, Mulato II	Collins, 2010; Rao *et al.*, 1996

2.3 Diseases of *Brachiaria* grasses

Several diseases found damaging *Brachiaria* species, those include foliar blight (*Rhizoctonia solani*), leaf rust (*Uromyces setariae-italicae*) and leaf spot (*Dreschslera* sp.). Other diseases such as ergot, smut, bacterial wilt, viral and phytoplasma were reported (Lenné and Trutmann, 1994).

2.3.1 Foliar blight

Foliar blight is caused by a fungus, *Rhizoctonia solani*. Production of *Brachiaria* is affected up to 50% due to this foliar disease. Isolates of *Rhizoctonia solani* anastomosis group (AG)-1 IA were found to be predominantly associated with *Brachiaria* foliar blight in Colombia (Alvarez *et al.*, 2013). *Rhizoctonia solani* has several strains which differ in hosts and pathogenicity. Cultivated crops such as maize, rice, soybean, potato, sugarcane, strawberry, tomato, bean, sorghum and ornamental plants were reported as hosts of this fungus where it causes a destructive loss.

Isolates of *Rhizoctonia* from some crops including sugarcane and sorghum cause disease in several species of *Brachiaria* grass (Alvarez *et al.*, 2013). *Rhizoctonia solani* is a soil-borne pathogen that can survive in soils indefinitely and it has worldwide distribution (Alvarez *et al.*, 2014). Outbreaks on big areas occurred in Brazil and it was reported as a serious disease of forage grasses in Latin America.

Most of the *Brachiaria* species are affected by this pathogen when humidity and temperature are high as they have an impact on disease development (Alvarez *et al.*, 2013). Disease symptoms include water-soaked lesions which become dark and turn light brown on leaves. Sclerotia and white mycelium can be observed on leaves. Using resistant cultivars is a cultural method which is effective for leaf blight disease control (Miles *et al.*, 1996). *Bipolaris cynodontis* is among other pathogens that are associated with leaf blight for *Brachiaria* grass.

2.3.2 Leaf rust

Uromyces setariae-italicae and *Puccinia levis var. panici sanguinalis* are among the causal agents of leaf rust disease in *Brachiaria* grass species (Lenné, 1990; Lenné and Trutmann, 1994). It was reported as one of the main diseases affecting *Brachiaria* grass.

Studies conducted in Central and South America showed susceptible cultivars to *Uromyces setariae-italicae* which include Llanero, CIAT 679, CIAT 6369 and CIAT 16126 (Lenné and Trutmann, 1994). Apart from *Brachiaria* genera, *Uromyces setariae-italicae* has a wide host range such as *Cyrtococcum, Eriochloa, Melinis, Ottochloa, Panicum, Paspalidium, Setaria.* Rust disease is widely distributed.

Symptoms of rust include yellowish to blackish pustules on leaves. It is difficult to identify rust diseases at the beginning of infection (Lenné, 1990; Wang *et al.*, 2015). Several management options to control this disease include establishment of hedges -allowing sunlight- to prevent wind movement that can disseminate rust spores, acceleration of *Brachiaria* growth by application of nitrogen fertilisers; use of rust-free planting materials, plant at appropriate time since rust is favoured by rainfall, use of diverse *Brachiaria* genotypes, avoid burnings, early cutting of *Brachiaria g*rass (between four and eight weeks).

2.3.3 Leaf spot

Leaf spot is caused by fungal species such as *Bipolaris* species including *Bipolaris cynodontis*, *Bipolaris orizae*, *Bipolaris maydis*, *Bipolaris saccharicola*, *Bipolaris zeicola*, *Bipolaris setariae* and *Bipolaris secalis*. Apart from *Brachiaria* grass, this disease attacks cultivated and wild plants including *Axonopus* spp., *Cynodon dactylon, Stylosanthes guianensis*, oat, rye, sorghum, *Panicum maximum* and rice. Lesions with eye shape which become black to brown leading to leaf death are major symptoms of leaf spot disease. Use of resistant cultivars to control leaf spot diseases was reported (Cook *et al.*, 2005).

2.3.4 Ergot

Ergot is a fungal disease caused by *Claviceps purpurea* and it was reported on *Brachiaria* grass in different countries including Africa, Australia, and South America (Ramirez *et al.*, 2015). It causes serious loss in seed production. Symptoms are characterised by infected inflorescence where the fungal structure (sphacelium) replaces the ovary. Sphacelium develops into sclerotium which is like seed grain of the host but bigger than seeds. *Claviceps* spp. overwinters as sclerotinia in the soil or in seed mixture (Ramirez *et al.*, 2015). Fungicide is applied in controlling ergot disease.

2.3.5 Smut

Smut disease affecting *Brachiaria* grass was found to be in association with *Ustilaginoidea virens* (Kamidi *et al.*, 2016). Apart from cereals and grasses, smut was reported to infect onions. Black spore mass in infected inflorescence is the major symptom of this disease. Use of resistant genotypes and seed dressing are control options of smut disease (Agrios, 2005).

2.3.6 Bacterial blight

Bacterial blight disease caused by *Burkholderia glumae* was reported on several genotypes of *Brachiaria* grass in Colombia (Alvarez and Latorre, 2017). Symptoms of bacterial blight include, necrosis, chlorotic streaks and yellowing of flag-leaf margins. It was reported that serious damages are associated with long periods of hot and dry weather. It is difficult to find the diseased plant during cool and wet periods. Use of resistant and clean planting materials are the most practical options for management of bacterial blight disease management.

2.3.7 Viral and phytoplasma diseases

Viral diseases of *Brachiaria* grass are caused by different viral pathogens from *Potyvirus* subgroup. They are worldwide distributed and affect several cultivated and wild plants (Cook *et al.*, 2005). Typical symptoms are mosaic on leaves causing its early senescence. Use of resistant varieties and clean planting materials are effective for control of viral diseases (Miles *et al.*, 1996). Chemical spray can be used in seed production programmes to control vectors. Adam (2008) reported *Phytoplasma* causing diseases on several *Brachiaria* species in Kenya. They are transmitted through infected materials and vectors. *Phytoplasma* can infect other plants such as *Cynadon dactylon* and symptoms include chlorosis of leaves, short internodes and stunting. Management options include use of clean materials and chemical application to control vectors especially in case of seed production.

Diseases affecting *Brachiaria* grass and respective symptoms, as well as susceptible cultivars are presented in Table 2.2.

Table 2.2: Documented diseases and associated pathogens affecting *Brachiaria* grass

Diseases	Associated pathogens	Susceptible and/or *Brachiaria* host (Species)	Symptoms on affected plants	Reference
Leaf blight	*Rhizoctonia solani, Bipolaris cynodontis*	*Brachiaria brizantha*	Necrotic lesions on leaves	Alvarez *et al*., (2013, 2014); Macedo and Barreto, 2006; Miles *et al*.,1996
Leaf rust	*Puccinia levis var. panici sanguinalis*; *Uromyces setariae-italicae*	*Brachiaria humidicola*	Red to orange powder (uredospores) on leaves and stems; leaf necrosis	Agrios, 2005; Lenné, 1990; Lenné and Trutmann, 1994; Raul, 1996
Ergot	*Claviceps* species	*Brachiaria brizantha*	Presence of honeydew on flowers	Miles *et al*., 1996 ; Ramirez *et al*., 2015
Smut	*Ustilaginoidea virens*	*Brachiaria holosericeae*	black spore masses (sori) on inflorescence	Cook *et al*., 2005 ; Kamidi *et al*., 2016
Leaf spot	*Drechslera* sp.	*Brachiaria* spp.	Spots on leaves, brown and purple lesions	Agrios, 2005; Cook *et al*., 2005
Bacterial blight	*Xanthomonas* species, *Erwinia Chrysanthemi pv. Zeae; Burkholderia glumae*	*Brachiaria* spp.	chlorotic streaks on leaves, wilting and necrosis of leaves	Alvarez and Latorre, 2017; Lenné, 1990; Miles *et al*., 1996
Viral diseases	Sugarcane mosaic virus (SCMV)	*Brachiaria* spp.	Mosaic on leaves	Miles *et al*., 1996;
	Maize dwarf mosaic virus (MDMV)	*Brachiaria* spp	Mosaic and stunting	Miles et al., 1996
	Guinea grass mosaic virus (GGMV)	*Brachiaria* spp.	Mosaic on leaves	CGKB, 2009; Miles *et al*., 1996
	Johnson grass mosaic virus (JGMV)	*Brachiaria miliiformis, Brachiaria praetervisa*	Mosaic, ring spots, stunting	CGKB, 2009
	Digitaria striate mosaic virus (DSMV)	*Brachiaria subquadripara*	Chlorotic and strike on leaves	CGKB, 2009
	Maize streak virus (MSV)	*Brachiaria* spp.	Chlorotic and streak lines on leaves	CGKB, 2009; Miles *et al*., 1996
Phytoplasma	Phytoplasma	*Brachiaria* spp.	Small leaves, short internodes	Adam *et al.*, 2015

2.4 Methods of diagnosing diseases of plants

Disease prevention and management require rapid and accurate identification of associated pathogens. Different methods for plant diseases diagnosis are available and the choice depends on several factors including specificity, speed, sensitivity, and cost-effectiveness (Narayanasamy, 2011). Methods for disease diagnosis include conventional (observation of disease symptoms, use of microscopy, test with indicator seedling, isolation on culture media), serological and molecular techniques. The general diagnosis method for fungi is based on fungal mycelium morphology, structure of fruiting bodies and spores under microscope and compares them with existing literature (Agrios, 2005).

Agrios (2005) showed the easiest way of bacteria identification through isolation and growth on nutrient media followed by re-inoculation of susceptible hosts and testing pathogen association with the symptoms (confirming Koch's postulate). Other techniques for disease diagnosis include molecular and serological techniques such as immunomagnetic separation (IMS), immunofluorescence colony (IFC) staining, and enzyme-linked immunosorbent assay (ELISA). ELISA kits can be found and can be used for some fungal, bacterial, and viral diseases. Several molecular based techniques including the polymerase chain reaction (PCR), and genome sequencing have been increasingly used in pathogen identification and they are more sensitive and rapid than the conventional techniques. Different primers targeting different regions have been reported and widely used in disease identification (Bhunjun *et al*., 2020; Manamgoda *et al*., 2012).

2.5 Factors that affect development of diseases on *Brachiaria* grass

Several prerequisite factors influencing disease development relate to the environment, host and pathogens. Parameters such as spore production rate, rate of growth of the pathogen, the environmental conditions, the presence of hosts that are susceptible, and time are very important for the degree and rapidity of spread of diseases (Stubbs *et al*., 1986). Dispersal mechanisms include wind, water, insects, nematodes, fungi and activities of human beings.

2.5.1 Environmental factors

Environmental conditions including rainfall, temperature and relative humidity are key important factors and play a key role in disease surveillance and development.

Rainfall is essential in disease development as it provides the free moisture necessary for the process of infection (Stubbs *et al*., 1986). Germination of rust spores requires free moisture on the surface of plants and specific temperatures. Temperature affects the number of spores produced in the field and wind was reported as the major factor involved in moving at the long distance the inoculum of most of the foliar disease wide spreading. Rust diseases can spread over long distances through wind.

2.5.2 Host related factors

The host factors influence the degree to which the disease spreads. Disease development also depends on how the host is susceptible, the size, distribution and genetic diversity of host populations. While most of the diseases attack plants at any time at any growth stage, some other diseases including smut and ergot were reported to affect plants at specific growth stages.

2.5.3 Pathogen related factors

The abundance of inoculum, virulence and reproductive ability were reported as main components for disease spread. Different factors affecting the survival of disease inoculum include dormancy, structure of spores and mechanisms of dispersion. A minimum number of pathogen spores were reported as the first requirement for establishment of diseases even under favourable conditions.

2.6 Management of diseases of *Brachiaria* grass

A range of strategies have been used elsewhere to control different diseases affecting *Brachiaria* grass. Using resistant cultivars has been proven to be an appropriate and effective option for disease control. It has been used in controlling pasture diseases and researchers developed *Brachiaria* hybrids which can resist to pests and diseases. Some other methods include planting season, spacing and fertiliser application are effective in disease management. Management options include host-pest/pathogen resistance and good agricultural practices. Use of chemical spray was reported to be important in seed production to produce clean planting materials and they can also be used in management of diseases before sawing and pasture establishment through seed dressing and root split treatment (Lenné and Trutmann, 1994). Avoidance through use of clean materials is one of the best strategies to avoid any kind of diseases (fungi, bacteria, virus and phytoplasma).

CHAPTER THREE:

DISTRIBUTION, INCIDENCE AND SEVERITY OF DISEASES OF BRACHIARIA GRASS IN RWANDA

3.1 Abstract

Brachiaria grass, also known as *Urochloa* grass is a very important perennial fodder grass originating from Africa. Despite its importance, diseases are among major constraints that affect its performance. The study aimed at assessing the geographical distribution, the extent of diseases of *Brachiaria* grass and documenting knowledge of farmers on diseases of *Brachiaria* grass in Rwanda. Field surveys were conducted in different agro-ecological zones in five districts (Bugesera, Huye, Nyamagabe, Nyagatare and Rwamagana) in the dry (from June to August) and wet (from September to December) seasons in both years 2018 and 2019. The demographic information and farmers' knowledge of *Brachiaria* diseases symptoms, prevalence and their effect on yield were collected using structured questionnaires. Incidence and severity of the diseases were assessed whereby the severity was recorded using disease rating scale, established for each specific disease. The results of the study demonstrated that leaf spot, leaf blight and leaf rust diseases were widely distributed in Rwandan agro-ecologies. *Brachiaria* grass showed symptoms of all three diseases (leaf spot, leaf blight and leaf rust) in all surveyed locations and in both dry and wet seasons, with exception of leaf spot which was absent in Eastern savana agro-ecological zone (Nyagatare district) during the period of June to August 2018, while ergot disease was found only in Nyagatare district during the same period. Both disease incidence and severity of all three foliar diseases were significantly different ($p \leq 0.05$) by district, growing seasons and the interaction between district and growing season, exception was found on rust incidence in 2018 where the effect of season and the interaction of district × season was not significant. The highest disease incidence was recorded in Huye (72%) and Rwamagana (42.7%) for leaf blight, in 2018 and 2019, respectively. The highest incidence was recorded in Nyamagabe (48%) and Bugesera (66%) for leaf rust and leaf spot respectively. The findings of this study indicate preliminary information for future studies on identification of causal agents associated with major *Brachiaria* diseases in Rwanda.

Key words: Agro-ecologies, leaf blight disease, leaf rust disease, leaf spot disease, Rwanda

3.2 Introduction

Brachiaria grass *(Urochloa* grass) is a very significant perennial tropical fodder and of increasing importance grown on about 99 million hectares in Brazil only (Jank *et al.*, 2014). The eastern Africa represents the centre of diversity of all *Brachiaria* species with known forage values (Keller-Grein *et al.*, 1996). *Brachiaria* is a genus of plants in the tribe *Paniceae*, subfamily *Panicoideae* and *Poaceae* family (Jungmann *et al.*, 2009). It has around 100 reported species with wide distribution in the tropical regions, principally in Africa (Renvoize *et al.*, 1996). Among them, seven species have been cultivated to produce forage mostly in Asian, American, Australian, and the Southern Pacific tropical regions: *Brachiaria ruziziensis* Germain & Evrard, *Brachiaria mutica* (Forssk.) Stapf, *Brachiaria arrecta* (Hack. ex. Th. Dur & Schinz) Stent, *Brachiaria decumbens* Stapf, *Brachiaria brizantha* (A. Rich.) Stapf, *Brachiaria mutica* (Forssk.) Stapf, *Brachiaria humidicola* (Rendle) Schweick, and *Brachiaria dictyoneura* (Keller-Grein *et al.*, 1996). Until recently, little attention was given to the promotion and use of *Brachiaria* grass as a good quality forage to improve natural pastures that are still dominant in Africa due to other forages that were mostly used in existing livestock production systems (Ndikumana and Leeuw, 1996). Consequently, not much attention was given to research on species with good quality forage especially in Africa.

Cultivation of feeds and forages is key for improvement of the low livestock productivity in Sub-Saharan Africa and disease and pests of concern require efforts to contain. Livestock keepers in the African region were inspired to cultivate and disseminate good quality (nutritious) forage that includes *Brachiaria* grass with aim to solve the problem of increase in production of livestock associated with diminishing forage availability caused by either frequent or prolonged droughts due to variability of climate change, overgrazing, degradation, and continuous rangeland dwindling natural pasture. Importantly, different Institutions including Kenya Agricultural and Livestock Research Organization (KALRO), Rwanda Agriculture and Animal Resources Development Board (RAB) and International Livestock Research Institute (ILRI) have put enormous effort on researchable issues and developmental activities on *Urochloa* grass. Under these aspirations, ILRI implemented different research-development programmes on *Brachiaria* grass across the Sub-Saharan Africa region. Through those research and development programmes, technologies of *Brachiaria* grass for Africa have been developed and they were integrated successfully into existing farming systems with a mixing of crops and livestock productions.

Brachiaria grass significantly improved the forage availability and contributed to alleviate forage shortage in dry growing periods. Livestock productivity has been documented, while the sale of *Brachiaria* hay and planting materials have been new income generation opportunities for livestock farmers (Ghimire *et al.*, 2015; Maass *et al.*, 2015). A large number of dairy keepers in the East African region depends on traditional natural pastures and Napier grass, which has been the major fodder in the region (Klapwijk *et al.*, 2014). This fodder has adversely affected by the outbreak of Napier stunt and smut diseases leading to low levels of livestock productivity (Farrell, 1998; Lukuyu *et al.*, 2009; Nyiransengimana *et al.*, 2015; Umunezero *et al.*, 2016). Previous studies have indicated the high smut disease incidence and severity in fields of Napier grass in Rwandan agro-ecologies (Nyiransengimana *et al.*, 2015). Improved *Brachiaria* grasses that are recently introduced were proven to make additional benefits as additional options of forages and contributed to feed availability for livestock keepers especially in season with low rainfall (semi to dry growing seasons). It is very important to note that some of the species of *Brachiaria* grass have been widely adapted significantly to several environmental conditions in the African region (Ndikumana and de Leeuw, 1996; Njarui *et al.*, 2016). However, expanding area under cultivation for *Brachiaria* grass in the Sub-Saharan Africa region requires much attention since the plant centre origin is also taken as the variability centre for plant pests and pathogens (Jennings and Cock, 1977). Therefore, this can lead to exposing cultivars of *Brachiaria* grass to natural diseases and pests in the African continent. Since 2013, when improved *Brachiaria* varieties were introduced by ILRI, various *Brachiaria* diseases including leaf rust, leaf spot, leaf blight, and smut have been reported (Nzioki *et al.*, 2016). It is important to note that present documentation related to diseases of *Brachiaria* grass in the African region is not adequate, even unavailable for Rwanda. As cultivation of feeds and forages is key for improvement of the low livestock productivity in Sub-Saharan Africa, as well as in Rwanda and diseases and pests of concern require efforts to contain, there was need to understand the status of diseases (incidence and severity) of *Brachiaria* grass as an improved forage for production of livestock in Rwanda. The broad objective of this study was to increase livestock productivity and improve income of farmers through sustainable management of diseases affecting *Brachiaria* grass in Rwanda. Thus, the specific objectives of the study were to determine prevalence, incidence and severity of diseases of *Brachiaria* grass in distinct agro-ecological zones of Rwanda and to document knowledge and perceptions of farmers on diseases of *Brachiaria* grass and their effect on yield.

3.3 Materials and methods

3.3.1 Survey sites

On-farm surveys were conducted in *Brachiaria* fields in five distinct districts of Rwanda (Figure 3.1). They were situated in five different agro-ecological zones with variability in terms of altitude, rainfall and temperature (Table 3.1). The importance of livestock in a given agro-ecological zone and the number of dairy farmers with *Brachiaria* grass in their fields, were used as criteria to select the surveyed districts. Field disease surveys were conducted in consecutive years of 2018 and 2019 during dry and wet growing seasons that differ in terms of rainfall, humidity and temperature. The growing dry season is composed of three months including June, July and August with July as the driest month. The wet season is composed of four months including September, October, November and December where the wettest month is November. Data of total rainfall and average temperature during both growing seasons of the years 2018 and 2019 are presented in the table below (Table 3.1). For the first on-farm disease survey in the year 2018, only 15 *Brachiaria* grass fields were surveyed in each district and these fields were maintained in the second disease surveys during the year of 2019. Geographical coordinates of surveyed fields were taken with global positioning system (GPS), then the quantum geographic information system (GIS) software was used to plot them in the map of Rwanda (Figure 3.1).

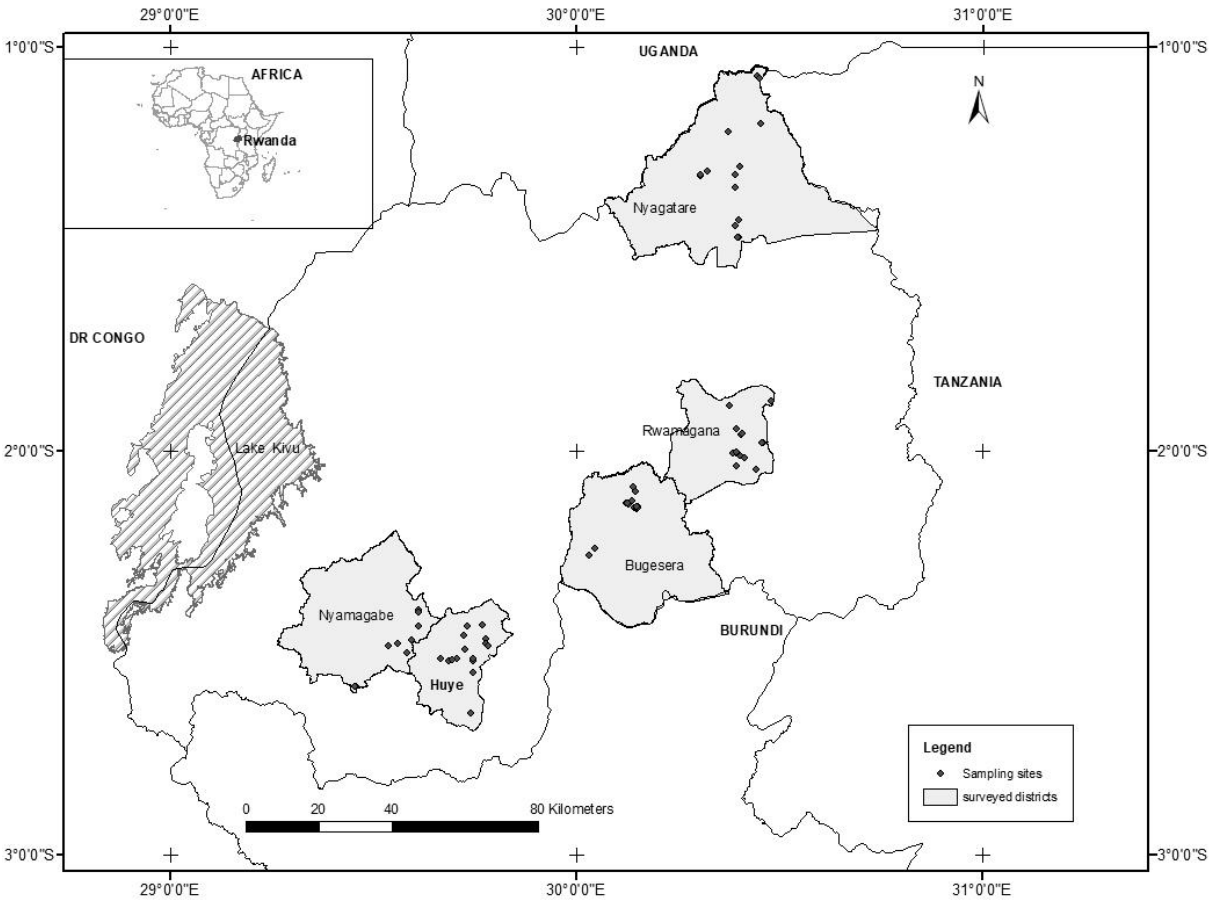

Figure 3.1: Location of survey districts for *Brachiaria* grass diseases. Selected fields for survey in each surveyed district are indicated by black dots

Table 3.1: Ecological characteristics of survey districts in Rwanda in 2018 and 2019

Survey district	Agro-ecological zone	Altitude (m. asl)	Average temperature (°C)				Total rainfall (mm)			
			2018		2019		2018		2019	
			Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
Bugesera	Mayaga and Bugesera	1440	20.9	22.3	22.8	22.3	67.4	239.0	74.7	602.3
Huye	Central Plateau and Granitic Ridges	1700	19.5	19.8	19.8	20.7	98.3	417.3	126.3	541.4
Nyagatare	Eastern Savanna	1575	20.8	19.7	21.4	19.6	105.2	353.3	210.5	542.6
Nyamagabe	Congo Nile Watershed Divide	2400	18.8	20.0	19.2	20.1	107.8	434.2	222.4	929.0
Rwamagana	Eastern Plateau	1300	21.0	21.9	21.2	22.6	21.2	515.1	12.4	475.4

3.3.2 Determination of knowledge of farmers of symptoms of *Brachiaria* grass diseases

On-farm surveys were conducted for farmers growing *Brachiaria* grass. The target population was farmers who had established *Brachiaria* grass plot in their farms. A three-stage purposive sampling procedure was used to determine a representative sample. At the first stage, districts which had the highest number of cows were selected and the list of farmers with established *Brachiaria* grass plot in their farms was established. The number of *Brachiaria* growers among the districts varied between 18 and 26. For the second stage, only farmers with at least one-month old *Brachiaria* field were selected among the targeted population. At the third stage, for the purpose of uniformity, a sample size of 15 farmers in every district was selected to facilitate the comparison between districts and the selection was based on plot size (Miles and Huberman, 1994). Therefore, 75 farmers were interviewed in all survey districts. Data on the level of education and age of the respondents, weed infestation level, area under cultivation of *Brachiaria* grass, types of cropping systems, farmers' knowledge of diseases of *Brachiaria* grass and the extend of above ground biomass reduction caused by diseases were collected using an open interview and a structured questionnaire. Interviewed farmers estimated the biomass reduction or *Brachiaria* yield loss in their fields using a four levels scale which includes: below 5%, from 5 – 25%, 25– 50%, and more than 50%. The questionnaire used in data collection is in appendix I.

3.3.3 Assessment of prevalence, incidence and severity of *Brachiaria* grass diseases

During on-farm surveys, assessment of disease prevalence, disease incidence and disease severity were conducted in every field. The prevalence of the disease was defined for each surveyed district by taking the number of fields where symptoms of a specified disease were present, and dividing by the total number of fields surveyed, and then expressed as percentage (Nutter *et al.*, 2006). To assess *Brachiaria* grass disease incidence and disease severity, twenty (20) stools were used and these were selected from four quadrats of one square meter each and they were randomly selected from each *Brachiaria* field. Within one quadrat, observations on disease incidence and severity were done considering five (5) stools of *Brachiaria* grass chosen following "X" shape-pattern. The incidence of disease was calculated by taking the number of *Brachiaria* stools showing disease symptoms and dividing by the total number of all assessed stools and then expressed as percentage (Agrios, 2005; Nutter *et al.*, 2006).

Disease severity was recorded as the infection level of the given disease on individual *Brachiaria* stools using the established disease scoring scale shown below (Table 3.2).

Table 3.2: Scoring scale used in determining severity of different diseases

Name of disease	Rating scale	Description of the disease	Reference
Leaf blight	0	No disease symptom	CIAT, 2004
	1	0.1 – 1.9% of leaf symptoms	
	2	2 – 5.9% of leaf symptoms	
	3	6 – 15.9% of leaf symptoms	
	4	16 – 19.9 % of leaf symptoms	
	5	20 – 100% of leaf symptoms	
Rust	0	No infection	CIMMYT, 1985;
	1	5% showing rust infection on plant	Peterson *et al.*,
	2	10% showing rust infection on plant	1948;
	3	20% showing rust infection on plant	
	4	40% showing rust infection on plant	
	5	60% showing rust infection on plant	
	6	100% showing rust infection on plant	
Leaf spot	0	Free from infection	Modified from
	1	1% showing leaf lesions or very few lesions	Stubbs *et al.*, 1986
	2	5% showing leaf lesions or light lesions	
	3	25% showing leaf lesions or moderate lesions	
	4	50% showing leaf lesions	
	5	80 % showing leaf lesions or heavy lesions	
Ergot	1	No visible honeydew	Menzies, 2004
	2	Honeydew limited within the glumes	
	3	Honeydew showing from the florets in small drops	
	4	Honeydew running down the Spike in large drops	
Viral diseases	0	Healthy plants	Koyshibayev and Muminjanov, 2016
	1	Weak infection of plant parts	
	2	Moderate infection, no severe infection of the plant	
	3	Severe damage of plant parts and death	

3.3.4 Data analysis

Data collected through interviews of farmers were analysed using SPSS 22.0 software (Statistical package for social sciences). To indicate the effect of seasons and agro-ecology as major factors in order to understand what is happening between different agro-ecological zones and between different seasons, the status of disease through time was considered by analysing the effect of seasons and agro-ecology whereby data on incidence and severity were subjected to the analysis of variance (ANOVA) at interaction between season and district using GenStat for Windows 20th Edition software (VSN International, 2019). Results were presented and discussed based on the significance of that interaction ($p \leq 0.05$). Mean values for disease incidence and disease severity were separated using the Least significant difference (LSD) mean separation test at $p \leq 0.05$.

3.4 Results

3.4.1 Characteristics of *Brachiaria* farms and farmers

All interviewed farmers planted *Brachiaria* grass on less than 0.5 ha and they were on hillside (96%) and marshland (4%). Compared to other cultivars, it was found that Mulato II cultivar was mostly grown by farmers in all the surveyed districts (62% of the farmers) while 16% of the farmers were growing Basilisk followed by 9.5%, 7%, 4%, 1.5% of farmers who planted Piata, Xaraes, MG4, and Cayman cultivars respectively. *Brachiaria* grass was planted as a monoculture for about 82.7% of the fields. Field conditions showed diversity in terms of field management where some fields were properly maintained and others were highly infested with weeds. These observations were found across surveyed districts and growing seasons. The overall conditions of *Brachiaria* fields towards infestation of weeds was 38.3% (no weeds), 32% (low weed infestation), 20% (medium infestation of weeds) and 10.7% (high infestation of weeds). For the respondents, the women were represented at 40% and only 67.1% of respondents were educated at primary level (Table 3.3).

Table 3.3: Information on age, gender and educational level of respondents during survey on *Brachiaria* grass diseases

Survey district	Age (years)	Farmer's gender (%)		Education level (%)		
		Male	Female	Primary	Secondary	University
Huye (n =15)	41.7	40.0	60.0	40.0	53.3	6.7
Nyamagabe (n =15)	50.2	80.0	20.0	40.0	53.3	6.7
Bugesera (n =15)	45.5	60.0	40.0	93.3	6.7	0.0
Rwamagana (n =15)	48.0	60.0	40.0	93.3	6.7	0.0
Nyagatare (n =15)	46.1	61.5	38.5	69.2	23.1	7.7
Mean	46.3	60.3	39.7	67.1	28.8	4.1

n = The total number of interviewed farmers in each district

3.4.2 Farmers' knowledge on *Brachiaria* disease symptoms and their effect on yield

Farmers (28%) highlighted the presence of diseases in their *Brachiaria* grass fields in all surveyed districts. The highest prevalence of the diseases was reported by farmers at Bugesera district, and it was (60%) (Table 3.4). Most farmers estimated disease associated losses of less than 5%, with exception of some farmers at Bugesera district who indicated the loss of up to 50%. Leaf yellowing was the most common and the most recognised symptoms and it was indicated by 17.3% of the farmers. Results showed that a big number of interviewed farmers (about 73%) did not know about the symptoms of *Brachiaria* grass diseases (Table 3.4).

Table 3.4: Knowledge of farmers of *Brachiaria* diseases and estimated biomass reduction in surveyed districts in Rwanda

Surveyed district	Percentage of farmers with knowledge of disease symptoms						Prevalence	Biomass reduction (%)	
	Bad growth	Drying of leaves	Holes on leaves	Yellowing of leaves	Yellowing of leaves and drying	Symptoms not known		Below 5	25 – 50
Huye	0.0	0.0	0.0	0.0	13.3	86.7	20.0	0.0	20.0
Nyamagabe	0.0	0.0	0.0	6.7	13.3	80.0	20.0	0.0	20.0
Bugesera	6.7	0.0	0.0	53.3	0.0	40.0	60.0	40.0	20.0
Rwamagana	0.0	6.7	0.0	6.7	0.0	86.7	13.3	13.3	0.0
Nyagatare	0.0	0.0	6.7	20.0	0.0	73.3	26.7	26.7	0.0
Mean	1.3	1.3	1.3	17.3	5.3	73.3	28.0	16.0	12.0

3.4.3 Prevalence, incidence and severity of *Brachiaria* grass

On-farm disease surveys showed a wide distribution of leaf spot, leaf blight, leaf rust, and virus-like diseases in *Brachiaria* grass' fields in Rwanda, while ergot disease was present in Nyagatare district during the dry growing season of the year 2018 only (Table 3.5). Leaf blight, leaf rust and leaf spot diseases were detected on *Brachiaria* grass fields across all surveyed districts and both dry and wet growing seasons, but leaf spot was not found in Nyagatare District during the dry season of the year 2018 (Table 3.5). Compared to other districts, the prevalence of leaf blight was greater at Rwamagana, Nyagatare, and Huye during the dry growing season of the year 2018. Likewise, leaf rust prevalence was consistently high (87%) at Rwamagana district during the wet growing season of the years 2018 and 2019. The prevalence of leaf spot disease was the highest at Huye District in the dry growing season of the year 2019. The Virus-like diseases prevalence was found to be low in both years of 2018 and 2019 and growing seasons, and ergot disease was recorded, at very low prevalence, in Nyagatare District in the dry growing season of the year 2018 (Table 3.5). Prevalence data of *Brachiaria* grass diseases by grown cultivars showed that Cayman was the less cultivated and registered high prevalence (100%) of leaf rust, leaf blight and leaf spot in two dry and wet growing seasons, with exception in dry season of the year 2018 for leaf rust and dry season of the year 2019 for leaf spot where the prevalence was zero (Table 3.6).

Necrotic lesions on *Brachiaria* leaves, often drying from the tip of the leaf, indicated leaf blight disease symptoms (Figure 3.2a). The presence of yellowish or brownish pustules mainly on the upper surface of leaves indicated symptoms of leaf rust disease (Figure 3.2b). Black spots or necrotic purple spots with whitish centre on upper surface of leaves showed the presence of leaf spot disease (Figure 3.2c1-2). Ergot disease symptoms were indicated by the presence of honeydew on the inflorescence (Figure 3.2d) whereas virus-like disease (Figure 3.2e1-3) was recognised by chlorosis, reduced size of leaves and stunting of the whole plant of *Brachiaria* grass.

Table 3.5: Prevalence (%) of diseases of *Brachiaria* grass in growing seasons of the years 2018 and 2019 in surveyed districts

Growing season	Surveyed district	2018					2019				
		Leaf blight	Leaf rust	Leaf spot	Ergot disease	Virus-like disease	Leaf blight	Leaf rust	Leaf spot	Ergot disease	Virus-like disease
Dry	Bugesera	60.0	80.0	8.0	0.0	0.0	73.0	80.0	93.0	0.0	6.0
	Huye	100.0	20.0	2.0	0.0	6.0	73.0	86.0	100.0	0.0	27.0
	Nyagatare	100.0	60.0	0.0	6.0	6.0	66.0	86.0	73.0	0.0	17.0
	Nyamagabe	80.0	80.0	6.0	0.0	0.0	53.0	80.0	46.0	0.0	6.0
	Rwamagana	100.0	60.0	6.0	0.0	0.0	80.0	80.0	80.0	0.0	17.0
	Mean	88.0	60.0	5.0	1.0	3.0	69.0	83.0	78.0	0.0	15.0
Wet	Bugesera	80.0	80.0	93.0	0.0	0.0	27.0	67.0	87.0	0.0	0.0
	Huye	93.0	20.0	27.0	0.0	6.0	60.0	67.0	87.0	0.0	27.0
	Nyagatare	67.0	60.0	20.0	0.0	0.0	67.0	53.0	60.0	0.0	0.0
	Nyamagabe	80.0	67.0	27.0	0.0	0.0	54.0	87.0	74.0	0.0	0.0
	Rwamagana	93.0	87.0	60.0	0.0	0.0	60.0	87.0	87.0	0.0	0.0
	Mean	83.0	63.0	45.0	0.0	1.0	53.0	72.0	79.0	0.0	6.0

Table 3.6: Prevalence (%) of *Brachiaria* grass diseases by grown cultivars by farmers in surveyed districts during the growing seasons in the years 2018 and 2019

Growing Season	Cultivar	2018			2019		
		Leaf blight	Leaf rust	Leaf spot	Leaf blight	Leaf rust	Leaf spot
Dry	Mulato II (n = 47)	93	60	27	72	87	83
	Piata (n =7)	100	100	100	57	71	86
	Toledo (n = 5)	100	50	100	80	80	60
	Basilisk (n = 12)	60	60	60	50	67	67
	MG4 (n = 3)	100	100	0	100	100	100
	Cayman (n = 1)	100	0	100	100	100	0
	Mean	88	60	44	69	83	79
Wet	Mulato II (n = 47)	87	57	34	64	74	83
	Piata (n = 7)	86	71	71	43	100	100
	Toledo (n = 5)	80	60	60	40	100	60
	Basilisk (n = 12)	67	67	50	33	25	67
	MG4 (n = 3)	67	100	100	0	100	33
	Cayman (n = 1)	100	100	100	100	100	100
	Mean	83	63	45	53	72	79

Numbers in parentheses refer to the number of surveyed fields.

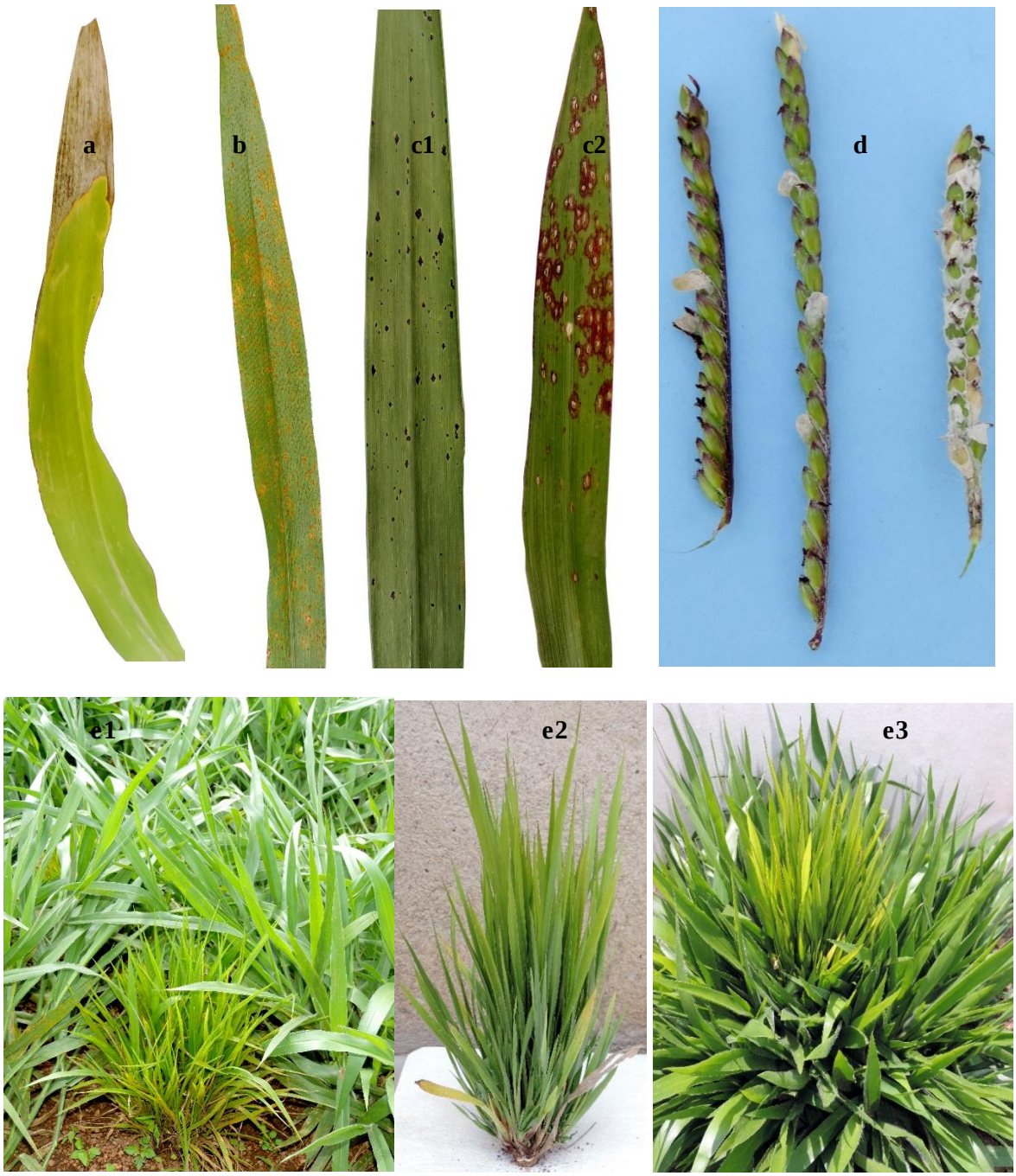

Figure 3.2: Symptoms of the major diseases infecting *Brachiaria* grass in surveyed districts. (a) shows leaf blight disease, (b) shows leaf rust disease, (c1-2) shows leaf spot disease, (d) shows ergot disease, and (e1) indicate virus-like disease with affected stool, diseased uprooted stool indicated by (e2) and stool showing many small size and stunted leaves indicated by (e3).

The results showed a significant variation ($p \leq 0.05$) for the incidence of leaf spot, leaf rust, and leaf blight diseases among five districts in 2018 and 2019 survey periods. Exception was recorded with leaf rust in the year 2018. The effects of growing season and district × season interactions were evident for incidence of all three diseases (leaf spot, leaf rust, and leaf blight) in survey periods ($p \leq 0.05$) (Figures 3.3, 3.4). Compared to other four districts, leaf blight incidence was significantly higher at Huye district for the year 2018 but this was also true for Rwamagana district during the year 2019. Compared to the year 2019, the year 20218 registered high leaf blight incidence irrespective of district location and season characteristics. Nyamagabe district registered high incidence of leaf rust compared to other districts in both growing seasons in 2018. Leaf rust incidence was the highest in the dry growing season of the year 2019 regardless of the survey districts. The significant and highest leaf spot incidence was found in Bugesera district during both seasons of the year 2018 and in the dry season of the year 2019.

As of disease incidence, the severity of leaf spot, leaf rust and leaf blight diseases revealed significant variation by surveyed districts, growing season and district × season interaction ($p < 0.05$) (Table 3.6). Leaf blight disease was highly severe at Rwamagana district in the dry growing season for both years of 2018 and 2019, while leaf rust severity was the highest at Nyamagabe and Huye Districts in the dry seasons of both years, 2018 and 2019, respectively. The severity of leaf spot was the highest at Bugesera in the dry season of both years (2018 and 2019) and in the wet growing season of the year 2018.

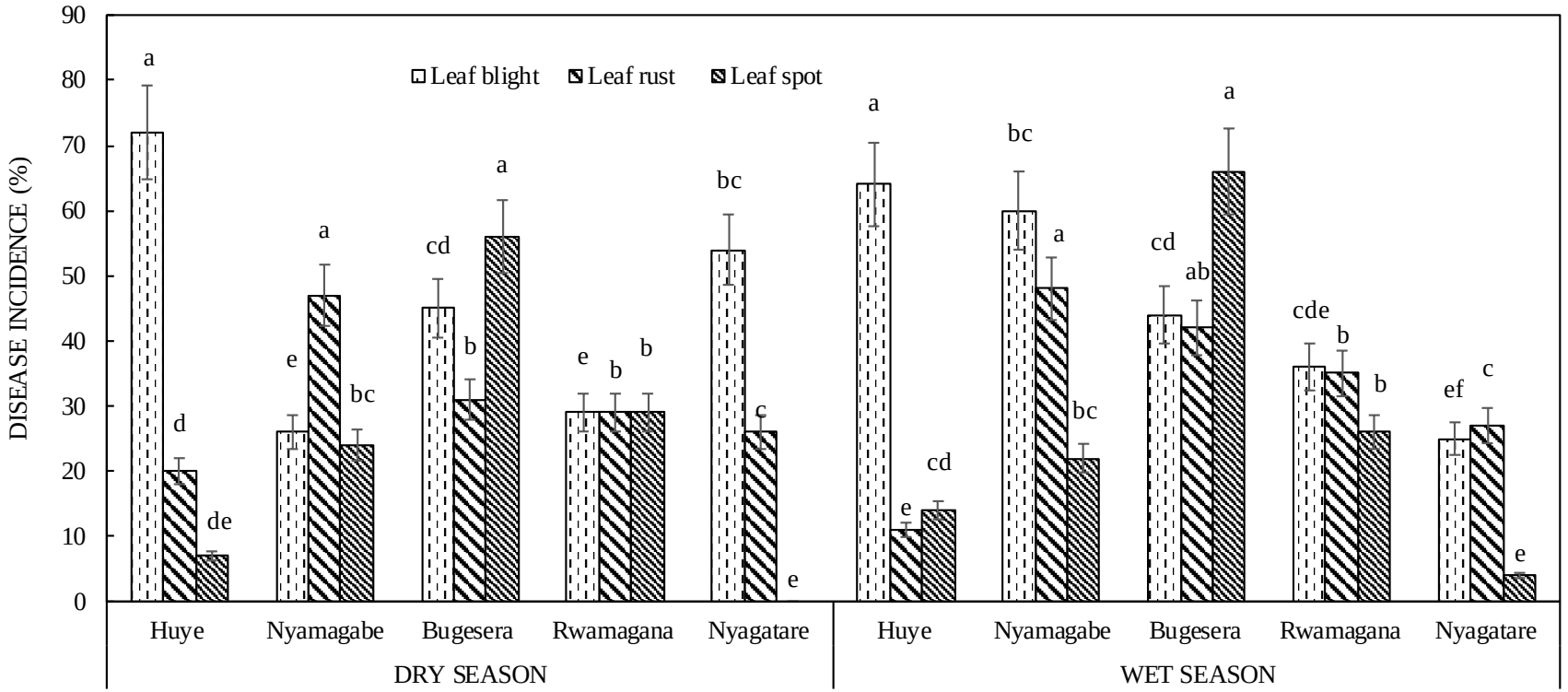

Figure 3.3: Incidence of foliar diseases affecting *Brachiaria* grass in surveyed districts in 2018. Bars with the same letters for each disease are not statistically different at $p \leq 0.05$. Plotted error bars show standard errors of the means. Separation of means was done using LSD.

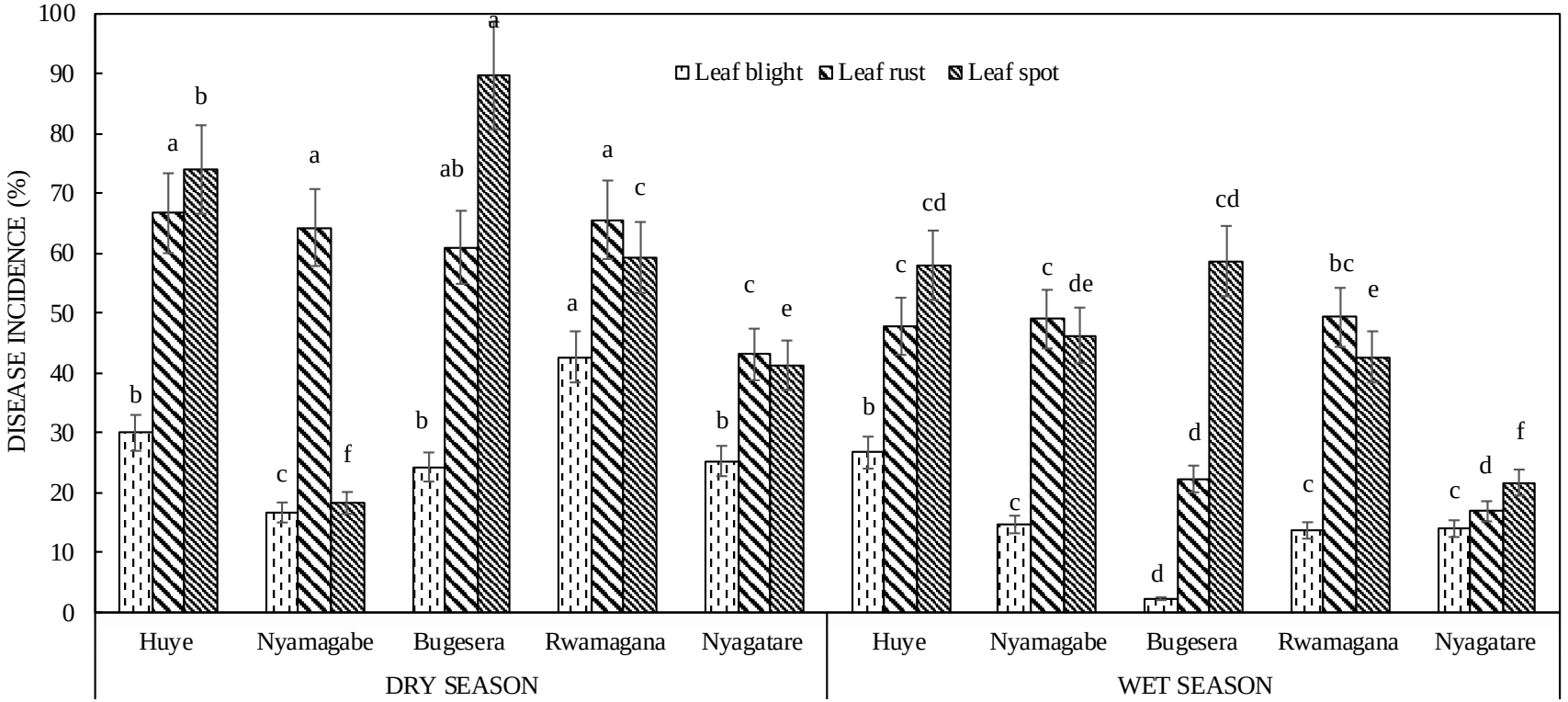

Figure 3.4: Incidence of foliar diseases affecting *Brachiaria* grass in surveyed districts in 2019. Bars with the same letters for each disease are not statistically different at $p \leq 0.05$. Plotted error bars show standard errors of the means. Separation of means was done using LSD.

Table 3.7: Severity of foliar diseases infecting *Brachiaria* grass in surveyed districts

Season	District	2018			2019		
		Leaf blight (%)	Leaf rust (%)	Leaf spot (%)	Leaf blight (%)	Leaf rust (%)	Leaf spot (%)
Dry	Bugesera	25.4^{b}	11.7^{bc}	37.0^{a}	5.2^{def}	13.2^{cd}	39.4^{a}
	Huye	36.6^{a}	16.5^{ab}.	2.8^{cd}	7.9^{b}	27.0^{a}	27.1^{b}
	Nyamagabe	12.4^{c}	22.1^{a}	9.0^{c}	6.3^{bcd}	20.5^{b}	5.4^{g}
	Nyagatare	23.6^{b}	9.7^{cde}	0.0^{d}	5.3^{cde}	9.8^{de}	17.5^{d}
	Rwamagana	38.4^{a}	10.8^{bcd}	3.4^{cd}	12.0^{a}	17.0^{bc}	16.8^{d}
Wet	Bugesera	9.0^{cd}	8.3^{cde}	19.8^{b}	0.4^{h}	5.4^{ef}	15.1^{de}
	Huye	22.8^{b}	3.4^{f}	3.9^{cd}	7.1^{bc}	11.5^{cd}	22.8^{c}
	Nyagatare	5.1^{d}	4.5^{ef}	0.8^{d}	3.4^{fg}	4.0f	6.0^{g}
	Nyamagabe	20.7^{b}	12.3^{bc}	6.3^{c}	3.5^{efg}	15.0^{bcd}	12.1^{ef}
	Rwamagana	7.4^{cd}	5.6^{def}	5.7^{c}	2.7^{g}	19.3^{b}	11.2^{f}
Source of variation		*p* values					
Season		<.001	<.001	<.001	<.001	<.001	<.001
District		<.001	<.001	<.001	<.001	<.001	<.001
Season × district		<.001	<.001	<.001	<.001	<.001	<.001

Mean values with the different superscript letters within the column are statistically different at 0.05 probability level. Separation of means was done using LSD.

3.5 Discussion

The present study indicates the distribution, the status of diseases of *Brachiaria* grass in main districts growing *Brachiaria* grass in Rwanda. Likewise, it indicates for the first time as new knowledge for the scientific community the understanding of farmers about the symptoms of leaf diseases infecting *Brachiaria* grass and the estimation on biomass reduction due to the presence of diseases. The findings of this study give initial knowledge and relevant documentation to inform all other future studies on diseases that can affect *Brachiaria* grass in Rwanda. The study revealed the widespread reality of leaf blight, leaf rust and leaf spot diseases on *Brachiaria* grass across different agro-ecological zones in Rwanda, and infrequent and more seasonal occurrences of virus-like and ergot diseases. These findings corroborate with other previous studies in other countries including Kenya where all diseases discovered in this study have been documented by several authors (Cook *et al.*, 2005; Lenné and Trutman, 1994; Nzioki *et al.*, 2016; Valério *et al.*, 1996;). Importantly, all three diseases (leaf spot, leaf rust and leaf blight) were consistently found with different infection levels in all five survey districts including Bugesera, Huye, Nyamagabe, Nyagatare and Rwamagana. However, leaf spot prevalence was zero at Nyagatare district in the dry growing season of the year 2018. The variability level of both disease incidence and disease severity suggests the endemic nature and wide distribution of *Brachiaria* grass diseases in Rwanda.

Most farmers interviewed did not recognise diseases of *Brachiaria* grass. They were also not informed about symptoms of diseases and had no understanding about the extent of biomass reductions that are associated with the presence of diseases. Even though, disease surveys confirmed disease presence in all five surveyed districts in Rwanda, few farmers were able to recognise diseases affecting *Brachiaria* grass in their fields (28%). These findings are supported by Kiros-Meles and Abang (2007) who reported that a limited number of farmers have a good understanding to know diseases affecting crops. In contrary to the study's findings, a lot of Kenyan farmers were able to recognise the symptoms of Napier stunt disease which affects Napier grass (Khan *et al.*, 2014). This understanding of these farmers could be attributed to the fact that Napier grass was highly popularised for beef and dairy farmers in Kenyan farming systems, coupled with a clear reduction of the productivity of Napier grass due to stunt disease.

It is evident that pests and diseases have been indicated as one of the essential constraints of *Brachiaria* grass production in the Sub-Saharan Africa region (Kiros-Meles and Abang, 2007).

Consequently, the introduction and promotion of this fodder require education and information of farmers on diseases of *Brachiaria* grass, possible yield loss caused by diseases and management options of diseases. It is essential to take an inventory of indigenous options for the management of diseases (Mahapatro and Sreedevi, 2014) and apply those into the cropping system while developing new or appropriate crop protection measures.

The findings from this study showed that leaf spot, leaf rust, and leaf blight diseases that are affecting *Brachiaria* grass were widely spread and distributed in different agro-ecological zones of Rwanda. This diverse distribution may be due to different factors such as the existence of local *Brachiaria* grass in surveyed districts and wild relatives that serve as alternate and/or collateral hosts to the causal pathogens and prevalence of favourable climatic conditions for the development and spread of these diseases. This study also revealed differences in the prevalence, incidence and severity of the diseases among survey districts. The variation in disease parameters could be attributed to different reasons including differences in host and pathogen genotypes, differences in agroclimatic conditions among the survey districts, farmers' agricultural practices, and other biotic and abiotic factors. For instances, the variability in the total amount of rainfall in Rwanda between the years 2018 and 2019 could led to the observed differences. The range of annual rainfall of the surveyed districts (Bugesera, Huye, Nyamagabe, Nyagatare and Rwamagana) was between 966 mm and 1833 mm in 2018 but it was between 1232 mm and 2009 mm in 2019 (National Institute of Statistics of Rwanda, 2019; Rwanda Meteorology Agency, 2019). Another cause could be the seasonal rainfall variability between the districts which was between 65 mm and 108 mm for the dry growing season of the 2018 while it was between 54 mm and 222 mm for the dry growing season of the year 2019. Likewise, it was noted that rainfall was between 239 and 515 mm for the wet season of 2018 while it was between 466 and 929 mm for the wet season of 2019. Even though, the difference between seasons and districts in terms of daily temperature was negligeable, the difference in amount of rainfall had probably an effect on different environmental factors including relative humidity may put a specific disease in favourable or in difficult conditions for its development. The wet conditions with high moisture content, caused by higher rainfall regimes, that prevailed in some districts, might have contributed to the spreading and growing of some fungi.

Differences in environmental conditions might have played key role in variability of incidence between the year 2018 and the year 2019, whereby incidence of leaf rust and leaf spot were clearly higher in the year 2019 compared to the year 2018. Importantly, temperature is one of the key actors which plays essential role in making host plants to be susceptible to rust disease.

Stem rust including *Pg3* and *Pg4* has a function resistance genes that fails at temperature more than 20°C, while wheat leaf rust (*Lr2a*) has a resistant gene that confers resistance when the temperature is more than 25°C (Das *et al.*, 2017; Martens *et al.*, 1967). The range of temperature between 15 to 25°C has been reported as the optimal temperature for teliospore germination and basidiospore formation in Asian grapevine leaf rust pathogen (*Phakopsora euvitis*) (Edwards, 2015). Another justification is that the average daily temperature which was between 18.8 and 22.8°C in all surveyed districts during the period of the study might have influenced positively the development of the rust pathogen (*Phakopsora apoda)* affecting *Brachiaria* grass.

The evidence of widespread distribution of leaf spot, leaf rust, leaf blight diseases in all main *Brachiaria* growing districts indicates their importance that need much attention for sustainable production of *Bracharia* grass, specifically for livestock farmers in the east African countries including Rwanda. The increase of area under cultivation with *Brachiaria* grass in a wider geographical region should put much attention on the spread and distribution for both existing and other emerging diseases challenges. Disease with low prevalence should also attract attention. For example, even though ergot disease was found in Nyagatare District only, it has high potential to widely spread in bigger agro-ecological zones when planting materials are transported to the farms, that may lead to low *Brachiaria* grass productivity and quality reduced and affects health of animals (Vermeulen *et al.*, 2012; Young *et al.*, 1983). Likewise, it is very important to note that the symptoms of virus-like diseases that have been reported in this study may negatively affect the growth of plants and their productivity and forage quality (Valerio *et al.*, 1996). Therefore, it is imperative to identify causal agents associated with diseases of *Brachiaira* grass for development of effective management options against diseases targeting to the African smallholder farmers for the prevention of disease outbreaks and associated economic losses.

3.6 Conclusions

This is the first study that highlights distribution, incidence and severity of diseases affecting *Brachiaria* grass in Rwanda. The current study indicates the evidence that major foliar diseases affecting *Brachiaria* grass in Rwanda include leaf spot, leaf blight and leaf rust and they are widely spread and distributed across districts. In addition, the study indicated that farmers had limited knowledge of diseases affecting *Brachiaria* grass and associated yield loss. Diseases have high potential to cause severe yield loss of *Brachiaria* grass leading to low productivity when prevention measures are not taken. Furthermore, under conducive environmental conditions that favour disease development, these endemic diseases can be the cause of epidemic. Moreover, there is a challenge of relying on the centre of diversity of *Brachiaria* grass that corresponds to high diversity of pathogen. This may lead currently to grown cultivars of *Brachiaria* at maximum vulnerability. Thus, it is recommended to conduct regular diseases' surveillance, diagnosis of associated pathogens, and put in place effective options for disease management and advisory systems. Diagnosis of causal agents and development of skills for farmers on management of diseases in the fields are very essential for the sustainability of *Brachiaria* production in Rwanda.

CHAPTER FOUR:
CAUSATIVE RELATIONSHIP BETWEEN FUNGAL PATHOGENS ISOLATED FROM BRACHIARIA GRASS AND LEAF SPOT AND LEAF RUST DISEASES IN RWANDA

4.1 Abstract

Brachiaria grass is one of the nutritious fodder crops grown across the tropics and subtropics. However, it is attacked by different diseases which have negative impact on biomass yield and herbage qualities. Although several diseases including leaf rust and leaf spot have been reported from all *Brachiaria* growing regions of East Africa, there is a lack of precise information about the relationships between each specific disease and associated micro-organisms. The broad objective of the study was to increase livestock productivity and improve income of farmers through sustainable management of diseases affecting *Brachiaria* grass in Rwanda. Specifically, the study intended (i) to identify the causative agents of two major diseases (Leaf spot and leaf rust) and (ii) to evaluate the pathogenicity of different isolates on *Brachiaria* cultivars that showed high disease susceptibility. Leaf samples with symptoms of disease were collected from farmers' fields in wet season of the year 2018. Fungi associated with major diseases including leaf rust, leaf blight and leaf spot were isolated and identified based on morphological and molecular characteristics. The pathogenicity test was performed by inoculating one-month old seedlings of *Brachiaria humidicola* cv. Humidicola using hand rubbing and the conidial suspension of 10^5 spores ml^{-1} for leaf spot. Mulato cultivar and the concentration of 10^6 spores ml^{-1} was used for pathogenicity test of leaf rust. Molecular identification revealed *Phakopsora apoda* as the only fungus associated with leaf rust. Dominant fungi associated with leaf blight symptoms were *Fusarium* spp., *Epicoccum* spp. and *Nigrospora* spp. while *Bipolaris secalis* was the dominant fungal species associated with leaf spot symptoms. Morphological, molecular identification and symptoms reproduced on inoculated *Brachiaria* seedlings confirmed *Bipolaris secalis* and *Phakopsora apoda* as causal agent of leaf spot and leaf rust, respectively. All isolates of *Bipolaris secalis* were pathogenic to seedlings of *Brachiaria humidicola* cv. Humidicola and produced typical leaf spot symptoms and Koch's postulates were confirmed. Similar results were also obtained with *Phakopsora apoda* isolates where leaf rust symptoms were consistently reproduced on inoculated seedlings of Mulato cultivar for all rust isolates. The results of this study provide information to assist for development of effective disease management options of leaf spot and leaf rust in Rwanda.

Key words: *Bipolaris secalis*, pathogenicity, internal transcribed spacer, *Phakopsora apoda*.

4.2 Introduction

Brachiaria grass remains one of the nutritious forages originating from Africa which is also its centre of biodiversity. It belongs to the *Poaceae* family and it is appreciated by several farmers in Sub-Saharan region due to its high biomass production with high nutrient content, drought tolerance and its adaptation to low fertility soils. Improvement of this genus was done outside Africa, especially in America and Australia (Maass *et al*., 2015). Seven species of African origin including *B. arrecta, B. dictioneura, B. brizantha, B. decumbens, B. mutica, B. humidicola* and *B. ruziziensis* are used as forages. The development of improved cultivars outside Africa led to different challenges and little attention has been considered towards biotic stress, especially *Brachiaria* grass foliar diseases. With the increase of improved *Brachiaria* cultivation in East Africa including Rwanda, there have been reports on the occurrence of diseases including leaf spot, leaf rust and leaf blight diseases (Nzioki *et al*., 2016; Uzayisenga *et al*., 2020). Different species in the genus *Bipolaris* were reported to be associated with leaf spot disease affecting several plants worldwide (Manamgoda *et al*., 2012; 2014; Sun *et al*., 2020). The genus *Bipolaris* includes several species that affect plants especially grasses and has a worldwide distribution (Manamgoda *et al*., 2012). The genus *Bipolaris* is a dematiaceous, filamentous described for the first time by Van Tieghem in 1876. It belongs to Ascomycota, Dothideomycetes, Pleosporales, in Pleosporaceae family (Manamgoda *et al*., 2014).

Bipolaris causes different symptoms on several plant-hosts that include wheat, rye, rice, sorghum, maize, corn, switchgrass, oat, barley and bermudagrass (Fajolu, 2012; Sun *et al*., 2020; Vu *et al*., 2011, 2013). The widespread of leaf spot and leaf rust on *Brachiaria* grass was reported in Rwanda and symptoms were characterised by necrotic or black spots, purple spots with the centre which is whitish on upper leaf surface and the presence of yellowish or brownish pustules for leaf spot and leaf rust respectively (Uzayisenga *et al*., 2020). Catastrophic losses caused by *Bipolaris* species were reported on rice and maize worldwide. In 1970, the southern corn leaf blight led to animal starvation in USA and UK (Manamgoda *et al*., 2014). Different reports have described the phenotype of *Bipolaris* species as characterised by the presence of white or pale grey mycelium to dark grey depending on age of colonies (Manamgoda *et al*., 2014). Apart from DNA sequence analysis, morphological characters were reported to be important in taxonomy (Ramesh *et al*., 2021). ITS sequence analysis was successively used in identification and confirmation of *Bipolaris* species including *Bipolaris cynodontis, Bipolaris micropus* and *Bipolaris setariae* (Da Cunha *et al*., 2012).

Basic Local Alignment Search Tools (BLAST) search and comparison of ITS and GAPDH sequences were reported to be used in identification of several species of *Bipolaris* (Ramesh *et al.,* 2021; Sun *et al.*, 2020). Leaf rust disease was reported as one of the major diseases affecting *Brachiaria* grass and reduces the biomass both quantitatively and the qualitatively. The biomass reduction is up to 100% of the yield loss and the reduction of crude proteins of *Brachiaria* leaves was 49 – 53%. The availability of other nutrients was shown to be highly affected even when the affected leaf area was below 5% (Lenné and Trutman, 1994). Leaf rust disease management options include establishment of hedges, acceleration of *Brachiaria* growth by application of nitrogen fertilisers; use of rust-free planting materials, plant at appropriate time since leaf rust is favoured by rainfall, use of diverse *Brachiaria* genotypes, avoid burnings and early cutting of *Brachiaria g*rass (Alvarez *et al.*, 2014; CIAT, 2004).

Although leaf spot and leaf rust affecting *Brachiaria* grass were reported in East Africa, there is little information documenting causal agent associated with each disease. The broad objective of the study was to isolate and confirm causative relationship between fungal pathogens (*Bipolaris secalis* and *Phakopsora apoda*) and leaf spot and leaf rust diseases. The specific objectives of the study were (i) to identify the causative agents of two major diseases (Leaf spot and leaf rust) and (ii) to evaluate the pathogenicity of different isolates on *Brachiaria* cultivars that showed high disease susceptibility.

4.3 Materials and methods

4.3.1 Collection of samples and isolation of associated pathogens

Brachiaria grass with leaf spot symptoms were sampled from surveyed fields in different districts. Three representative samples were collected in different fields for each disease symptom per district. Photographs of surveyed plots and disease symptoms were taken and samples were coded. They were kept in paper bags and they were put in an ice cooling box before transportation to the Plant Pathology laboratory at Rubona research station of RAB. Tap water was used to properly wash the samples, and these were cut into pieces of 3 to 5 mm length having tissue with disease symptoms and the one adjoining without symptoms.
Thereafter, samples were put in 1% sodium hypochlorite (NaOCl) solution for surface disinfection for three minutes, then samples were properly rinsed two times using sterile distilled water then blot dried.

Washed and dried tissue pieces were transferred to Potato Dextrose Agar (PDA) supplemented with ampicillin (100 µg ml^{-1}) and plates were incubated at 22°C for time interval between 24 – 48 hours in darkness (Narayanasamy, 2011). The subcultures of growing colonies were done into new PDA plates and cultures that were pure were obtained by hyphal tip transfer to fresh PDA medium (El-Morsi and Abdel-Monaim, 2015). Fungal isolates were grown on Whatman FTA cards and kept at - 80°C for long-term preservation. Leaf rust samples were handled in way that, leaves with rust symptoms were taken and put in paper bags, then dried at normal room temperature for 48 hours. Brushes were used to remove the rust spores from *Brachiaria* leaves and the spores were collected on aluminium foil, put in Eppendorf tubes and stored in darkness at - 4ºC for use in further studies (Guo *et al.*, 2016).

4.3.2 Identification of fungal species associated with symptoms of *Brachiaria* diseases

Microscopic examination at 40x magnification was used to check the size and form of spores for initial identification of fungi associated with *Brachiaria* diseases and the confirmation was done by DNA (Deoxyribonucleic Acid) analysis. Except for rust, isolates of other fungi, were grown on Potato Dextrose Agar (PDA) at 22ºC for 1 to 3 weeks. Thereafter, sterilised scalpel blade was used to harvest the fresh mycelium which was then transferred into 1.5 ml Eppendorf tube. The fresh fungal mycelium or spores of rust that was harvested was then put in liquid nitrogen to break all structures and it was ground into fine powder using sterilised mortar and pestle. QIAGEN DNeasy kit was used to extract genomic DNA of isolates from 100 mg of the ground samples, respecting the manufacturer's instructions. To check the DNA concentration and DNA integrity, respectively, NanoDrop spectrophotometer ND-1000 (NanoDrop Technologies) and agarose gel electrophoresis were used. The final DNA was stored at - 20°C for further use. The amplification of internal transcribed spacer (ITS) region in ribosomal DNA of all DNA from fungal isolates except rust isolates were done and specific primers of rust were used for amplification of genomic DNA extracted from rust isolates for identification of microorganisms associated with *Brachiaria* diseases. For the confirmation of causal pathogens, multigene analysis was used where ITS and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sequence analysis were used for leaf spot and rust primers and 28S large subunit of nuclear ribosomal RNA (LSU) were used for the confirmation of the causal agent of leaf rust. Primers and PCR conditions used are described in Table 4.1.

Table 4.1: Primer name, sequences and PCR conditions used in amplification of genomic DNA of fungal isolates

No	Primer name/Target DNA	Primer Sequence	Fungal disease considered	PCR conditions	PCR reactions	Source
1	ITS1F/ITS4 – Internal Transcriber Spacer DNA	5′-TCCGTAGGTGAACCTGCGG-3′/ 5′-TCCTCCGCTTATTGATATGC-3′	Leaf spot and leaf blight	4 minutes of denaturation at 94°C, followed by 35 cycles of 94°C for 45 seconds, 56.7°C for 45 seconds and 72°C for 45 seconds, with final extension of 72°C for 10 minutes and hold at 4°C.	25 µl total volume: 3 µl of diluted genomic DNA containing at least 20 ng, 12.5 µl of premix, 0.5 µl of ITS1F, 0.5 µl of ITS4 and 8.5 µl of water for molecular biology	White *et al*., 1990
2	ITS1rustF10d/ rust1	5′-TGAACCTGCAGAAGGATCATTA-3′/5′-GCTTACTGCCTTCCTCAATC-3′	Leaf rust	4 minutes of initial denaturation at 94°C, followed by 35 cycles of 94°C for 45 seconds, 59.5°C for 45 seconds, and 72°C for 45 seconds, with the final extension of 72°C for 10 minutes and hold at 4°C at the end.	25 µl total volume: 3 µl of diluted genomic DNA containing at least 20 ng, 12.5 µl of premix, 0.5 µl ITS1rustF10d, 0.5 µl of rust 1 and 8.5 µl of water for molecular biology	Barnes and Szabo, 2007

No	Primer name/Target DNA	Primer Sequence	Fungal disease considered	PCR conditions	PCR reactions	Source
3	gpd-1/gpd-2 – GAPDH (glyceraldehyde-3-phosphate dehydrogenase)	5′-CAACGGCTTCGGTCGCATTG-3′/ 5′-GCCAAGCAGTTGGTTGTGC-3′	Leaf rust and leaf spot	Initial denaturation step at 95°C for 3 minutes followed by 34 cycles of 30 seconds of denaturation at 95°C, 30 seconds of annealing temperature at 52°C, 1 minute of elongation at 72°C, final extension of 10 min at 72°C, and hold at 4°C at the end.	25 µl total volume: 3 µl of diluted genomic DNA containing at least 20 ng, 12.5 µl of premix, 0.5 µl of gpd 1, 0.5 µl of gpd 2 and 8.5 µl of water for molecular biology	Berbee *et al.*, 1999 ; Cheng *et al.*, 2020; Manamgoda *et al.*, 2014
4	LR5/LROR – LSU (28S large subunit of nuclear ribosomal RNA)	5′-TCCTGAGGGAAACTTCG-3′/ 5′-ACCCGCTGAACTTAAGC-3′	Leaf rust	Initial denaturation step at 95°C for 3 minutes followed by 34 cycles of 30 seconds of denaturation at 95°C, 30 seconds of annealing temperature at 52°C, 1 minute of elongation at 72°C, final extension of 10 min at 72°C, and hold at 4°C at the end.	25 µl total volume: 3 µl of diluted genomic DNA containing at least 20 ng, 12.5 µl of premix, 0.5 µl of LR5, 0.5 µl of LROR and 8.5 µl of water for molecular biology	Chethana *et al.*, 2019 ; Schoch *et al.*, 2009

The confirmation of the presence of targeted products was checked by loading 3 µl of PCR product on 1.5% agarose gel and GelRed® staining (2.5 µl in 100 ml) for one hour at 70 Volt. Prior to DNA sequencing, the purification of PCR products was done using QIAquick PCR Purification Kit (QIAGEN) following the instructions of the manufacturer. After the purification, the sanger sequencing of the PCR products was done at MACROGEN Inc., Amsterdam, The Netherlands and the BecA-ILRI Hub (Nairobi, Kenya) using the same primers used for PCR amplification. The cleaning and consensus of raw DNA sequences were determined through alignment of nucleotide sequences produced by forward and reverse primers. Finally, the submission of consensus sequences at the National Centre for Biotechnology Information (NCBI) was done and Basic Local Alignment Search Tools (BLAST) programme (Altschul *et al*., 1990) was used for homology search and identification of species through comparison with reference sequences available in the database.

4.3.3 Morphological characterization of leaf spot and leaf rust pathogen isolates

A total of 12 isolates of leaf spot (three isolates per district) were characterised morphologically using the taxonomic key to species in *Bipolaris* developed by Manamgoda *et al*. (2014) where *Bipolaris secalis* was described to have 4 – 9 distoseptates. Origin of leaf spot samples, host cultivars and other collection details are presented in Table 4.2. Isolates were grown on Potato Dextrose Agar (PDA) medium amended with ampicillin (100 µg ml^{-1}) to avoid the growth of bacteria. To evaluate the radial growth and colony colour, 8 mm agar disc was cut from the edge of 10-day old PDA cultures and transferred to the centre of 90 mm Petri dishes containing 20 ml of PDA in three replicates for all 12 isolates. Thereafter, plates were incubated in growth chamber at 28°C in darkness. Radial growth and colony colour were recorded at 3 days, 5 days, 7 days, 10 days and 14 days of incubation. The measurement of radial growth was done using a ruler along two perpendicular lines drawn on the bottom of each Petri dish while naked eye was used for examination of colony colour, colony margin and colony texture. For the microscopic observations, conidia and conidiophores from 21 days' culture of isolate was visualised using the microscope Optika B – 350 at 40x magnification to evaluate physical features including the shape, the size and the colour; and the digital image of conidia and conidiophores were recorded using a camera installed on microscope. Measurements of the size of conidia and conidiophores were taken using a calibrated micrometre.

Since leaf spot isolates were morphologically similar, one isolate was arbitrarily selected from each district for evaluating the effect of *Brachiaria* leaf extract on morphology making a total of four *Bipolaris secalis* isolates for this study. Three media types (PDA, PDA supplemented with *Brachiaria* leaf extract and *Brachiaria* leaf extract agar) were compared for radial growth, colony colour and shape of four *Bipolaris* isolates. For preparing leaf extract, leaves of cultivars Mulato and Humidicola were collected from 6-week-old seedlings, rinsed, dried between filter papers and 150 g leaves (75 g for each cultivar) was ground in 400 ml of distilled water using sterile mortar and pestle. The homogenised extract was first passed through a 0.02 mm filter and centrifuged at 3,000 revolutions per minute (RPM) for 15 minutes to remove any residues. The filtrate of *Brachiaria* leaf extract was kept at - 20°C for further use. For media preparation, 40 ml of the *Brachiaria* leaf extract was added on 460 ml of PDA or water agar. Three replicates of 8 mm-diameter agar disks from 10-day old cultures were placed at the centre of every 90 mm-diameter Petri dish containing each medium type. Thereafter, plates that were inoculated were then incubated in growth chamber at 28°C as in dark place as darkness and the temperature of 28°C were reported to favor growth and conidia production of several *Bipolaris* species (Almaguer *et al*., 2012). Radial growth and colony colour were recorded after 5, 7, 10 and 14 days. The measurement of radial growth and examination of colony colour were performed as described earlier. For leaf rust, the colour, the shape and measurements of leaf rust spores were also recorded using the same microscope and the calibrated micrometre.

Table 4.2: Origin, host cultivars and other collection details of *Bipolaris secalis* isolates recovered from *Brachiaria* grass leaves with leaf spot symptoms

Isolate name	Cultivar	District	Altitude (m a.s.l)	GPS coordinates	
BS1BR	Basilisk	Bugesera	1392	E030°09'08.8"	S02°08'33.4''
BS2BR	Basilisk	Bugesera	1386	E030°08'59.9"	S02°08'24.5''
BS3BR	Basilisk	Bugesera	1455	E030°01'58.6"	S02°15'33.8''
BS4HY	Humidicola	Huye	1685	E29°46'.56.9″	S02°28'54.8″
BS5HY	Humidicola	Huye	1685	E29°46'.56.9″	S02°28'54.8″
BS6HY	Humidicola	Huye	1685	E29°46'.56.9″	S02°28'54.8″
BS7NR	Humidicola	Nyagatare	1346	E030°18'18.24″	S01°18'56.16″
BS8NR	Humidicola	Nyagatare	1346	E030°18'18.24″	S01°18'56.16″
BS9NR	Humidicola	Nyagatare	1346	E030°18'18.24″	S01°18'56.16″
BS10RN	Humidicola	Rwamagana	1517	E030°27'29.82″	S01°58'49.62″
BS11RN	Humidicola	Rwamagana	1521	E030°27.497'	S01°58.827'
BS12RN	Humidicola	Rwamagana	1521	E030°27.497'	S01°58.827'

4.3.4 Pathogenicity tests

A total of 12 *Bipolaris secalis* isolates with three isolates from each District (Bugesera, Huye, Nyagatare and Rwamagana) were tested for pathogenicity on one-month old seedlings of *Brachiaria humidicola* cv. Humidicola. The inoculum was prepared by growing isolates on water agar with leaf extract at 28°C for 14 days. Different authors reported the maximum growth, high number and good germination rate of conidia of different species of *Bipolaris* species at 28°C (Almaguer *et al.*, 2012). Spores were then washed using 0.04% tween in sterile distilled water (SDW) and the concentration of the spores was adjusted to 10^5 spores ml^{-1} using haemocytometer (Fajolu, 2012; Moges *et al.*, 2017). To facilitate the penetration of the fungus into host tissue, 1% carborandum was added in the inoculum. Three seedlings per isolate were inoculated using hand rubbing. Symptom development was observed daily up to four weeks and symptoms on inoculated plants were compared with those which occur in nature conditions. Seedlings for negative control were inoculated with the inoculum prepared in similar way but no fungal spores were added. To maintain high humidity, inoculated seedlings were covered in polyethylene bags for a time life of 48 hours (Falloon, 1976). The pathogen was re-isolated from artificially inoculated leaves of *Brachiaria humidicola* cv. Humidicola using the same procedure as done for the beginning. The morphological characteristics of the re-isolated fungi were compared with the original isolates based on morphological characteristics and 18S rDNA sequences.

For leaf rust, five isolates with one isolate from one of the five districts (Bugesera, Huye, Nyamagabe, Nyagatare, and Rwamagana) were used. The pathogenicity test was conducted using *Brachiaria* Hybrid cv. Mulato and the spore concentration was 10^6 spores ml^{-1}. Seedlings were similarly covered in plastic bags for a time life of 48 hours and leaf rust symptoms were checked on daily basis up to four weeks.

4.3.5 Phylogenetic data analysis

The CLC Genomics Workbench Version 8.0.3 software (https://digitalinsights.qiagen.com**)** was used to process data of DNA sequences from Sanger sequencing. The cladograms showing relationships among fungi isolates originating from leaves with individual symptoms were constructed using CLC Main Workbench - QIAGEN Bioinformatics.

The tree was constructed using the Neighbour joining method, nucleotide distance measured using Jukes-Cantor and 1000 bootstrap. Also, CLC Genomics Workbench software was used to perform sequence quality control through sequence trimming and assembling. Trimming was performed using quality scores of 0.05. The analyses did not consider all sequences with scores rating below 50%. The nucleotide sequences generated by the forward and reverse primers were checked, edited and consensus sequences were generated also using CLC Workbench version 8.0.3 software. Sequences were aligned using the same software and gaps were considered as missing data. The dendrograms showing the phylogenetic relatedness among the isolates were constructed using CLC Genomics Workbench 8.0.3 version software. The statistical validity of the tree was tested using the neighbour-joining method, jukes-canter nucleotide distance measure, percentage bootstrap support at each node with 1000 replicates, and a 30% threshold bootstrap value. To allocate identities to the test isolates, identification of species was done using the BLAST programme of the NCBI sequence database and the comparison of the GenBank database was based on the high similarity coverage, and identity. Sequences generated were separately analysed.

4.4 Results

4.4.1 Fungal species associated with diseases of *Brachiaria* grass

The association of *Phakopsora apoda* with rust symptoms was confirmed through molecular techniques. The sequence identity was 96%, while the e-value was zero. Even though the query sequence coverage was low (60%), while some variations in nucleotide sequences were noticed, all rust isolates corresponded to fungus *Phakopsora apoda*. A large number of fungi were isolated from leaf spot and leaf blight diseases symptoms. Sequence analysis revealed 69 fungi fitting to 14 genera associated with *Brachiaria* leaves with leaf blight symptoms. The most dominant genus included *Epicoccum* (33.2%) followed by *Nigrospora* (21.9%) and *Pestalotiopsis* (14.4%). Other genus including *Rhizopus Lasiodiplodia, Leptosphaeria, Fusarium, Didymella, Cochliobolous, Curvularia Arthrinium, Alternaria* occurred in the low frequency. A total of 23 fungal isolates that belong to 12 taxa were isolated from leaf spot symptoms of *Brachiaria* grass. Isolated fungi included *Pestalotiopsis microspore, Nigrospora* spp, *Nigrospora sphaerica, Chaetomium globosum, Alternaria arborescens, Didymella* sp., *Bipolaris secalis, Curvularia trifolii, Epicoccum* spp., *Epicoccum nigrum, Fusarium verticillioides,* and *Fusarium equiseti*. *Bipolaris secalis* was the predominant fungal species isolated from *Brachiaria* leaf spot (Table 4.3).

Table 4.3: Fungal species associated with leaf spot, leaf rust and leaf blight diseases affecting *Brachiaria* grass in Rwanda

Name of disease	Total number of isolates	Name of fungi species isolated	Frequency of isolated fungi (%)	Relationship between fungi species and the host	Reference
Leaf blight	69	*Nigrospora sphaerica*	6.0	Pathogenic	Liu *et al.*, 2015
		Nigrospora oryzae	8.7	Endophyte	Ghimire *et al.*, 2011 ; Sánchez Márquez *et al.*, 2008
		Nigrospora sp.	7.2	Endophyte	Sánchez Márquez *et al.*, 2008
		Pestalotiopsis microspora	10.1	Pathogenic, Endophyte	Jeon *et al.*, 2007 ; Lazarotto *et al.*, 2012
		Pestalotiopsis vismiae	2.9	Endophyte	Tejesvi *et al.*, 2007
		Pestalotiopsis sp	1.4	Endophyte	Tejesvi *et al.*, 2007
		Epicoccum sp	4.3	Endophyte	Sánchez Márquez *et al.*, 2008
		Epicoccum sorghinum	8.7	Endophyte	Sánchez Márquez *et al.*, 2008
		Epicoccum nigrum	18.8	Endophyte	Sánchez Márquez *et al.*, 2008
		Epicoccum nisorghi	1.4	Endophyte	Sánchez Márquez *et al.*, 2008
		Arthrinium phaeospermum	3.0	Endophyte, Pathogenic	Agut and Calvo, 2004; Jiang *et al.*, 2018
		Arthrinium sp	4.3	Saprobe	Agut and Calvo, 2004
		Cochliobolus kusanoi	1.4	Endophyte	Alurappa *et al.*, 2014
		Curvularia cf. brachyspora	1.4	Endophyte	Kameshwari *et al.*, 2015
		Alternaria arborescens	1.4	Endophyte	Ghimire *et al.*, 2011
		Curvularia aeria	1.4	Endophyte	Kamana and Hemalatha, 2018
		Didymella sp	7.2	Endophyte	Soltani and Moghaddam, 2014
		Leptosphaeria spegazzinii	2.9	Endophyte	Sánchez Márquez *et al.*, 2008
		Lasiodiplodia theobromae	2.9	Endophyte	Orlandelli *et al.*, 2012
		Fusarium equiseti	2.9	Endophyte	El-Nagerabi *et al.*, 2013 ; Sánchez Márquez *et al.*, 2008
		Rhizopus stolonifer	1.4	Endophyte	El-Nagerabi *et al.*, 2013

Name of disease	Total number of isolates	Name of fungi species isolated	Frequency of isolated fungi (%)	Relationship between fungi species and the host	Reference
Rust	9	*Phakopsora apoda*	100.0	Pathogenic	Adendorff and Rijkenberg, 1995 Gardner, 1984; McKenzie, 1998; Starr, 2004
Leaf spot	23	*Bipolaris secalis*	22.0	Pathogenic	Bernardi *et al.*, 2018; Sisterna, 1989;
		Didymella sp	4.3	Endophyte	Sánchez Márquez *et al.*, 2010
		Fusarium verticillioides	4.3	Pathogenic, endophyte	Bacon *et al.*, 2008
		Fusarium equiseti	13.0	Endophyte	Sánchez Márquez *et al.*, (2007, 2010)
		Alternaria arborescens	4.3	Endophyte	Sánchez Márquez *et al.*, 2008
		Curvularia trifolii	4.3	Pathogenic	Starr, 2004
		Nigrospora sphaereca	8.7	Endophyte	White and Backhouse, 2007
		Nigrospora oryzae	8.7	Endophyte	Ghimire *et al.*, 2011 ; Sánchez Márquez *et al.*, 2007
		Chaetomium globosum	4.3	Endophyte	Sánchez Márquez *et al.*, 2010
		Pestalotiopsis microspora	8.7	Pathogenic, endophyte	Jeon *et al.*, 2007 ; Lazarotto *et al.*, 2012 ; Tejesvi *et al.*, 2007
		Epicoccum nigrum	8.7	Endophyte	Fa´varo *et al.*, 2012 ; Sánchez Márquez *et al.*, 2010
		Epicoccum sp	8.7	Endophyte	Sánchez Márquez *et al.*, 2008

4.4.2 Morphological characteristics of *Bipolaris secalis* and *Phakopsora apoda* isolates

All 12 isolates on PDA medium showed similar colony colour, colony margin and texture of colonies. The colony colour was whitish to brownish at the bottom with irregular edges until the first 10 days and they became blackish with whitish and irregular edges at the beginning of 14 days. The surface colony colour was whitish to greyish with abundant and dense aerial mycelium for all isolates. The margin of colonies was irregular and the texture was cottony. Conidia were solitary and straight with 5 to 6 distosepta (mean = 6) and their size ranged from 61 – 77 µm in length and 12 – 14 µm in width (mean = 70 x 13 µm). Conidiophores were cylindrical and septate. The size of the conidiophore ranged from 268 – 284 µm in length and 6 – 8 µm in width (mean = 275 x 7 µm) (Table 4.4).

Table 4.4: Size of conidia and conidiophores of *Bipolaris secalis* isolates

Isolate	Number of septa	Size of conidia (µm)		Size of conidiophores (µm)	
		Length	Width	Length	Width
BS1BR	5.4 ± 0.5^{a}	76.9 ± 5.3^{c}	13.3 ± 1.1^{a}	270.0 ± 5.2^{cd}	7.8 ± 0.5^{a}
BS2BR	5.4 ± 0.5^{a}	61.4 ± 2.0^{e}	11.6 ± 1.1^{a}	273.8 ± 4.9^{bcd}	5.6 ± 0.6^{c}
BS3BR	6.0 ± 0.7^{a}	68.3 ± 5.1^{de}	12.6 ± 1.3^{a}	279.0 ± 4.8^{bcd}	6.4 ± 0.7^{bc}
BS4HY	6.0 ± 0.3^{a}	71.1 ± 2.3^{de}	13.4 ± 0.7^{a}	281.0 ± 4.8^{bcd}	6.5 ± 0.4^{bc}
BS5HY	5.8 ± 0.7^{a}	77.3 ± 5.1^{a}	13.8 ± 1.1^{a}	269.2 ± 1.8^{cd}	7.3 ± 0.4^{bc}
BS6HY	5.0 ± 0.3^{a}	61.5 ± 2.0^{e}	11.5 ± 1.1^{a}	272.4 ± 3.5^{bcd}	5.8 ± 0.6^{c}
BS7NR	5.8 ± 0.4^{a}	68.3 ± 5.3^{de}	12.9 ± 1.3^{a}	278.0 ± 3.7^{bcd}	6.3 ± 0.7^{bc}
BS8NR	5.8 ± 0.4^{a}	71.0 ± 2.2^{de}	13.1 ± 0.6^{a}	275.0 ± 2.7^{bcd}	6.6 ± 0.5^{bc}
BS9NR	5.4 ± 0.6^{a}	76.9 ± 5.2^{b}	13.3 ± 1.1^{a}	268.0 ± 5.1^{d}	7.6 ± 0.4^{b}
BS10RN	6.2 ± 0.8^{a}	61.6 ± 2.0^{e}	11.6 ± 1.0^{a}	279.0 ± 4.3^{bcd}	5.6 ± 0.6^{c}
BS11RN	5.6 ± 0.7^{a}	69.0 ± 5.1^{de}	12.3 ± 1.3^{a}	282.0 ± 3.4^{ab}	6.5 ± 0.6^{bc}
BS12RN	6.2 ± 0.5^{a}	70.6 ± 2.2^{de}	13.1 ± 0.8^{a}	284.0 ± 1.9^{a}	6.9 ± 0.5^{bc}
Mean	5.7 ± 0.2	69.5 ± 1.2	12.7 ± 0.3	276.0 ± 1.2	6.6 ± 0.2

± is followed by standard error of the mean. Values with the same letters within the column are not statistically different at the probability level of 0.05.

Morphological characteristics of *Bipolaris secalis* isolates on different media amended with *Brachiaria* leaf extract showed noticeable difference in growth pattern and colony colour for

all isolates (Figure 4.1). Tested isolates showed a similar pattern of mycelial growth on a given medium type. On leaf extract agar, the mycelia were present on the surface and whitish colour on both sides whereas mycelia were greyish and cottony on colony surface and blackish with whitish edge on the reverse side of the colony on PDA and PDA supplemented with leaf extract at 14 days. Conidiophores and conidia are shown in Figure 4.2.

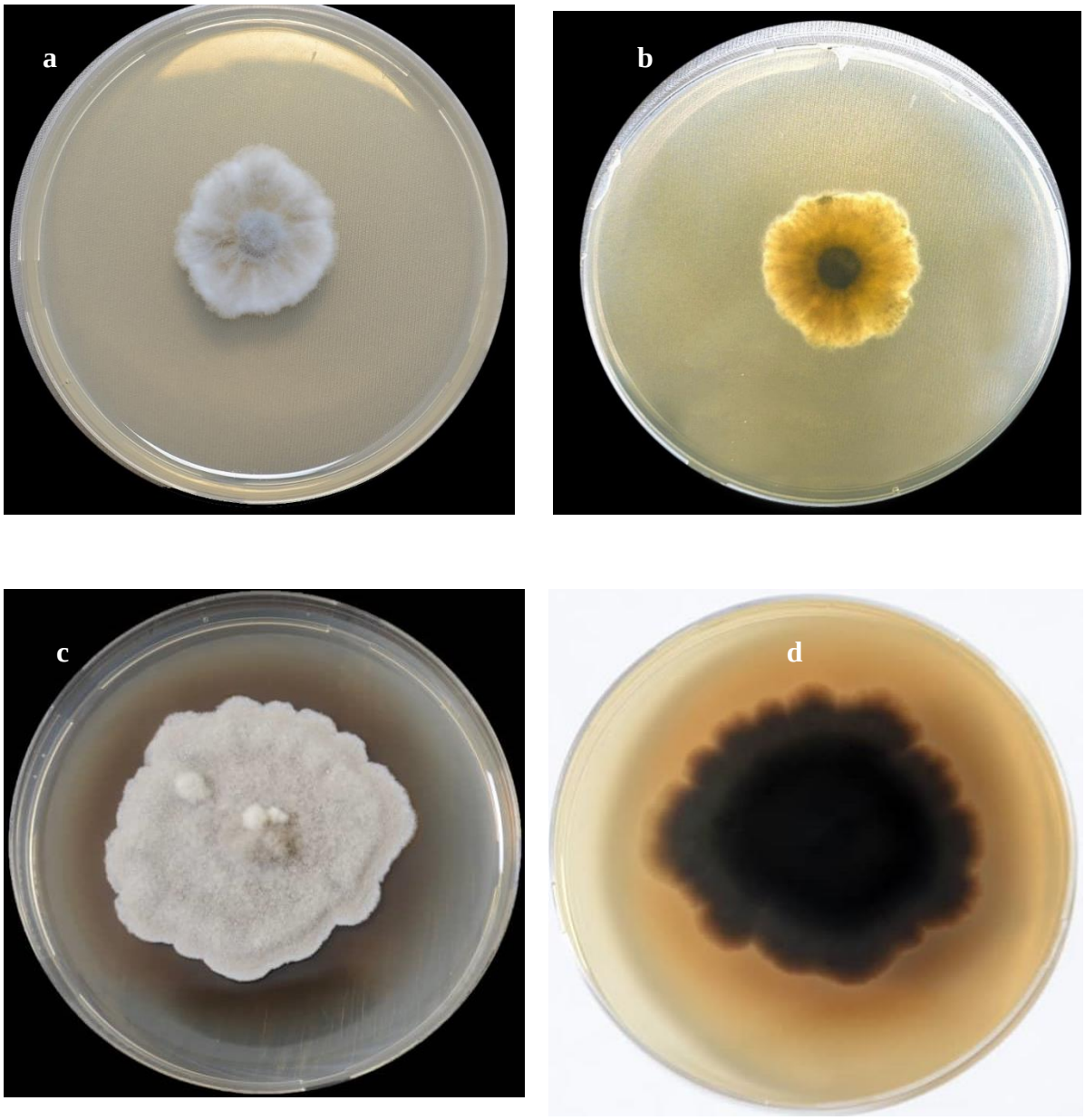

Figure 4.1: Phenotypic characteristics of *Bipolaris secalis* isolates on PDA. a: Surface colony at three days; b: Reverse colony at 3 days; c: Surface colony at 14 days of incubation, d: Reverse colony at 14 days.

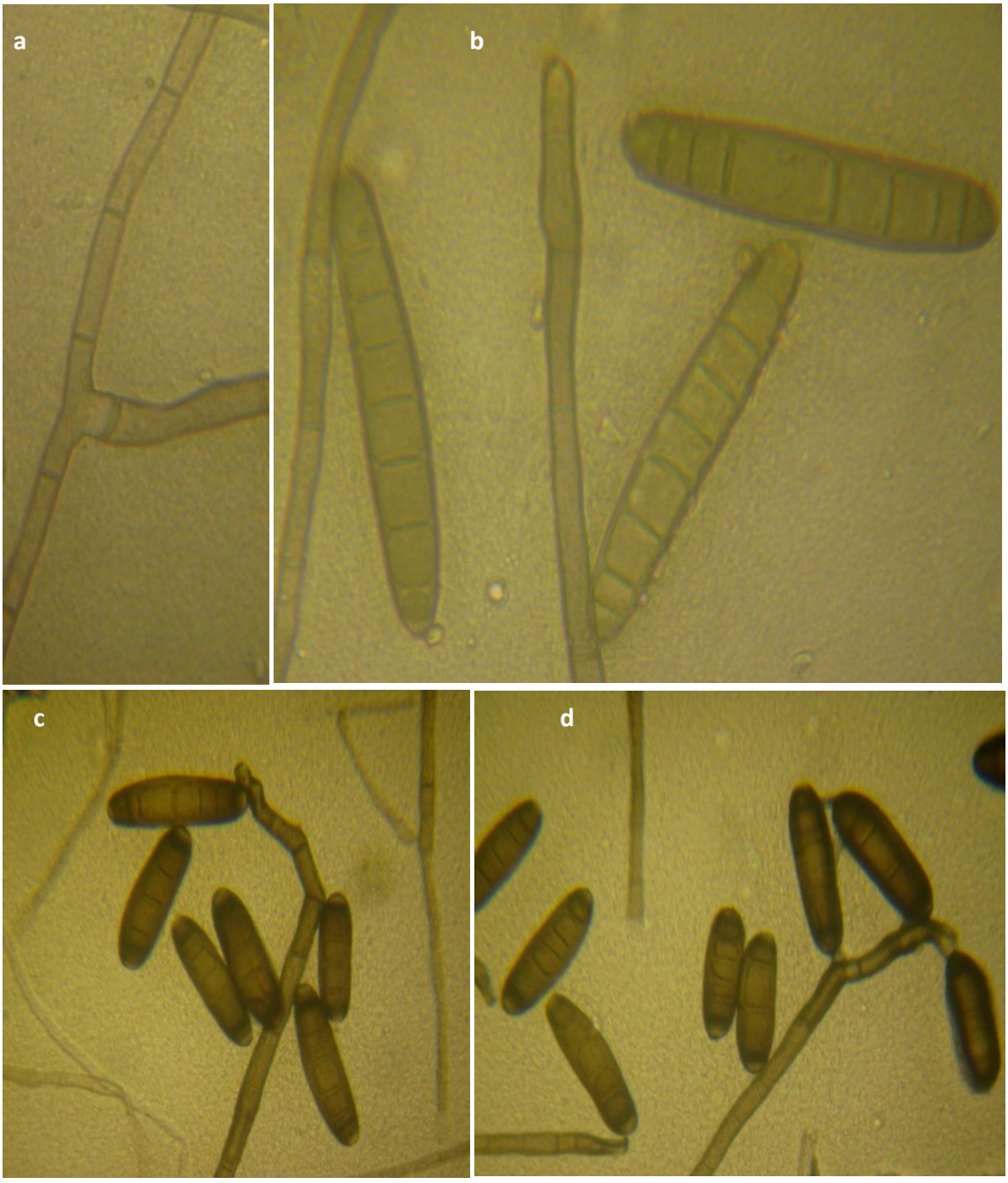

Figure 4.2: Conidiophores and conidia of *Bipolaris secalis* isolated from *Brachiaria* grass in Rwanda. a: Conidiophore, b – d: Conidiophores and conidia. Images of conidia and conidiophores were taken by camera installed on an OPTIKA B-350 microscope system. Conidia were obtained from fungi grown on PDA at 28ºC for three weeks.

The radial growth of *Bipolaris secalis* isolates varied depending on time, isolate and media type (Figure 4.3). The average radial growth of tested *Bipolaris secalis* isolates ranged from 3.5 to 5.1 cm at 14 days.

The radial growth of the causal organism on three media types was variable with maximum growth on *Brachiaria* leaf extract agar followed by PDA supplemented with *Brachiaria* leaf extract and PDA.

The PDA supplemented with *Brachiaria* leaf extract led to the higher radial growth for all the isolates than on PDA. The linear growth of the causal organism on three different media was variable with maximum at agar supplemented with leaf extract followed by PDA supplemented with leaf extract. Agar medium supplemented with leaf extract led to the higher radial growth of isolates than the radial growth of *Bipolaris secalis* isolates on PDA supplemented with leaf extract (Figure 4.4).

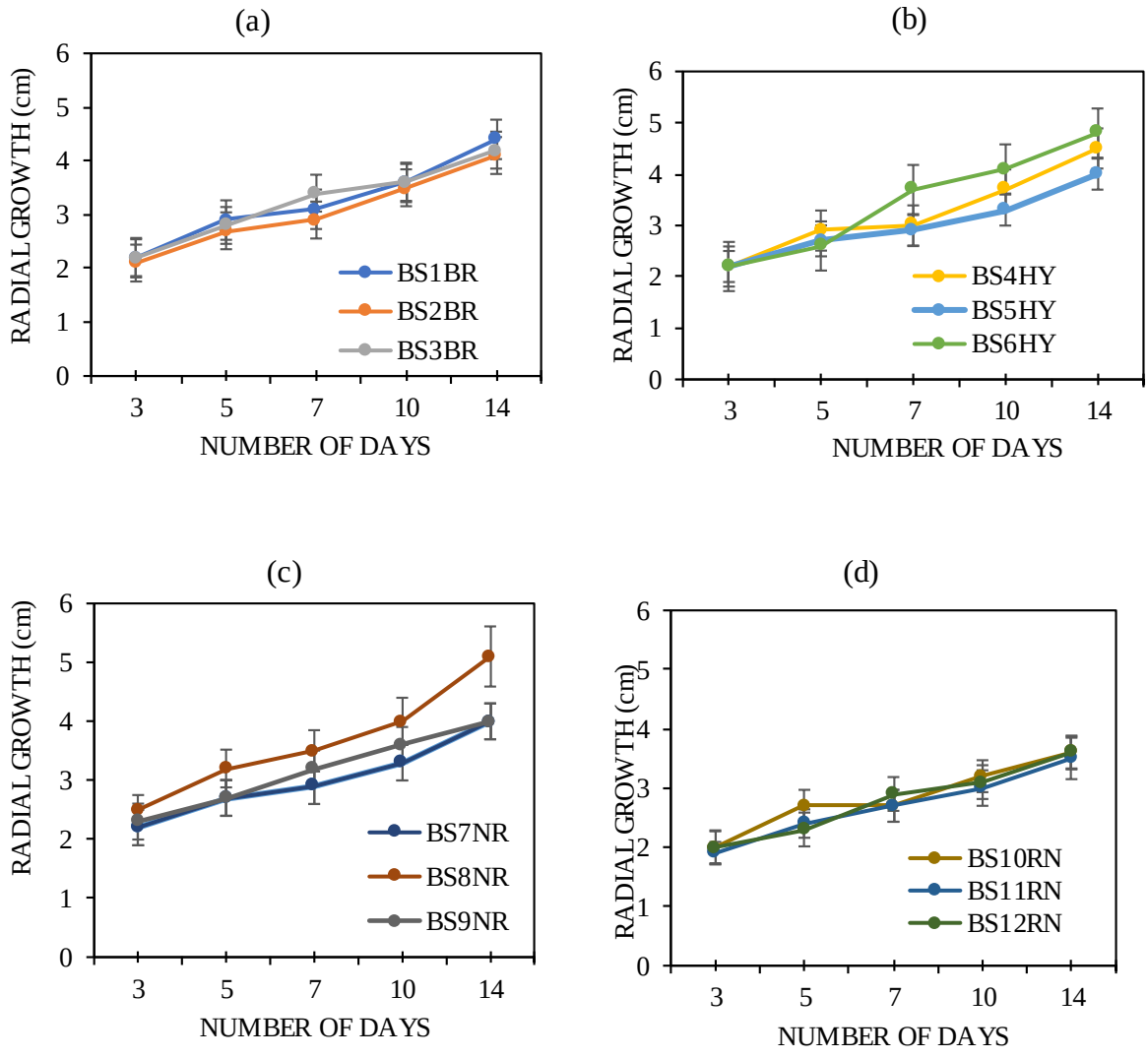

Figure 4.3: Radial growth of *Bipolaris secalis* isolates on PDA medium over time (in days). a) BS1BR – BS3BR = Isolates from Bugesera, b) BS4HY – BS6HY = Isolates from Huye, c) BS7NR – BS9NR = Isolates from Nyagatare and d) BS10RN – BS12RN = Isolates from Rwamagana. Error bars indicate standard errors of the mean.

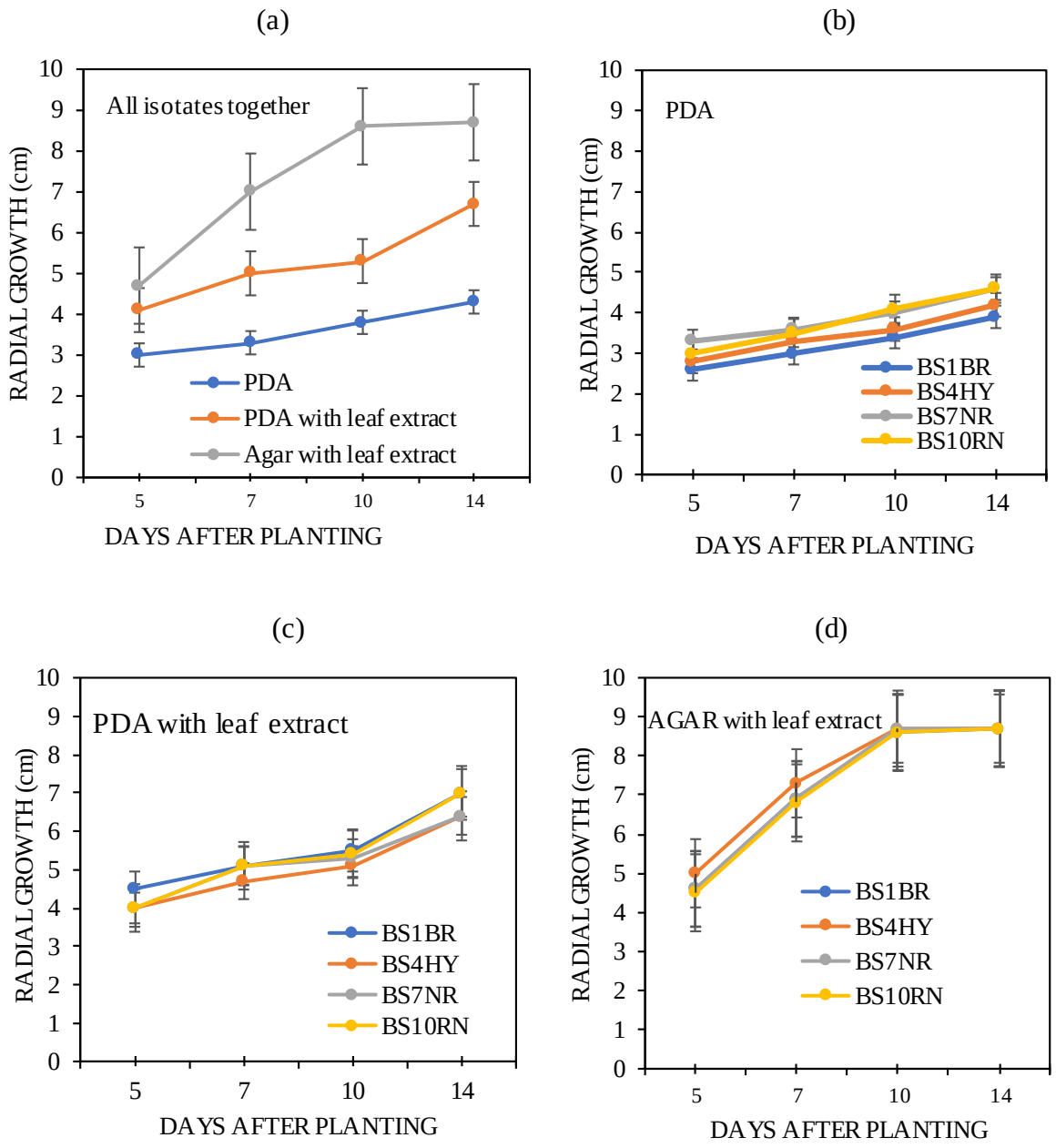

Figure 4.4: Radial growth (cm) of *Bipolaris secalis* isolates at different days after planting on PDA and water agar with or without amendment. BS1BR = Isolate from Bugesera, BS4HY = Isolate from Huye, BS7NR = Isolate from Nyagatare and BS10RN = Isolate from Rwamagana. a) All isolates are considered together, b) Isolates grown on PDA, c) Isolates grown on PDA with leaf extract and d) Isolates grown on Agar with leaf extract. Error bars indicate standards errors of the mean.

For leaf rust, pustules with yellowish or brownish colours were mostly found on the adaxial surface of leaves. Spore was ellipsoidal or circle, yellowish or brownish with the length of 23 – 29 µm and 16 – 17.5 µm of width (Table 4.5; Figure 4.5).

Table 4.5: Size of spores of *Phakopsora apoda* isolates

Isolate	Size of rust spore (µm)	
	Length	Width
Bugesera	23.8 ± 2.4^{ab}	16.7 ± 1.7^{a}
Huye	23.3 ± 1.7^{ab}	15.8 ± 0.8^{a}
Nyamagabe	29.2 ± 2.4^{a}	15.8 ± 2.0^{a}
Nyagatare	22.5 ± 1.7^{b}	18.3 ± 1.7^{a}
Rwamagana	26.7 ± 1.7^{ab}	17.5 ± 1.1^{a}
Mean	25.1 ± 0.9	16.8 ± 0.7

± is followed by standard error of the mean. Values with the same letters within the column are not statistically different at the probability level of 0.05.

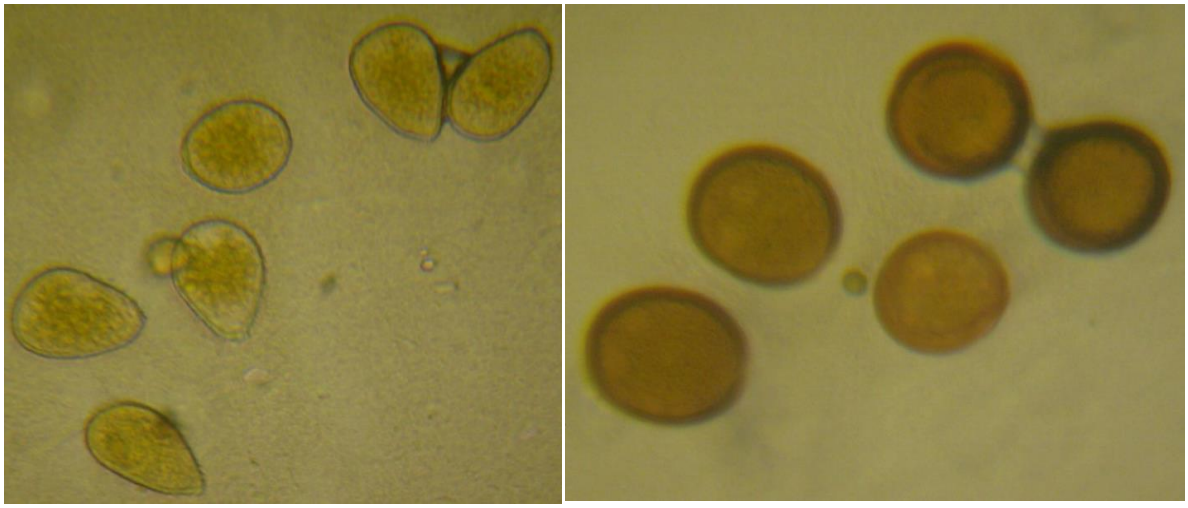

Figure 4.5: Spores of *Phakopsora apoda* isolated from *Brachiaria grass* in Rwanda. They were photographed using a camera installed on an OPTIKA B-350 microscope.

4.4.3 Molecular identification and phylogenetic relationships of *Bipolaris secalis* and *Phakopsora apoda* isolates

The molecular identification using ITS and GAPDH sequences confirmed the results of physical characterisation. Both primer sets yielded good amplification of both target regions for all 12 isolates. The ITS primers amplified the size of DNA amplicons varying between 532 and 582 bp while GAPDH primers produced amplicon size between 443 and 530 bp. The details about these amplicons following BLAST search in the NCBI fungal database are presented in Tables 4.6 and 4.7**.**

The ITS sequences of 11 isolates matched to genebank accession MH864688.1 and one isolate from Bugesera matched to the genebank accession number KU554556.1 and respective sequences were submitted to NCBI under the gene accession number from MW159734 to MW159745 (Table 4.6). GAPDH sequences of all 12 isolates matched to the genebank accession number KJ415409.1. The phylogenetic analysis based on ITS sequences for 12 isolates revealed them in two major clades. The first clade was composed of one isolate from Nyagatare district (BS9NR) whereas the second clade included 11 isolates (Figure 4.6). These 11 isolates were further divided into three sub-clades. The phylogenetic analysis based on GAPDH sequences led to nine major clades (Figure 4.7).

For leaf rust isolates, rust specific primers amplified the size of DNA between 1291 bp and 1381 bp while the DNA size varied between 874 bp to 882 bp when the amplification was done using LSU primers. The identity of the sequence was 96%, sequence coverage of 60% with e-value equal to zero for rust primers and the sequence identity was 94.97% to 95.37%, sequence coverage of 99% to 100% and e-value of 0 for LSU primers. All sequences recovered from five isolates of leaf rust matched to the genebank accession number MG461668.1 for both rust specific and LSU sequences (Table 4.8).

Table 4.6: Twelve *Bipolaris secalis* isolates, 18S rDNA sequence characteristics, homology search results, and genebank accession number

Code of isolate	Identified species	Length (bp)	Genebank accession number	Sequence cover age (%)	e value	Sequence identity (%)	Accession number referring to identity
BS1BR	*Bipolaris secalis*	532	MW159734	98	0	98.09	KU554556.1
BS2BR	*Bipolaris secalis*	561	MW159735	100	0	99.46	MH864688.1
BS3BR	*Bipolaris secalis*	565	MW159736	98	0	97.20	MH864688.1
BS4HY	*Bipolaris secalis*	573	MW159737	97	0	97.54	MH864688.1
BS5HY	*Bipolaris secalis*	567	MW159738	100	0	99.64	MH864688.1
BS6HY	*Bipolaris secalis*	563	MW159739	99	0	99.47	MH864688.1
BS7NR	*Bipolaris secalis*	559	MW159740	100	0	98.76	MH864688.1
BS8NR	*Bipolaris secalis*	562	MW159741	97	0	99.30	MH864688.1
BS9NR	*Bipolaris secalis*	582	MW159742	100	0	99.30	MH864688.1
BS10RN	*Bipolaris secalis*	562	MW159743	99	0	99.29	MH864688.1
BS11RN	*Bipolaris secalis*	574	MW159744	100	0	99.47	MH864688.1
BS12RN	*Bipolaris secalis*	569	MW159745	100	0	98.75	MH864688.1

The isolates were obtained from *Brachiaria* leaves showing typical symptoms of leaf spot disease. ITS primers were used to identify each isolate. NR = Nyagatare; BR = Bugesera; RN = Rwamagana; HY = Huye

Table 4.7: Twelve *Bipolaris secalis* isolates, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sequence characteristics, homology search results

Isolate code	Identified species	Sequence Length (bp)	Sequence coverage (%)	e-value	Sequence identity (%)	Matching NCBI accession number
BS1BR	*Bipolaris secalis*	530	99	0	98.69	KJ415409.1
BS2BR	*Bipolaris secalis*	498	100	0	99.00	KJ415409.1
BS3BR	*Bipolaris secalis*	501	100	0	99.20	KJ415409.1
BS4HY	*Bipolaris secalis*	482	100	0	99.79	KJ415409.1
BS5HY	*Bipolaris secalis*	520	98	0	97.14	KJ415409.1
BS6HY	*Bipolaris secalis*	444	100	0	99.55	KJ415409.1
BS7NR	*Bipolaris secalis*	483	100	0	99.59	KJ415409.1
BS8NR	*Bipolaris secalis*	483	100	0	99.59	KJ415409.1
BS9NR	*Bipolaris secalis*	443	100	0	99.77	KJ415409.1
BS10RN	*Bipolaris secalis*	458	100	0	99.35	KJ415409.1
BS11RN	*Bipolaris secalis*	529	98	0	97.38	KJ415409.1
BS12RN	*Bipolaris secalis*	487	100	0	99.59	KJ415409.1

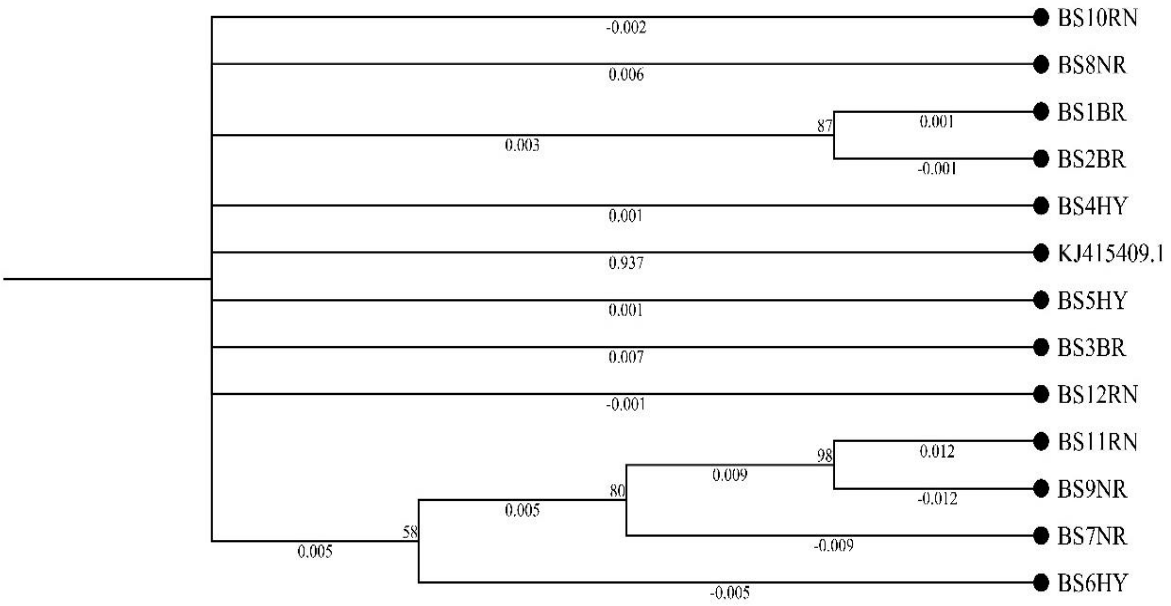

Figure 4.6: Phylogenetic relationship among 12 isolates of *Bipolaris secalis* based on 18S rDNA sequences. The tree was constructed using Neighbour joining method and nucleotide distance measured using Jukes-Cantor and bootstraps were analysed at 1000 replicates.

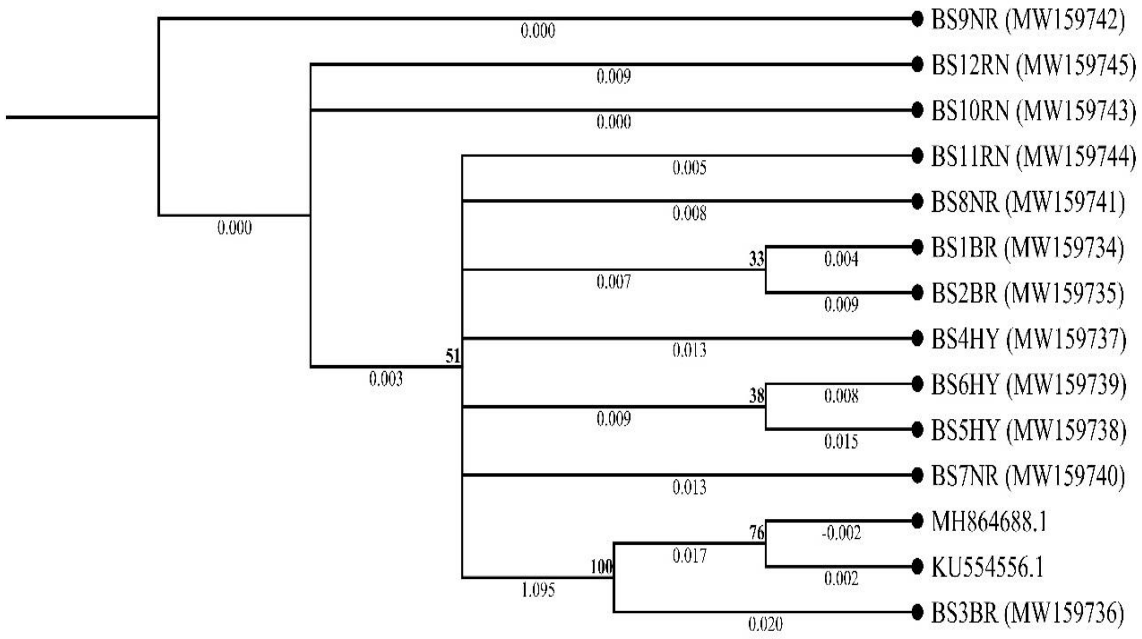

Figure 4.7: Phylogenetic relationship among 12 isolates of *Bipolaris secalis* based on glyceraldehyde-3-phosphate dehydrogenase sequences. The tree was constructed using Neighbour joining method, and nucleotide distance measured using Jukes-Cantor and bootstraps were analysed at 1000 replicates.

Table 4.8: Five *Phakopsora apoda* isolates, rust primer and LSU sequence characteristics, homology search results

Primer	Isolate	Identified species	Sequence length (bp)	Sequence coverage (%)	e-value	Sequence identity (%)	Matching NCBI accession
Rust specific	Bugesera	*Phakopsora apoda*	1370	60	0	96	MG461668.1
	Huye	*Phakopsora apoda*	1381	60	0	96	MG461668.1
	Nyamagabe	*Phakopsora apoda*	1374	60	0	96	MG461668.1
	Nyagatare	*Phakopsora apoda*	1291	60	0	96	MG461668.1
	Rwamagana	*Phakopsora apoda*	1378	60	0	96	MG461668.1
LSU	Bugesera	*Phakopsora apoda*	882	100	0	95.37	MG461668.1
	Huye	*Phakopsora apoda*	882	100	0	95.25	MG461668.1
	Nyamagabe	*Phakopsora apoda*	868	100	0	94.72	MG461668.1
	Nyagatare	*Phakopsora apoda*	876	99	0	95.09	MG461668.1
	Rwamagana	*Phakopsora apoda*	874	99	0	94.97	MG461668.1

4.4.4 Pathogenicity of *Bipolaris secalis* and *Phakopsora apoda* isolates on susceptible *Brachiaria* seedlings

The results of the pathogenicity test revealed that all isolates investigated produced a typical leaf spot symptom on the leaves of *Brachiaria humidicola* cv. Humidicola seedlings. Inoculated plants showed leaf spot symptoms four days after inoculation for all isolates whereas leaves of seedlings used for negative controls did not show disease symptoms. All the isolates caused necrotic symptoms surrounded by purple colour (Figure 4.8a) and they were similar to those found in naturally infected leaves of *Brachiaria* grass (Figure 4.8b). Symptomatic leaves from the inoculation experiment were used to re-isolate causal agents. A total of four isolates each representing four districts were obtained, and their morphological examination and molecular identification confirmed them as *Bipolaris secalis*. These isolates when inoculated to healthy *Brachiaria humidicola* cv. Humidicola seedlings produced typical leaf spot symptoms and the postulates of Koch were fulfilled.

The same procedure was performed for inoculation and re-isolation of *Phakopsora apoda* isolates, and all five isolates were pathogenic to *Brachiaria* hybrid Mulato and caused similar symptoms as the ones observed in natural infection. The incubation period (IP) required for the first development of rust symptoms varied between 10 – 18 days after inoculation (Figure 4.9).

Figure 4.8: Pathogenicity of 12 *Bipolaris secalis* isolates from different districts of Rwanda on *Brachiaria humidicola* cv. Humidicola seedlings. a: Isolates BS1BR – BS3BR; BS4HY – BS6HY; BS7NR – BS9NR and BS10RN – BS12RN originate from Bugesera, Huye, Nyagatare and Rwamagana districts of Rwanda, respectively; C = Negative control. b: Leaf spot symptoms of *Bipolaris secalis* on naturally infected leaves of *Brachiaria humidicola* cv. Humidicola (Indicated by yellow-coloured arrows).

Figure 4.9: Pathogenicity of *Phakopsora apoda* isolates from different regions of Rwanda on *Brachiaria* Hybrid cv. Mulato. 1: Bugesera isolate; 2: Huye isolate; 3: Nyamagabe isolate, 4: Nyagatare isolate, 5: Rwamagana isolate, 6: Negative control, 7: Leaf rust symptoms caused by *Phakopsora apoda* on naturally infected leaves of *Brachiaria* hybrid cv. Mulato.

4.5 Discussion

The isolation and identification of microorganisms with three major diseases of *Brachiaria* grass indicated that apart from leaf rust which was associated with only one microorganism, *Phakopsora apoda*, leaf spot and leaf blight were associated with multiple microorganisms. Identification of rust pathogen isolates using molecular techniques matched *Phakopsora apoda* sequence on top in the database of NCBI query cover of 60%. The low percent query cover found in this study might be attributed to the rust fungi sequences from sequences that are unique and available in genebank. This calls for further investigation on these isolates of rust for reliable diagnosis and their exact taxonomic placement. *Phakopsora apoda* has been proven to cause rust disease infecting Kikuyu grass (Adendorff, 2014; Adendorff and Rijkenberg, 1995). Lenné (1990) and Marchi *et al.* (2007) indicated *Uromyces setariae-italicae* and *Puccinia levis* var. *panici-sanguinalis* as causal agents of rust disease affecting *Brachiaria* grass.

Another study by Brown and Vargas (1982) indicated that *Nigrospora* which is one among the microorganisms isolated from leaf tissues having leaf blight symptoms, has been the causal agent of *Nigrospora* patch disease in Kentucky Blue Grass.

Some studies conducted in Colombia have revealed that *Brachiaria* foliar blight disease is caused by *Rhizoctonia solani* (Alvarez *et al.*, 2013; Kelemu *et al.*, 1995;). It is important to note that, this study did not reveal any isolate that belonged to *Rhizoctonia* genus.

The analysis of the fungi community on leaf tissue with leaf spot symptoms indicated the association of 12 distinct fungal taxa where *Bipolaris secalis* was the dominant species. *Bipolaris secalis* has been shown as a pathogen of a native Mexican tree, Jangada Brava (*Heliocarpus americanus*) and rye (*Secale cereal*) (Sisterna, 1989). This study revealed that a lot of fungi that were isolated from leaf tissues having leaf spot and leaf blight symptoms were documented to be saprobes and endophytes in different hosts (Adendorff, 2014; Barnes and Szabo, 2007; Bernardi *et al.*, 2018; Ghimire *et al.*, 2011; Sánchez Márquez *et al.*, 2007; White and Backhouse, 2007).

This study elucidates the aetiology of leaf rust and leaf spot diseases of *Brachiaria* grass in Rwanda. Based on morphological characteristics, internal transcribed spacer (ITS) 18S rDNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sequences, and pathogenicity test, 12 fungi isolates examined in this study were identified as *Bipolaris secalis*. Previously, a study on surveillance of *Brachiaria* grass diseases in Rwanda indicated widespread distribution of leaf spot disease in the country and frequent association of *Bipolaris secalis* with the disease (Uzayisenga *et al.*, 2020). *Bipolaris* species have been reported to have a wider host range, specifically grasses (Manamgoda *et al.*, 2014; Marin-Felix *et al.*, 2017).

Likewise, analysis of sequences generated from rust and LSU primers confirmed *Phakopsora apoda* as the causal agent of *Brachiaria* grass. The ITS sequence of *Bipolaris secalis* generated in this study had sequence length varying from 532 bp to 582 while the length was from 443 to 530 bp for GAPDH sequences. It corroborates with the findings of Manamgoda *et al.* (2012) where ITS analysis of *Bipolaris* isolates led to 530 bp while GAPDH analysis led 495 bp (Bhunjun *et al.*, 2020). This work demonstrates the effectiveness of ITS and GAPDH sequences in the study of *Bipolaris secalis* isolates. Bhunjun e*t al.* (2020) reported GAPDH as the best marker to discriminate *Bipolaris* species and recommended its use in naming of *Bipolaris* species (Berbee *et al.*, 1999; Manamgoda *et al.*, 2014). The current study provides new etiological information about *Bipolaris secalis* and *Phakopsora apoda*, revealing these species for the first time as the causal agent of leaf spot and leaf rust, respectively, on *Brachiaria* grass in Rwanda.

From the literature, the descriptions of *Bipolaris secalis* and *Phakopsora apoda* reported by different authors are closely related to the findings of this study.

Morphological characteristics of conidia and conidiophores and the size are within the ranges reported for *Bipolaris secalis* (Bernardi *et al.*, 2018; Sisterna, 1989). *Bipolaris* species have been reported to have wider host range, specifically grasses (Manamgoda *et al.*, 2014; Marin–Felix *et al.*, 2017). The association of leaf spot disease with *Bipolaris secalis* as causal agent in Rwanda is relevant due to the presence of many factors such as conducive/favourable climatic conditions that facilitate the disease to develop and spread. Furthermore, local *Brachiaria* grass that are naturally present and the establishment of other potential hosts that can serve as alternate hosts to *B. secalis*. Jain *et al*. (2019) highlighted the role of environmental factors in development of foliar diseases. In a previous study conducted in Rwanda, 12 different fungi species with *Bipolaris secalis* as the most frequent fungi were found to be associated with *Brachiaria* grass disease symptoms. The leaf spot disease caused by *Bipolaris* species has been reported on several grasses including panicum, wheat, and rice. Some reports confirmed that it is a pathogen of *Secale cereale* (Sisterna, 1989) and Jangada Brava (*Heliocarpus americanus* L.) (Bernardi *et al.*, 2018). This species was also reported to cause disease on genus *Pennisetum*. Zhae

All isolates of *Bipolaris secalis* exhibited similar morphological characteristics with whitish edges and greyish colour on surface on PDA media. These findings suggest that all isolates used in our study are closely related. Similar features were reported by other authors (Manamgoda *et al.*, 2014). The radial growth of all isolates was contrasted with the findings of Sisterna (1989). In this study, the growth was lower than in the study of Sisterna (1989). This variation may be due to the difference between strains used in the study. The results of this study agree with other authors who reported the slow growth of *Bipolaris* species (Yamaguchi and Mutsunobu, 2010).

The optimum growth and sporulation of all isolates were recorded on media supplemented with leaf extract indicating that leaf extract brought additional substances which favoured the growth of isolates. Authors indicated that media type and their chemical compositions significantly affected the mycelia growth rate and the production of conidia of *Phoma exigua*. Plant exctrat based culture media were indicated to enhance the fungal growth and sporulation of different species (Uppala *et al.*, 2019; Zhao and Shamoun, 2006).

Morphological grouping of fungal species based on characteristics of colony and mycelial growth was documented in different studies (Jaiswal *et al.*, 2007; Mann *et al.*, 2014). The ITS sequencing data revealed two distinct clusters of *Bipolaris secalis* isolates with several subgroups within one of the two genetic groups, showing a low level of genetic diversity within *Bipolaris secalis*. Similar results of cluster analysis were reported. Isolates of *Bipolaris oryzae* were clustered in two different clusters with several subgroups within each genetic group using ITS sequence data (Archana *et al.*, 2014).

4.6 Conclusions

This is the first study that characterised the causal agents of *Brachiaria* grass diseases (Leaf spot and leaf rust) in Rwanda using morphological features, molecular analysis and pathogenicity tests. Referring to the colony, conidial morphological characteristics, analysis of ITS and GAPDH sequences, and pathogenicity test *Bipolaris secalis* was confirmed to be the causal agent of *Brachiaria* leaf spot disease in Rwanda. All *Bipolaris secalis* isolates recovered from *Brachiaria* grass leaves with leaf spot symptoms were similar in morphology, had a high ITS & GAPDH sequence identity and were constantly pathogenic to *Brachiaria humidicola* cv. Humidicola seedlings. Similarly, morphological features, rust and LSU sequence analysis of five isolates of leaf rust confirmed *Phakopsora apoda* as the causal agent of leaf rust of *Brachiaria* grass in Rwanda. All rust isolates caused rust symptoms on *Brachiaria* Hybrid cv. Mulato and they were similar with re-isolates. The information generated in this study will be highly important for the development of management strategies and will contribute to future studies on different aspects of *Bipolaris secalis* and *Phakopsora apoda*.

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CHAPTER FIVE:
SEQUENCING AND GENOMIC CHARACTERIZATION OF BIPOLARIS SECALIS

5.1 Abstract

Bipolaris genus includes devastating fungal pathogens with a wide range of hosts including forages and agricultural crops. *Bipolaris secalis* causes leaf spot disease of *Brachiaria* grass in Rwanda. However, no information is available about genome and genetic variability of *Bipolaris secalis*, which is very important to develop an effective disease control strategy. The objective of this study was to determine the whole genome sequence and genomic characterisation of *Bipolaris secalis*. Twelve *Bipolaris secalis* isolates were sequenced for whole genome in illumina platform, short reads were assembled. De novo assembly isolate BS7 was performed by various k-mer using Platanus-allee and it was used as a reference genome for other 11 isolates. The best k-mer was selected by the status of assembled results like the number of contigs, contig sum, N50 and the genome size was estimated. Eleven isolates were re-sequenced and mapped to the isolate BS7. Illumina sequencing of BS7 produced the estimated genome size of 34,813,291 bp with an average GC content of 50.01%, organised into 108 contigs, with the longest contig of 2,265,317 bp, the N50 of 1,032,497 bp and L50 of 12. The self-mapping of BS7 was 97.69% as only 48,559,991 reads mapped to the total filtered reads of 49,706,737. While mapping sequences of 11 isolates to BS7 the final mapping rate was between 80 and 95%, consisting of 28,950,637 – 15,611,348 total mapped reads. The high mapping rate was found in isolates BS2, BS6, BS8 and BS11. The phylogenomic tree analysis revealed three different clades where one clade grouped three isolates with the reference (BS7) while the remaining clades isolates clusted together with four isolates each. The genomic data generated in this study will serve as unique resource for further studies on *Bipolaris secalis* as well as it will contribute to identification of novel sources of genetic resistance against leaf spot disease and formulating new strategies for the disease control.

Key words: *Bipolaris secalis*, *Brachiaria*, contigs, Illumina platform, phylogenomic tree, reads, sequence, whole genome.

5.2 Introduction

Brachiaria grass is among significant plants contributing to animal nutrition qualitatively and quantitatively. It is native to Africa and it is one of the preferred forages with wide distribution in tropical and sub-tropical regions of eastern and western hemispheres (Renvoize *et al.*, 1996). Agricultural and environmental merits of *Brachiaria* grass including drought resilience, shade tolerance, nutritious to livestock, soil fertility improvement, high biomass yields, high nutrient use efficiency, mitigation of adversity of climate changes and effective bioagents for pests and parasitic weed management were reported (Khan *et al.*, 2016; Maass *et al.*, 2015; Subbarao *et al.*, 2009). However, the performances of *Brachiaria* grass are affected by several foliar diseases including leaf spot.

Brachiaria leaf spot caused by a fungus *Bipolaris secalis* is one of the emerging threats to *Brachiaria* grass in Rwanda (Uzayisenga *et al.*, 2020). The genus *Brachiaria* has about 100 species among which eight species were introduced, evaluated and integrated into the mixed crop-livestock system in Rwanda (Ghimire *et al.*, 2015; Mutimura *et al.*, 2016, 2018). The challenges caused by diseases in *Brachiaria* production system was reported in East Africa including Rwanda (Nzioki *et al.*, 2016; Uzayisenga *et al.*, 2020). Effective management of any plant disease requires good understanding of the biology and genetics characteristics of causal agent. Genetic variability of fungi including *Bipolaris* species have been studied using molecular techniques (Caligiorne *et al.*, 1999). Several molecular methods used include Random Amplified Polymorphic DNA (RAPD) (Kumar *et al.*, 2011), PCR, Restriction Fragment Length Polymorphism (RFLP) (Weikert-Oliveira *et al.*, 2002) and Repetitive Extragenic Palindromic-PCR genomic fingerprinting (rep-PCR) (Nazari *et al.*, 2015). The MycoBank database mentions *Bipolaris secalis* belongs to Ascomycota, Dothideomycetes, Pleosporales, Pleosporaceae.

Many species in genus *Bipolaris* are plant pathogens worldwide and the genome of several *Bipolaris* were published including *B. cookie, B. maydis, B. sorokiniana, B. zeicola, B. oryzae* and *B. victoriae* with respective genome size of 36,171,030 bp; 36,456,735 bp; 34,417,436 bp; 31,267,936 bp; 31,362,097 bp and 32,829,575 bp respectively (Zaccaron and Bluhm, 2017). However, the genome of *Bipolaris secalis* needs to be studied.

The genome information can help in understanding the mechanisms and the history of changes in size of genome and evolution of plant pathogens including *Bipolaris secalis*. *Bipolaris secalis* was first reported in *Brachiaria* fields in Rwanda in 2020 (Uzayisenga *et al*., 2020). Despite the importance of *Brachiaria* grass, several studies can be limited due to the lack of the reference genomes of *Bipolaris secalis*. *Brachiaria* grass is affected by a wide range of fungal diseases including leaf spot and it was documented as one of the major diseases of *Brachiaria* grass in East Africa (Nzioki *et al.*, 2016; Uzayisenga *et al*., 2020). Molecular basis of *Bipolaris secalis* causing leaf spot on *Brachiaria* grass is poorly understood. The objective of this study was to avail whole genome sequence of *Bipolaris secalis* and examine genetic diversity of *Bipolaris secalis* isolates originating from four *Brachiaria* grass growing districts of Rwanda.

5.3 Materials and methods

5.3.1 Isolate collection and culturing

For isolates used for the whole genome sequencing and assembly, symptomatic samples were collected in four districts of Rwanda (Bugesera, Huye, Nyagatare and Rwamagana) in 2018 – 2019. Identity of isolates were confirmed through macroscopic and microscopic observations as well as through ITS and GAPDH sequence analysis prior to whole genome sequencing. All isolates were confirmed as pathogens of *Brachiaria* grass and causal agent of leaf spot disease. Fungal isolation was conducted using PDA medium and single spore cultures were maintained at - 80°C for further use. Fungal isolates were subcultured on PDA amended with ampicillin (100 mg L^{-1}) for 3 days in darkness. Thereafter, isolates were grown for four days in Potato Dextrose Broth (PDB) amended with ampicillin and chloramphenicol (100 mg L^{-1} of each antibiotic) at 28°C with shaking at 100 RPM.

5.3.2 DNA extraction

Genomic DNA (gDNA) was extracted from mycelia obtained from single spore culture for each of 12 *Biporalis secalis* isolates (BS1, BS2, BS3, BS4, BS5, BS6, BS7, BS8, BS9, BS10, BS11 and BS 12). The mycelium was harvested by filtration, rinsed with sterile distilled water and dried between sterile filter papers prior to DNA extraction. For good DNA quality for short read sequencing, Qiagen DNeasy Plant Mini Kit was used for DNA extraction.

The manufacturer protocol was used except that the mycelia were mixed with sterilized carborundum (0.01%) for easy break of cells. The grinding was done in liquid nitrogen using sterile mortar and pestle and next steps of DNA extraction used 20 mg of the fine ground powder of each sample.

5.3.3 Determination of DNA concentration, purity and integrity

The DNA concentration and purity were evaluated spectrophotometrically using a NanoDrop 2000 (Thermo Scientific, Waltham, MA) and the A260/280 and A260/230 absorbance ratios obtained were recorded to ensure and keep DNA with good purity. Only samples having 1.8 – 2.0 for A260/280 value and the concentration above 20 ng/µl were used for sequencing. Integrity of DNA was evaluated with gel electrophoresis using 1% (w/v) agarose in 0.5 × TAE buffer followed by staining using GelRed® at 70 Volt for one hour. DNA fragment and distribution were visualized under UV light and the 1 kb Plus ladder was used for estimation of fragment sizes.

5.3.4 DNA library preparation and genomic DNA sequencing

DNA samples of 12 *Bipolaris secalis* isolates were frozen and submitted to Macrogen Inc., Seoul, Korea for genome sequencing. The quantity and quality of original DNA samples were checked by PicoGreen method using Victor 3 fluorometry (Fluorescence based quantification) and by determining the gDNA integrity (DNA Integrity Number/DIN) using TapeStation and gDNA Screen Tape respectively prior to construction of the library. Highly degraded DNA is indicated by a low DIN value (DIN ~1) while highly intact gDNA gives a high DIN value (DIN ~10) (Corcoll *et al.*, 2017). The library was constructed using TruSeq Nano DNA (350) Library kit (350 bp insertion) and the amount of at least 0.100 µg of the total input of gDNA was used. Final libraries were undergone quality check (QC) through distribution on an Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip and quantity PCR (qPCR) for template size and template quantity, respectively. The DNA library was sequenced using illumina platform to give 151 bp reads in paired – end sequencing. The de novo assembly was done for one isolate (BS7) and the remaining isolates (11 isolates) were re-sequenced based on BS7 as it was sharing similar features with most of the isolates.

5.3.5 Data processing, de novo genome assembly and genome assembly validation

Reads pre-processing was performed and quality control of raw reads were done using FastQC (v0.11.5). Total bases, GC content, total reads, number of contigs, N50 and the quality of the reads were generated for BS7 after filtering and removing any contamination. The adapter trimming and quality filtering were done using Trimmomatic tool for reducing bias in the analysis (Bolger *et al.*, 2014) and only contigs which had the first hit *as Bipolaris sorokiniana* or *Bipolaris victoriae* were maintained for further analysis. The genome size was estimated using k-mer analysis before assembly (Marcais and Kingsford, 2011). The assembled genome was validated using self-mapping strategy and Benchmarking Universal Single Copy Ortholog (BUSCO) analysis. Therefore, filtered reads were aligned against the assembled genome and their insert size was estimated for validation (Simao *et al.*, 2015).

5.3.6 Genome mapping and single nucleotide polymorphism (SNP) analysis

Re-sequenced data of 11 isolates were mapped to the assembly of BS7 which was used as the reference genome to check genetic variations among all isolates. Therefore, processed reads of re-sequenced isolates were mapped to the reference assembly (BS7) using Burrows-Wheeler Aligner (BWA) and the mapping ratio was determined. The algorithm BWA-MEM was selected because it is optimized for aligning reads greater than 70 bp, has an advantage of providing split alignment and it is better in terms of speed and accuracy than other algorithms. After mapping, Sambamba and SAMTools were used to remove duplicated reads and identify single nucleotide polymorphisms, respectively (Danecek *et al.*, 2021; Tarasov *et al.*, 2015).

5.3.7 Phylogenomic analysis of *Bipolaris secalis*

The Phylogenomic tree was constructed to show relationships between 12 *Bipolaris secalis* isolates collected from Huye, Bugesera, Rwamagana and Nyagatare districts of Rwanda using UGENE software (Okonechnikov *et al.*, 2012). PhyML Maximum Likelihood tree building method was used and the nucleotide substitution model was HK85 for optimizing for tree topology and substitution rate for tree searching. Branches were supported using 100 bootstraps.

5.4 Results

5.4.1 Quality and quantity of original DNA and libraries

The total amount of DNA varied between 0.12 and 0.29 while the DIN value of DNA samples ranged from 6.4 and 7.7 (Table 5.1) indicating that all samples met minimum requirements for TruSeq Nano (350) library preparation. Similarly, all libraries passed QC and their concentration ranged from 6.18 ng/µl to 89.16 ng/µl and the library size was between 564 bp and 638 bp (Table 5.2).

Table 5.1: Quality and quantity of original DNA of *Bipolaris secalis* isolates

Nº	Isolate ID	District of isolation	Concentration (ng/µl)	Final volume (µl)	Total amount (µg)	DIN
1	BS1	Bugesera	10.68	18	0.19	6.5
2	BS2	Bugesera	12.56	20	0.25	7.6
3	BS3	Bugesera	10.77	19	0.20	7.3
4	BS4	Huye	11.86	18	0.21	7.9
5	BS5	Huye	10.95	15	0.16	6.7
6	BS6	Huye	11.42	24	0.27	7.7
7	BS7	Nyagatare	9.92	21	0.20	6.4
8	BS8	Nyagatare	7.33	17	0.12	7.2
9	BS9	Nyagatare	12.12	10	0.12	7.4
10	BS10	Rwamagana	10.24	24	0.24	7.9
11	BS11	Rwamagana	10.7	27	0.27	6.6
12	BS12	Rwamagana	11.07	27	0.29	6.6

Table 5. 2: Quantity of final libraries for whole genome sequencing

N°	Isolate ID	Location	Concentration (ng/µl)	Concentration (nM)	Size (bp)
1	BS1	Bugesera	81.92	211.1	597
2	BS2	Bugesera	6.36	15.34	638
3	BS3	Bugesera	66.07	168.86	602
4	BS4	Huye	80.49	202.01	613
5	BS5	Huye	83.58	208.4	617
6	BS6	Huye	6.18	15.27	622
7	BS7	Nyagatare	76.72	209.26	564
8	BS8	Nyagatare	6.72	16.54	625
9	BS9	Nyagatare	65.96	173.75	584
10	BS10	Rwamagana	74.36	196.24	583
11	BS11	Rwamagana	7.52	18.58	623
12	BS12	Rwamagana	89.16	233.28	588

5.4.2 Genome characteristics and assembly of BS7 and other *Bipolari*s species

All *Bipolaris secalis* isolates were sequenced using illumina platform. The sequencing depth for BS7 was 170X. De novo assembly was done for BS7 and led to the estimated genome size of 34,813,291 bp. The contig numbers were 108 corresponding to 78 contigs belonging to both *B. sorokiniana* and *B. victoriae*, and 14 and 16 contigs corresponding to *B. sorokiniana* and *B. victoriae*, respectively. Furthermore, the total contigs sum of 34,813,291, N50 of 1,032,497 bp, the shortest contig of 1,024 bp, the longest contig of 2,265,317 bp, the average length of 185,046 bp, L50 of 12 and the GC content of 50.01% were observed (Table 5.3). The percentage of self-mapping of BS7 was 97.69% for mapped reads and 2.31% of filtered reads were unmapped. The BUSCO results showed a high completeness of *Bipolaris secalis* BS7 genome assembly.

Available genomes from other *Bipolaris* species show that full genomes have 16 chromosomes with genome size ranging from 31 to 37 MB, with an L50 of about 6 or 7 scaffolds/contigs (Table 5.4).

Table 5.3: Genome size and contigs of *Bipolaris secalis* isolate BS7

Characteristics	Value
Genome size (bp)	34,813,291
Sequencing depth	170
Genome repeat length	3,877,833
Total read bases	7,461,957,959
Total reads	49,682,374
Contigs sum (bp)	34,813,291
Genome contig number	108
Contig N50 (bp)	902,022
Longest contig (bp)	2,265,317
Shortest contig (bp)	1,024
GC (%)	50.01
L50	12

Table 5 4: Genome characteristics of other *Bipolaris* species

Species	Genebank assembly accession	Date	Assembly level	Assembly method	Size	Genome coverage	Sequencing technology	Total number of scaffolds	Scaffold N50	Scaffold L50	Number of contigs	Contig N50	Contig L50	Chromosomes and Plasmids
B. maydis ATCC 48331	GCA_000354255.1	April, 2013	Scaffold	Allpaths-LG v. 2011	32,929,167	67.5x	Illumina	207	964,089	13	903	83,684	122	
B. maydis C5	GCA_000338975.1	February, 2013	Scaffold	Phrap v. 0.990319		36.46x	Sanger	68	1,842,487	7	88	1,168,586	12	0
B. maydis. Strain: BM1	GCA_019454015.1	August, 2021	Contig	Canu v. 2.1	36,230,473	251x	Illumina HiSeq; PacBio Sequel				27	1,859,413	7	1
B. oryzae ATCC 44560	GCF_000523455.1	January, 2014	Scaffold	AllPaths-LG v. r41043	31,362,097	191x	Illumina	619	134,117	68	671	131,724	71	
B. oryzae Isolate: TG12bL2	GCA_001675385.1	June, 2016	Scaffold	Velvet v. 1.1.05	31,674,030	229x	Illumina HiSeq	1,640	74,921	131	2,737	34,580	267	0
B. zeicola 26-R-13	GCF_000523435.1	January, 2014	Scaffold	AllPaths-LG v. r41043	31,267,936	200x	Illumina	844	110,153	82	882	105,171	86	
B. zeicola Strain: GZL10	GCA_016906865.1	February, 2021	Contig	SMARTdenovo v. 1.0	36,143,178	193x	PacBio RSII; Illumina HiSeq				23	2,045,011	7	
B. cookei	GCA_002286855.1	September, 2017	Scaffold	SPAdes v. 3.1	36,169,199	900x	Illumina Hi-Seq	320	378,688	31	531	288,149	43	1
B. sorokiniana ND90Pr	GCA_000338995.1	February, 2013	Scaffold	Newbler v. 2.5	34,409,167	42x	Sanger; Illumina	154	1,789,485	7	504	243,393	43	
B. sorokiniana BS112	GCA_000338995.1	March, 2019	Contig	MaSuRCA v. 2.7	37,377,538	80x	Illumina; ONT; IonTorrent				43	2,114,703	7	0

Species	Genebank assembly accession	Date	Assembly level	Assembly method	Size	Genome coverage	Sequencing technology	Total number of scaffolds	Scaffold N50	Scaffold L50	Number of contigs	Contig N50	Contig L50	Chromosomes and Plasmids
B. Strain: BRIP10943a	GCA_008452735.1	September, 2019	Chromosome	Canu v. 1.5	36,921,586	37x	PacBio RSII	22	2,135,441	7	22	2,135,441	7	16
B. sorokiniana, Strain: BRIP27492a	GCA_008452725.1	September, 2019	Chromosome	Canu v. 1.5	35,237,195	40x	ONT	19	2,128,958	7	19	2,128,958	7	16
B, sorokiniana	GCA_008452715.1	September, 2019	Chromosome	Canu v. 1.5	36,241,382	36x	ONT	21	2,111,142	7	21	2,111,142	7	16
B. sorokiniana (ascomycetes), Strain: WAI2406	GCA_008452705.1	September, 2019	Chromosome	Canu v. 1.5	36,886,791	35x	ONT	21	2,203,058	7	21	2,203,058	7	16
B. victoriae FI3 (ascomycetes)	GCA_000527765.2	September, 2020	Contig	Canu v. 1.6	33,973,299	37x	ONT				21	2,461,692	6	

5.4.3 Mapping of re-sequenced dataset of 11 isolates of *Bipolaris secalis* and variants

The remaining 11 isolates (re-sequenced) were assembled and aligned to the isolate BS7 genome assembly to have a general overview of genomic similarity among 12 *Bipolaris secalis* isolates. The results obtained when mapping dataset of 11 isolates to BS7 showed the final mapping ratio in the range of 80 – 95%. The high mapping ratio was found in BS2, BS6, BS8 and BS11. Table 5.5 shows the overall read mapping ratio which is the ratio of mapped reads to the total reads of the sample. The highest percentage of mapped reads was 95% with the mean depth varying from 52.76 to 107.75. The percentage of unmapped reads varied from 5% to 20%; four isolates had more than 90% and seven isolates had around 80% of mapped reads. The number of variants varied between 1,003 and 10,744 (Tables 5.5).

Table 5.5: Statistics for short read re-sequenced *Bipolaris* isolates, mapping and variants relative to the reference isolate BS7

Isolates	Reference genome length (bp)	Total reads	Mapped reads to the reference	Percentage mapped reads	Unmapped reads	Percentage unmapped reads	Variants	Mean depth
BS1	34,813,291	19,478,765	15,767,790	81	3,710,975	19	10,740	61.50
BS2	34,813,291	30,348,124	28,950,637	95	1,397,487	5	1,029	92.34
BS3	34,813,291	33,345,860	26,835,968	80	6,509,892	20	6,585	89.26
BS4	34,813,291	27,446,033	22,144,257	81	5,301,776	19	9,847	75.09
BS5	34,813,291	19,392,904	15,611.348	81	3,781,556	19	6,018	52.76
BS6	34,813,291	28,792,246	27,321,947	95	1,470,299	5	1,003	88.23
BS8	34,813,291	25,624,114	24,124,788	94	1,499,330	6	1,051	77.88
BS9	34,813,291	27,556,251	22,249,782	81	5,306,469	19	6,226	74.93
BS10	34,813,291	34,337,707	28,821,963	84	5,555,744	16	10,744	107.75
BS11	34,813,291	28,670,176	26,876,250	95	1,793,926	6	1,516	87.17
BS12	34,813,291	23,381,605	19,136,752	82	4,244,853	18	10,742	73.69

5.4.4 Phylogenomic tree analysis

The phylogenomic tree was constructed using 12 isolates of *Bipolaris secalis*. One population was formed and divided into three different clades. The first clade grouped three isolates (BS10, BS1, and BS12) together with the reference (BS7). Four isolates clusted together (BS11, BS8, BS6 and BS2) and (BS11, BS8, BS6 and BS2) for the second and the third clade, respectively. The two last clades had a relatively long branch length that separated them from the first (0.107) while the second and third cluster were not very distant from each other (Figure 5.1).

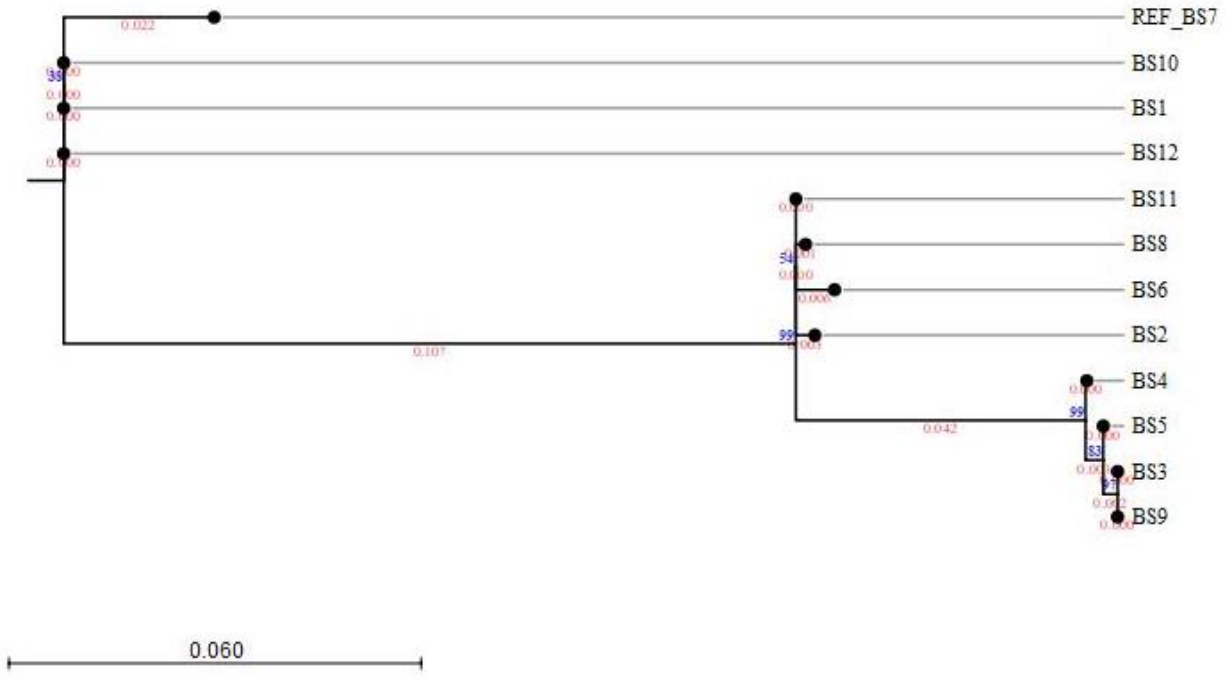

Figure 5.1: Phylogenomic tree showing clustering of 12 *Bipolaris secalis* isolates from four different districts of Rwanda

5.5. Discussion

This is the first report documenting the first *de novo* genome assembly of *Bipolaris secalis* isolate BS7 using Illumina platform. In addition, 11 isolates collected in four different districts were re-sequenced and they were mapped to BS7. The estimated genome size of *Bipolaris secalis* BS7 isolate was 34,813,291 bp. The size of the assembled genome is within the range of the genome size reported for other *Bipolaris* species. Zaccaron and Bluhm (2017) reported the whole genome of *Bipolaris cookie* (36,171,030 bp), *Bipolaris maydis* (36,456,735 bp), *Bipolaris sorokiniana* (34,417,436 bp), *Bipolari zeicola* (31,267,936 bp), *Bipolari oryzae* (31,362,097 bp), *Bipolaris victoriae* (32,829,575 bp). The genome size found in this study is within the range of reported fungal genomes which are naturally diverse according to species and several other factors can lead to the change of the genome size. They vary from 8.97 Mb to 177.57 Mb and the average size is 36.91 and 46.48 Mb for Ascomycota and Basidiomycota, respectively (Mohanta and Bae, 2015). *Bipolaris secalis* belongs to Dothideomycetes and the very high variability of the genome size within species (21.88 Mb to 74.14 Mb) under Dothideomycetes were reported (Ohm *et al*., 2012). The BUSCO analysis indicated the completeness of the genome sequence of *Bipolaris secalis* at 97.69%. Aggarwal *et al*. (2019) reported that the BUSCO evaluation of assembly *Bipolaris sorokiniana* genome sequence predicted its completeness at around 97.6%.

The *de novo* assembly of BS7 led to 108 contigs (*Bipolaris sorokiniana* and *Bipolaris victoriae*: 78; *Bipolaris sorokiniana*: 14; *Bipolaris victoriae*: 16), with the longest contig of 2,265,317 bp, the N50 of 1,032,497 bp and L50 of 12. The dataset obtained in this study are within the similar range with the dataset from other studies on other *Bipolari*s species. The N50 of *Bipolaris sorokiniana* was 1,654,800 bp; *Fusarium equiseti* (6,178,397 bp); *Fusarium oxysporum* (4,490,135 bp). The observed difference may be due to the use of different technologies and platforms in assembly. In this study, Illumina platform was used and several authors used the assembly dataset from the same platform combined with Pacific Biosciences (PacBio) or Oxford Nanopore Technology (ONT). Even though PacBio and ONT platforms offer long reads and more accurate results, the results of this study indicated the effectiveness of Illumina platform. The reads of 11 re-sequenced isolates were mapped to the reference genome to check the variations. Sequences of four isolates mapped at more than 90% and seven remaining sequences mapped at around 80%. This indicated high level of genetic similarity between isolates.

The number of variants were varying between isolates, and it varied from 1,003 to 10,744. The difference between the number of variants shows the presence of Single-nucleotide polymorphisms (SNPs)at substantial level. The number of variants found in this study was lower than the number of its sister *Bipolaris sorokiniana* which had 93,122; 88,672 and 4,450 for variants, SNPs and deletions respectively (Aggarwal *et al.*, 2019). The high-level variability may be explained by the fact that isolates were collected in different locations with varying agro-ecologies and the reference genome was collected in Nyagatare district. Several authors reported the genetic variations between *Bipolaris oryzae* isolates and the fate of variability between *Bipolaris* isolates due to parasexual recombination was reported (Nazari *et al.*, 2015; Safari Motlagh and Anvari, 2010). Furthermore, the whole genome sequencing and genome variability among isolates from several *Bipolaris* species including *Bipolaris victoriae*, *Bipolaris sorokiniana* and *Bipolaris zeicola* were reported (Condon *et al.*, 2013; Ohm *et al.*, 2012).

5.6. Conclusions

In this study, the whole genome of *Bipolaris* isolates was sequenced and the genetic variation between 12 isolates collected from four districts of Rwanda was evaluated. *Brachiaria* leaf spot caused by *Bipolaris secalis* is an emerging disease *in Brachiaria* growing districts in Rwanda. Due to lack of genomic information for *Bipolaris secalis*, it was important to make this sequencing effort using Illumina platform and this study reports for the first time the whole genome of *Bipolaris secalis*. The whole genome size of *Bipolaris secalis* is 34,813,291 bp. The genome of *Bipolaris secalis* was assembled for the first-time using Illumina platform. Comparison of the *de novo* assembled genome of *Bipolaris secalis* isolate BS7 to other whole genome re-sequenced of isolates from four districts of Rwanda indicated comparable qualities in terms of total reads, the GC content and the number of variants identified. The genome sequence obtained here should accelerate genomic and molecular studies of *Bipolaris secalis* and other species from the same genus and should provide a good reference draft sequence for further sequencing and genome analysis of other similar species. In addition, these results will form part of the molecular toolbox for *Bipolaris* species management and will contribute to the lack of information on *Bipolaris secalis* genomics. Therefore, there is need to evaluate further studies on effective management options for *Brachiaria* grass diseases in Rwanda.

CHAPTER SIX:
REACTION TO FOLIAR DISEASES AND AGRONOMIC PERFORMANCE OF IMPROVED BRACHIARIA GRASS CULTIVARS

6.1 Abstract

Diseases have been found as one of the major constraints of *Brachiaria* productivity in Africa. Improved *Brachiaria grass* cultivars were evaluated for leaf rust, leaf spot and leaf blight diseases and their agronomic performances under field experiments in two distinct agro-ecological zones of Rwanda. Analysis of variance (ANOVA) was performed for data on disease parameters and agronomic traits of *Brachiaria* grass and Pearson correlation analysis was used to determine the relationships between agronomic traits and the extent of the diseases expressed as Area under disease progress curve (AUDPC). Results showed that cultivars differed significantly ($p \leq 0.05$) for their response to leaf blight, leaf rust and leaf spot diseases. Cultivars Basilisk, Marandu, MG4, and Xaraes exhibited moderately resistant to resistant response to all three diseases but Cayman and Cobra were highly susceptible to leaf rust. The effects of interaction between site, cultivar and harvest was statistically significant for diseases and agronomic traits ($p \leq 0.05$). Cultivars also differed significantly for biomass production and dry matter content ($p \leq 0.05$). Cultivars Marandu, Xaraes, Cayman, Cobra and Piata were the highest biomass producers and had highest dry matter content. The AUDPC for leaf rust and leaf spot had negative and significant correlation with dry matter yield. This study concludes a satisfactory level of resistance in Basilisk, Marandu, MG4 and Xaraes to all three foliar diseases in Rwanda. Moderate to resistant cultivars identified under this study can be used in promotion of *Brachiaria* grass in Rwanda and neighbouring countries. It is also important to conduct further study on other disease management options for sustainable production of *Brachiari*a grass.

Key words*:* Area under disease progress curve, *Brachiaria* (*Urochloa*) grass, disease resistance, leaf blight, leaf rust, leaf spot

6.2 Introduction

Brachiaria (*Urochloa)* grass was documented as one of the best quality forages in the tropical and sub-tropical regions (Renvoize *et al.*, 1996). *Brachiaria* grass possesses several characteristics of agricultural and environmental significance. These include high biomass yield, nutritious to livestock, drought and shade tolerance, soil fertility improvement, high nutrient use efficiency, mitigation of the climate change adversity and effective bioagents for pests and parasitic weed management (Khan *et al.*, 2016; Maass *et al.*, 2015; Subbarao *et al.*, 2009). The genus *Brachiaria* counts arround 100 species where among them seven perennial species of African origin have been exploited for fodder production, and they have been cultivated in Asia, Australia, the South Pacific, and South America at various scales (Jank *et al.*, 2014; Stür *et al.*, 1996). *Brachiaria* species are a common and valuable constituent of natural vegetation in East Africa (Boonman, 1993) but their use as sown pasture for animal production is very recent in the region (Maass *et al.*, 2015; Njarui *et al.*, 2016). A broad adaptation, excellent animal performances and high biomass yields are among the major factors that promote a wider and rapid adoption of *Brachiaria* grass across the tropics and subtropics. However, different abiotic and biotic factors including diseases were reported to affect the productivity of *Brachiaria* grass, causing high biomass reduction / yield losses (Hernandez *et al.*, 2017; Nzioki *et al.*, 2016). Diseases of *Brachiaria* grass, their symptoms, geographical distribution, and management options have been reviewed by Valerio *et al.* (1996). These diseases have the potential to reduce forage yields and quality, therefore have negative impact on livestock productivity.

Brachiaria grass is one of the preferred forages by livestock keepers in Rwanda and in other East African countries (Maass *et al.*, 2015; Mutimura and Everson, 2012). As mentioned earlier, its production is being constrained by diseases such as leaf rust, leaf spot, and leaf blight that are reported to cause economic loss (Alvarez *et al.*, 2014; Maass *et al.*, 1996; Lenné and Trutman, 1994; Miles *et al.*, 1996; Rao *et al.*, 1996). For example, yield losses caused by leaf rust can reach up to 100 per cent, and leaf rust can reduce leaf crude protein content to between 49 and 53% and subsides availability of other nutrients (Lenné and Trutman, 1994). Similarly, leaf blight reduces forage biomass yield by about 50% in the tropics (Alvarez *et al.*, 2013).

Recent studies have shown that *Brachiaria grass* diseases (leaf spot, leaf rust and leaf blight) are widely spread and distributed in Kenya and Rwanda (Nzioki *et al.*, 2016; Uzayisenga *et al.*, 2020). Therefore, sustainability of *Brachiaria* grass production in Africa depends on how well these diseases are managed.

Many pastures including *Brachiaria* grass are considered low-value crops, often cultivated in large acreage and management of diseases using chemicals is too costly and not safe for livestock and the environment. Therefore, disease management efforts in *Brachiaria* grass should focus on low-cost control measures like host-plant resistance that is effective, economical, easy to apply and safe for the environment. Currently, some improved *Brachiaria* grass cultivars are available to address major production challenges like biomass yield, nutritive quality, drought tolerance, and pests and disease management (Alvarez *et al.*, 2014; Lenné and Trutman, 1994; Maass *et al.*, 2015). For instance, Mulato and Mulato II cultivars were developed in order to have spittlebug-resistance, high biomass yield and nutritive forage with high quality (Argel *et al.*, 2007; Miles *et al.*, 2004). Cultivar Cayman was developed for water logging tolerance (Pizarro *et al.*, 2013), and some *Brachiaria* hybrids were developed for foliar blight resistance (Alvarez *et al.*, 2014).

Demand for improved *Brachiaria* grass is high in Sub-Saharan Africa. Therefore, many livestock development initiatives were implemented by Research institutions in that region and international organizations and development agencies have been promoting *Brachiaria* grass in the continent as a nutritious and climate resilient forage. These programmes currently rely on a few improved cultivars initially developed for South America with an extremely narrow genetic base (Keller-Grein *et al.*, 1996). In Rwanda, evaluation of *Brachiaria* grass cultivars started in 2007 with the introduction of improved cultivars that included *Brachiaria* hybrid Bro2/0465, *Brachiaria brizantha* cv. Marandu, *Brachiaria* hybrid Bro2/1485, *Brachiaria decumbens* cv. Basilisk, *Brachiaria brizantha* cv. Xaraes, *Brachiaria* hybrid Bro2/1452, *Brachiaria* hybrid cv. Mulato and *Brachiaria* hybrid cv. Mulato II (CIAT, 2010; Mutimura and Everson 2012; Mutimura and Ghimire, 2021). In 2013, eight improved *Brachiaria* cultivars were introduced and evaluated for adaptation and biomass yield in different agro-ecological zones (Ghimire *et al.*, 2015). These cultivars were successfully integrated into a mixed crop-livestock system that subsequently improved forage availability and livestock productivity (Mutimura *et al.*, 2016, 2018).

All improved *Brachiaria* grass cultivars that are introduced and promoted in Africa were developed in South America and Australia. Some of these improved cultivars have shown broader adaptation, excellent agronomic performance and high livestock productivity, stimulating high demand for *Brachiaria* grass by farmers in Africa. However, many of these cultivars are susceptible to diseases such as leaf rust, leaf spot, foliar blight, and ergot in Kenya and Rwanda (Nzioki *et al.*, 2016; Uzayisenga *et al.*, 2020). Therefore, expansion of *Brachiaria* acreage without proper disease management measures will increase chances of disease outbreaks and crop failure. Therefore, the cultivation and scaling up of these improved cultivars over large geographical ranges in Africa requires some consideration of the existing and emerging diseases (Maass, 2015), thus warranting the need for routine evaluation of the existing and new cultivars against diseases. This study evaluated the response of nine improved *Brachiaria* grass cultivars to three major foliar diseases and assessed the effects of the foliar diseases on the agronomic performances of those cultivars in Rwanda.

6.3 Materials and methods

6.3.1 Biophysical characteristics of experimental sites

Field experiments were conducted in two different agro-ecological zones at Gashora and Rubona sites in the years 2019 and 2020 (Table 6.1). A previous study showed that leaf rust, leaf spot, and leaf blight of *Brachiaria* grass are endemic in both sites (Uzayisenga *et al.*, 2020). Rubona site has poor soils (Acrisols; FAO,1987), that are originating from granitic parent material with moderate agriculture value. Soil pH ranges from 5.6 to 5.9, organic matter (%) ranges from 2.1 to 2.4, total soil nitrogen (%) ranges from 0.15 to 0.16, extractable P (ppm): from 7.10 to11.17, exchangeable Ca, Mg and K ($cmol_c kg^{-1}$): from 2.02 to 3.15, 0.48 to1.00 and 0.02 to 0.18 respectively, and soil texture is sandy clay (64.4% sand) (Ndabamenye *et al.*, 2013). Soils in Gashora site are very weathered and dominated by humic and haplic Ferralsols with means of soil pH: 5.91, organic carbon (%): 1.67, organic matter (%): 2.84, available P (pmm): 8.00, clay (%): 21, silt (%): 14, sand (%): 65, and with sandy loam soil texture (Bucagu *et al.*, 2020).

Table 6.1: Biophysical characteristics of Gashora and Rubona experimental sites in Rwanda

Variables	Experimental sites	
	Gashora	Rubona
District	Bugesera	Huye
Agro-ecological zone	Mayaga and Bugesera	Central plateau and granitic ridges
Altitude (m a.s.l)	1333	1673
Latitude	S02˚11'17.8"	S02˚29'01.4"
Longitude	E030˚14'36.2''	E029˚46'-00.9"
Rainfall distribution (bimodal)		
First rains	March-May	March-May
Second rains	October-December	October-December
Total annual rainfall (mm)		
Mean	1038	1298
Minimum	891	1025
Maximum	1255	1993
Average annual temperature (˚C)		
Mean	20.3	19.5
Minimum	19.5	18.1
Maximum	22.0	20.5
Topography (% slope)	Gentle (2%)	Gentle (2%)
Soil textural classification	Sandy loam	Sandy clay
Soil types/parent material	Acrisol / shale and granitic rocks	Acrisol / granitic rocks
Agriculture value	Poor-moderate	Moderate

Source of climate data: RMA (Rwanda Meteorology Agency) 2020.

6.3.2 Plant materials

The *Brachiaria* cultivars used in this study were obtained from Rwanda Agriculture and Animal Resources Development Board's reseach fields (RAB). These cultivars were *Brachiaria brizantha* cv. Marandu (CIAT 6294), *B. brizantha* cv. MG 4 (CIAT 26646), *B. brizantha* cv. Piata (CIAT 16125), *Brachiaria brizantha* cv. Xaraes (CIAT 26110), *B. decumbens* cv. Basilisk (CIAT 606), *B. humidicola* cv. Humidicola (CIAT 16888), *Brachiaria* hybrid cv. Cayman (CIAT BR02/1752), *Brachiaria* hybrid cv. Cobra (CIAT BR02/1794), *Brachiaria* hybrid cv. Mulato (CIAT 36061) and *Brachiaria* hybrid cv. Mulato II (CIAT 36087). Characteristics of common *Brachiaria* species including cultivars used in this study are described under chapter 2 in the Table 2.1.

6.3.3 Field experimentation

Nine improved *Brachiaria* grass cultivars were evaluated in four replicates using randomised complete block design (RCBD) in Gashora and Rubona experimental sites. In addition to the main treatments, a leaf blight susceptible cultivar, Mulato (Alvarez *et al.*, 2014; Argel *et al.*, 2007), was planted as disease spreader rows four weeks prior to planting of test cultivars to check whether the disease was present naturally in the experimental sites and to trap early inoculum for the disease development. The test cultivars were surrounded by two spreader rows of Mulato and were planted at spacings of 50 cm and 25 cm between rows and between plants, respectively. Each test cultivar was planted on 3.5 m row, accommodating 14 plants per replicate at 25 cm spacing between plants within rows and 1 m spacing between rows, and a 2 m spacing was kept between replicates (Figure 6.1). Planting was done using two rooted tillers per hill. At planting, organic amendment using cattle manure at dose of 10 t ha^{-1} and inorganic fertiliser using NPK17-17-17 at dose of 100 kg ha^{-1} were used in the top soil (0-30 cm depth) in each planting hole. Two weeks after planting, Urea top-dressing was done at the rate of 50 kg N ha^{-1} in rows. These levels of organic amendment and inorganic fertiliser used at planting were as "basal dressing" across all cultivars and sites to avoid any crop failure during the vegetative growth and then affects the evaluation of diseases that were the major concern. Organic manure well decomposed was used to allow the mineral fertiliser to be well retained into the soil. This handling technique minimises nutrient leaching during the earlier growth period. Irrigation and weeding were performed manually as required.

All test cultivars in the experimental plots were subjected to standardisation cut at 5 cm above the ground level. It was done four weeks after planting to stimulate tillering and uniform regrowth. For each cultivar, six stools showing uniformity in appearance and growth were selected in each replication and tagged for assessing diseases and agronomic parameters. Border effect was eliminated during data collection by excluding at least one stool from each side of a row. The experiments covered three consecutive growing seasons: from March to July 2019, whis is wet to semi-dry season characterised by high rain intensity but shorter rains, from August to December 2019, which is dry to wet season, characterised by long rain- patterns and distribution with medium rain intensity, and from January to May 2020 which is wet season characterised by shorter rains with high rain intensity.

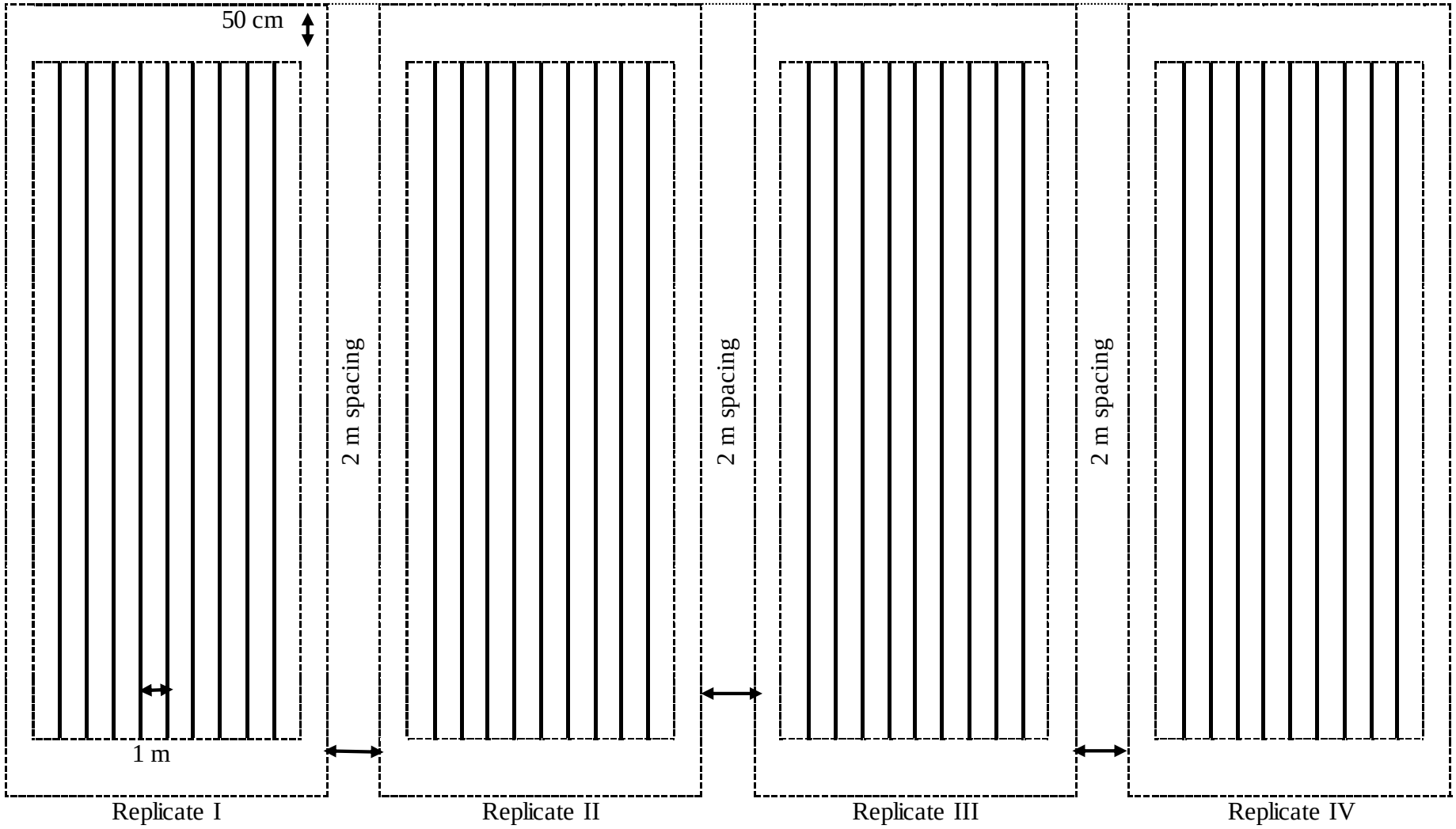

Figure 6.1: Field experimentation layout in Gashora and Rubona sites. ----------: Dotted lines indicate two spreader rows of Mulato. Test cultivars are indicated by full lines: ——

6.3.4 Assessment of the incidence and the severity of disease

Incidence and severity of the diseases were recorded every four weeks after the standardisation cut up to 20th week for the first, second and third seasons. A total of five assessments were made for each of the three consecutive seasons. Disease incidence was assessed on six tagged stools per cultivar in each replication and was determined as the number of stools showing disease symptoms, then converted to percentage of the total number of assessed stools. On the same stools, severity of leaf rust, leaf spot and leaf blight were assessed using the disease rating scales described in Table 3.2 under Chapter 3. The extent of the disease which refers to the area under disease progress curve (AUDPC) was computed using severity data collected over the five different time points in each season as described by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n-1}\left[\frac{(y_i + y_{i+1})}{2}\right](t_{i+1} - t_i)$$

where:

Y_i = Severity at i^{th} day observation,
t_i = Time in days at i^{th} observation of the disease at the i^{th} day evaluation,
n = The total number of observations.

The response of the test cultivars to leaf rust was determined based on infection types and AUDPC. The leaf rust infection types were recorded at eight-week-old stools for all three seasons including two harvests in 2019 and one harvest in 2020, using a five-category scale: immune, resistant, moderately resistant, moderately susceptible, and susceptible (Roelfs *et al.*, 1992). Similarly, the response to leaf spot and leaf blight was determined based on AUDPC data where lower AUDPC values correspond to resistance and higher AUDPC values correspond to susceptibility. The AUDPC values less than 3,500 correspond to resistance, values between 3,500 to 4500 correspond to moderately susceptible, and the values greater than 4,500 correspond to susceptible reaction (Kumari *et al.*, 2018; Magar *et al.*, 2015; Pantha *et al.*, 2017).

6.3.5 Evaluation of agronomic parameters

The data on agronomic parameters such as plant height and number of tillers per stool were collected at interval of four weeks following standardisation cut for each harvest. Plant height was measured from the ground at the base of the stool up to the longest leaf of every tagged stool (Rayburn and Lozier, 2007). At each harvest, the above ground biomass was harvested and dry matter (DM) yield and dry matter content were recorded. Harvesting was carried at 20 weeks' interval throughout the seasons. For determination of dry matter content, 200 g sub-sample from fresh biomass was kept in paper bags and dried in the oven at 105ºC for 24 hours. The total dry matter yield (Kg ha^{-1} DM) and the dry matter percentage were calculated following Wassie *et al.* (2018) and Oliveira *et al*. (2019).

6.3.6 Meteorological data of Gashora and Rubona sites

The rainfall and temperature data during the experimental period from March 2019 to May 2020 for Gashora and Rubona experimental sites was obtained from the Rwanda Meteorology Agency (RMA, 2020). Thirty-year average data available at https://www.besttimetovisit.com.pk/ was used for any missing monthly rainfall and temperature data.

6.3.7 Statistical analyses

Analysis of variance (ANOVA) using GenStat for Windows 20th Edition (VSN International, 2019) was performed to analyse data on disease parameters (incidence, severity and AUDPC) and agronomic traits (plant height, number of tillers, dry matter (DM) yield and the dry matter content). General Linear Model predictions using repeated measures (Littell *et al*., 1991) was used to account for the overall trend of disease incidence, disease severity, plant height, and number of tillers. All data were analysed to check interaction between the cultivar, site and harvest per season and the significance of interactions ($p \leq 0.05$) served as base to present analysed results. Least significant difference (LSD) test at $p \leq 0.05$ was used to separate the means of disease and agronomic parameters. As data were analysed at the interaction between cultivar, site and harvest per season; all cultivars, sites, and harvests/seasons were considered together to calculate Pearson correlations that allowed to illustrate the relationships among the agronomic traits (plant height, number of tillers, dry matter yield) and the AUDPC for leaf rust, leaf spot and leaf blight diseases.

6.4 Results

6.4.1 Meteorological data at Gashora and Rubona experimental sites

Monthly rainfall and monthly average temperature for Gashora and Rubona are presented in Figure 6.2. Sites were different in terms of total rainfall, and monthly rainfall differed between seasons (Figure 6.2). In the first season (March to July 2019), the total amount of rainfall at Gashora and Rubona were 331 mm and 545 mm, respectively. Rubona site received 1.65 times greater rainfall than Gashora site in the first growing season of 2019. During the growing season from August to December 2019, the total amount of rainfall at Gashora and Rubona were 492 mm and 590 mm, respectively. For the third season (January to May 2020), the total amount of rainfall was 551 mm and 810 mm for Gashora and Rubona, respectively. In all three seasons, Gashora had higher mean monthly temperature than Rubona (first season = 22.7 °C vs. 20.1 °C, second season = 23.4 °C vs. 20.5 °C, and third season = 21.0°C vs. 20.2°C). However, the differences in monthly temperatures between the sites were minimal for all three seasons.

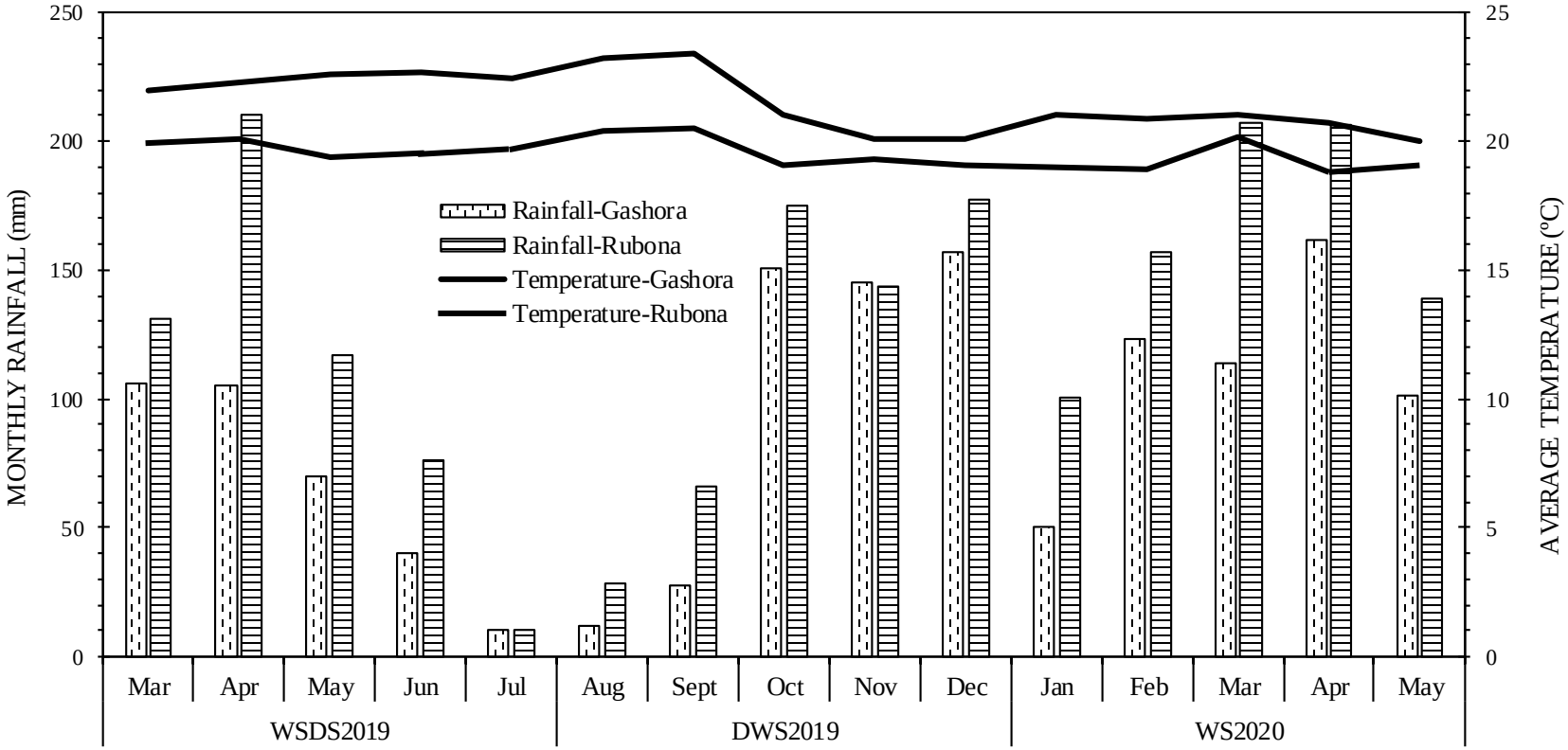

Figure 6.2: Monthly rainfall and monthly average temperature at Gashora and Rubona experimental sites for all harvests. The first harvest which is from March to July 2019 is wet to semi-dry season (WSDS), the second harvest from August to December 2019, which is dry to wet season (DWS), and the third harvest from January to May 2020, which is wet season (WS).

6.4.2 Responses of improved *Brachiaria* cultivars to foliar diseases under open field conditions

The spreader rows exhibited high leaf rust incidence and severity at Gashora and Rubona. However, leaf spot and leaf blight incidence and severity on spreader row were low in both experimental sites (data not shown). The effect of cultivar, site, harvest, and interaction of cultivar by site and harvest was significant for leaf rust incidence, severity and AUDPC ($p < 0.001$; Table 6.2). The leaf rust incidence among the cultivars for all sites and harvests together ranged from 31.9% to 100.0% with the lowest incidence in Basilisk at Gashora in the third harvest and the highest incidence in Cayman, Mulato II and Cobra, mostly at Rubona in all harvests. Similarly, leaf rust severity ranged from 5.4% to 81.8% with the lowest severity in Basilisk in the third harvest at Gashora and the highest in Cayman in the third harvest at Rubona. The AUDPC for leaf rust was the lowest in Basilisk (754) and MG4 (949) in the third harvest at Gashora and the highest in Cayman (10,177) in the first harvest at Rubona. Across all sites and harvests, the cultivars Cayman and Cobra were the most susceptible to leaf rust disease while Basilisk, Humidicola, Marandu, MG4, Piata and Xaraes were moderately resistant. Cultivar Mulato II was moderately susceptible.

There was significant effect of all treatments (except for site on incidence and AUDPC) and their interactions on incidence, severity and AUDPC of leaf spot disease ($p < 0.001$; Table 6.3). The incidence of leaf spot was the lowest in Cayman at Rubona in the first harvest and the highest in Humidicola at Rubona in the third harvest. The severity of leaf spot was the lowest in Cayman at Rubona in the first harvest and the highest in Humidicola at Rubona in the first harvest. The AUDPC for leaf spot ranged from 25 to 5,996 where Cayman had the lowest value at Rubona in the first harvest and Humidicola had the highest value at Gashora in the first harvest. All cultivars across the sites and harvests showed resistant reaction to leaf spot, except Cayman, Humidicola, and Piata which were moderately susceptible.

All treatments and their interactions had significant effect on leaf blight incidence, severity and AUDPC ($p \leq 0.001$; Table 6.4). Cultivar Humidicola had the lowest leaf blight incidence and severity in the first harvest at Gashora. Cultivar Cayman had the highest leaf blight incidence and severity in the third harvest at Rubona. Humidicola in the first harvest at Gashora and Cayman in the second harvest at Rubona had the lowest values of AUDPC (0.0%) for leaf blight disease, but Cayman had the highest value (2,317) in the third harvest at Rubona.

Table 6.2: Intensity of leaf rust and host reponse of nine improved *Brachiaria* cultivars to leaf rust at Gashora and Rubona experimental sites, in the years 2019 and 2020

Site	Harvest	Cultivar	Incidence (%)	Severity (%)	AUDPC	Host response
Gashora	First harvest (July 2019)	Basilisk	71.7^{ijk}	15.1^{wxyzA}	1,802^{stuv}	MR
		Cayman	95.0^{bc}	59.3^{c}	7,618^{c}	S
		Cobra	100.0^{a}	40.9^{e}	5,069^{e}	S
		Humidicola	62.8^{m}	14.1^{xyzAB}	1,792^{tuvw}	MR
		Marandu	83.9^{de}	19.6^{qrstuv}	2,437^{nopqr}	MR
		MG4	45.8^{gr}	13.8^{yzAB}	1,698^{uvw}	MR
		Mulato II	98.6^{ab}	28.8^{klmn}	3,441^{jkl}	MS
		Piata	70.3^{jk}	13.5^{zAB}	1,622^{uvwx}	MR
		Xaraes	82.2^{de}	22.1^{opqrs}	2,830^{mno}	MR
	Second harvest (December 2019)	Basilisk	57.2^{n}	18.0^{stuvwxy}	2,010^{rstu}	MR
		Cayman	80.6^{ef}	37.1^{efgh}	4,358^{fg}	S
		Cobra	77.2^{fgh}	24.7^{nop}	2,951^{lmn}	S
		Humidicola	63.3^{m}	17.7^{tuvwxyz}	2,108^{qrstu}	MR
		Marandu	52.8^{op}	15.1^{wxyzA}	1,691^{uvw}	MR
		MG4	58.6^{n}	18.4^{rstuvwx}	1,986^{rstu}	MR
		Mulato II	72.8^{ij}	21.9^{opqrst}	2,490^{nopqr}	MS
		Piata	55.6^{no}	15.8^{vwxyzA}	1,785^{tuvw}	MR
		Xaraes	63.6^{m}	18.5^{rstuvw}	2,146^{grstu}	MR
	Third harvest (May 2020)	Basilisk	31.9^{s}	5.4^{E}	754^{z}	R
		Cayman	75.6^{ghj}	36.2^{fgh}	4,319^{fg}	S
		Cobra	77.2^{fgh}	30.0^{ijkl}	3,469^{jkl}	S
		Humidicola	42.8^{gr}	7.9^{CDE}	1,063^{xyz}	R
		Marandu	50.8^{p}	10.6^{BCD}	1,233^{wxyz}	R
		MG4	42.2^{r}	7.2^{DE}	948^{yz}	R
		Mulato II	64.4^{lm}	19.8^{qrstuv}	2,354^{opqrs}	MS
		Piata	46.4^{q}	10.6^{BCD}	1,247^{vwxyz}	MR
		Xaraes	58.1^{n}	10.2^{BCD}	1,275^{vwxyz}	MR
Rubona	First harvest (July 2019)	Basilisk	65.0^{lm}	13.5^{zAB}	1,670^{uvw}	MR
		Cayman	100.0^{a}	70.5^{b}	8,927^{b}	S
		Cobra	98.3^{ab}	50.2^{d}	6,389^{d}	S
		Humidicola	86.1^{d}	20.6^{pqrst}	2,538^{mnopqr}	MR
		Marandu	73.3^{hij}	16.0^{uvwxyzA}	2,007^{rstu}	MR
		MG4	65.0^{lm}	11.9^{ABC}	1,403^{vwxy}	MR
		Mulato II	94.2^{c}	40.7^{e}	5,142^{e}	MS
		Piata	77.8^{fg}	18.0^{rstuyvwxy}	2,260^{pqrst}	MR
		Xaraes	68.1^{kl}	13.8^{yzAB}	1,656^{uvw}	MR
	Second harvest (December 2019)	Basilisk	98.3^{ab}	22.3^{opgr}	2,528^{mnopqr}	MR
		Cayman	100.0^{a}	58.2^{c}	7,108^{c}	S

Site	Harvest	Cultivar	Incidence (%)	Severity (%)	AUDPC	Host response
		Cobra	98.9^{ab}	39.9^{ef}	4,861^{ef}	S
		Humidicola	98.9^{ab}	25.5^{mno}	2,983^{lmn}	MR
		Marandu	98.6^{ab}	23.3^{opq}	2,649^{mnopq}	MR
		MG4	97.5^{abc}	20.3^{grstu}	2,323^{opqrst}	MR
		Mulato II	100.0^{a}	34.3^{ghi}	4,149^{gh}	S
		Piata	97.2^{abc}	29.6^{jklm}	3,462^{jkl}	MS
		Xaraes	99.4^{a}	23.4^{opq}	2,764^{mnop}	MR
	Third harvest (May 2020)	Basilisk	98.6^{ab}	33.5^{hij}	4,080^{ghi}	MR
		Cayman	100.0^{a}	81.8^{a}	10,177^{a}	S
		Cobra	100.0^{a}	38.2^{efg}	4,490^{fg}	S
		Humidicola	96.7^{abc}	28.6^{klmn}	3,490^{jkl}	MS
		Marandu	98.6^{ab}	31.9^{ijk}	3,726^{hij}	MR
		MG4	98.1^{abc}	25.7^{lmno}	3,073^{klm}	MR
		Mulato II	100.0^{a}	40.1^{ef}	4,799^{ef}	MS
		Piata	96.7^{abc}	39.6^{ef}	4,778^{ed}	MS
		Xaraes	98.6^{ab}	29.6^{jklm}	3,573^{ijk}	MR
Source of variation			*p* values			
Site			<.001	<.001	<.001	
Harvest			<.001	<.001	<.001	
Cultivar			<.001	<.001	<.001	
Site × harvest			<.001	<.001	<.001	
Site × cultivar			<.001	<.001	<.001	
Harvest × cultivar			<.001	<.001	<.001	
Site × harvest × cultivar			<.001	<.001	<.001	

AUDPC: area under disease progress curve, S: susceptible, MS: moderately susceptible, MR: moderately resistant and R: resistant. Values with different superscript letters within the columns are statistically different at $p \leq 0.05$. Mean separation was done using LSD.

Table 6.3: Intensity of leaf spot and host reponse of nine improved *Brachiaria* cultivars to leaf spot at Gashora and Rubona experimental sites, in the years 2019 and 2020

Site	Harvest	Cultivar	Incidence (%)	Severity (%)	AUDPC	Host response
Gashora	First harvest (July 2019)	Basilisk	50.0^{fghij}	16.7^{lm}	1,875^{jklmn}	R
		Cayman	18.3^{pqrst}	6.0^{vwx}	525^{stuvwxy}	R
		Cobra	25.6^{nopqr}	7.8^{tuv}	708^{rstuywx}	R
		Humidicola	89.7^{a}	48.4^{a}	5,996^{a}	S
		Marandu	6.9^{tu}	1.5^{zA}	121^{xy}	R
		MG4	35.3^{klmn}	16.5^{lm}	1,962^{ijklmn}	R
		Mulato II	26.7^{mnopq}	11.2^{pqr}	1,063^{opqrs}	R
		Piata	9.2^{stu}	2.1^{yzA}	175^{wxy}	R
		Xaraes	10.8^{stu}	4.2^{wxy}	500^{stuvwxy}	R
	Second harvest (December 2019)	Basilisk	64.7^{bcde}	25.3^{ef}	3,004^{ef}	R
		Cayman	70.6^{bc}	30.3^{d}	3,733^{cd}	MS
		Cobra	65.0^{bcde}	23.9^{fgh}	2,742^{efgh}	R
		Humidicola	87.8^{a}	30.8^{d}	3,725^{cd}	MS
		Marandu	39.7^{ijklm}	10.7^{pqrs}	1,138^{opqrs}	R
		MG4	50.6^{fghij}	14.0^{no}	1,567^{klmno}	R
		Mulato II	68.3^{bc}	27.6^{e}	3,242^{de}	R
		Piata	36.1^{klmn}	10.2^{qrst}	1,021^{opqrst}	R
		Xaraes	40.8^{ijkl}	8.3^{stuv}	996^{opqrst}	R
	Third harvest (May 2020)	Basilisk	64.7^{bcde}	23.9^{fgh}	2,821^{efg}	R
		Cayman	70.6^{bc}	24.5^{fg}	2,938^{ef}	R
		Cobra	65.0^{bcde}	15.2^{mn}	1,908^{jklmn}	R
		Humidicola	88.1^{a}	22.2^{ghi}	2,804^{efg}	R
		Marandu	20.0^{pqrst}	4.1^{wxy}	492^{stuvwxy}	R
		MG4	31.4^{lmnop}	6.2^{vw}	767^{pqrstuvwx}	R
		Mulato II	52.5^{efghi}	18.1^{kl}	2,183^{ghijk}	R
		Piata	35.8^{klmn}	7.6^{uv}	821^{pqrstuvw}	R
		Xaraes	15.8^{qrst}	3.2^{yz}	408^{tuvwxy}	R
Rubona	First harvest (July 2019)	Basilisk	51.1^{fghij}	12.6^{opq}	1,521^{lmno}	R
		Cayman	0.0^{u}	0.2^{A}	25^{y}	R
		Cobra	10.0^{stu}	3.1^{yz}	238^{vwxy}	R
		Humidicola	96.7^{a}	48.5^{a}	5,958^{a}	S
		Marandu	9.4^{stu}	3.6^{xyz}	317^{uvwxy}	R
		MG4	35.8^{lkmn}	8.3^{stuv}	971^{opqrst}	R
		Mulato II	15.0^{qrst}	6.2^{vw}	604^{stuvwxy}	R
		Piata	14.2^{qrst}	3.2^{yz}	296^{uvwxy}	R

Site	Harvest	Cultivar	Incidence (%)	Severity (%)	AUDPC	Host response
		Xaraes	40.8^{ijkl}	8.3^{stuv}	1,004^{opqrst}	R
	Second harvest (December 2019)	Basilisk	62.2^{bcdef}	12.9^{nop}	1,562^{klmno}	R
		Cayman	20.8^{opqrs}	6.0^{vwx}	638^{stuvwxy}	R
		Cobra	12.8^{rstu}	2.6^{yzA}	238^{vwxy}	R
		Humidicola	96.9^{a}	43.2^{b}	5,288b	S
		Marandu	33.6^{lmno}	7.5^{uv}	858^{pqrstuv}	R
		MG4	55.0^{defgh}	11.2^{pqr}	1,404^{mnop}	R
		Mulato II	26.7^{mnopq}	7.1^{uv}	738^{qrstuvwx}	R
		Piata	42.5^{hijkl}	13.1^{nop}	1,350^{nopqr}	R
		Xaraes	48.3^{ghijk}	11.6^{opqr}	1,400^{mnop}	R
	Third harvest (May 2020)	Basilisk	69.4^{bc}	19.8^{ijk}	2,367^{fghij}	R
		Cayman	38.6^{jklmn}	10.7^{pqrs}	1,383^{mnopq}	R
		Cobra	62.5^{bcdef}	16.7^{lm}	2,008^{ijklm}	R
		Humidicola	100.0^{a}	44.4^{b}	5,592^{ab}	S
		Marandu	68.6^{bc}	21.5^{hij}	2,558^{fghi}	R
		MG4	41.7^{ijkl}	9.1^{rstu}	921^{opqrstu}	R
		Mulato II	66.9^{bcd}	19.6^{jk}	2,467^{fghij}	R
		Piata	73.6^{b}	35.5^{c}	4,342^{c}	MS
		Xaraes	57.8^{cdefg}	17.8^{kl}	2,113^{hijkl}	R
Source of variation			*p* values			
Site			0.514	0.014	0.200	
Harvest			<.001	<.001	<.001	
Cultivar			<.001	<.001	<.001	
Site × harvest			<.001	<.001	<.001	
Site × cultivar			<.001	<.001	<.001	
Harvest × cultivar			<.001	<.001	<.001	
Site × harvest × cultivar			<.001	<.001	<.001	

AUDPC: area under disease progress curve, S: susceptible, MS: moderately susceptible and R: resistant. Values with different superscript letters within the columns are statistically different at $p \leq 0.05$. Mean separation was done using LSD.

Table 6.4: Intensity of leaf blight and host reponse of nine improved *Brachiaria* cultivars to leaf blight at Gashora and Rubona experimental sites, in the years 2019 and 2020

Site	Harvest	Cultivar	Incidence (%)	Severity (%)	AUDPC	Host response
Gashora	First harvest	Basilisk	5.8^{stuvwx}	1.3^{stuvwx}	96^{stuv}	R
		Cayman	6.4^{rstuvwx}	1.4^{rstuvwx}	108^{stuv}	R
		Cobra	10.8^{opqrstu}	2.6^{nopqrstuv}	258^{pqrstu}	R
		Humidicola	0.0^{x}	0.0^{x}	0^{v}	R
		Marandu	6.1^{rstuvwx}	1.2^{tuvwx}	92^{stuv}	R
		MG4	15.0^{lmnopqr}	3.4^{klmnopqr}	283^{nopqrst}	R
		Mulato II	9.2^{qrstuvw}	1.9^{pqrstuvwx}	175^{qrstuv}	R
		Piata	4.4^{tuvwx}	0.9^{uvwx}	92^{stuv}	R
		Xaraes	5.8^{stuvwx}	1.0^{uvwx}	104^{stuv}	R
	Second harvest	Basilisk	30.0^{efgh}	6.0^{efghi}	650^{efghijk}	R
		Cayman	34.4^{ef}	6.8^{ef}	683^{efghi}	R
		Cobra	36.1^{de}	7.2^{de}	792^{ef}	R
		Humidicola	5.3^{stuvwx}	1.1^{tuvwx}	83^{stuv}	R
		Marandu	25.8^{fghij}	5.2^{efghijkl}	542^{ghijklm}	R
		MG4	20.8^{ijklm}	4.2^{hijklmno}	388^{lmnopqr}	R
		Mulato II	30.8^{efg}	6.2^{efgh}	667^{efghij}	R
		Piata	25.6^{fghijk}	5.1^{efghijklm}	558^{fghijkl}	R
		Xaraes	18.9^{jklmno}	3.6^{jklmnopq}	392^{lmnopq}	R
	Third harvest	Basilisk	15.0^{lmnopqr}	3.0^{mnopqrstu}	425^{klmnop}	R
		Cayman	22.8^{ghijkl}	4.5^{ghijklmn}	508^{hijklmno}	R
		Cobra	28.9^{efghi}	6.4^{efg}	763^{efg}	R
		Humidicola	1.1^{vwx}	0.2^{wx}	21^{uv}	R
		Marandu	18.3^{jklmnop}	3.7^{jklmnopq}	429^{jklmnop}	R
		MG4	5.0^{stuvwx}	1.0^{uvwx}	150^{rstuv}	R
		Mulato II	15.8^{lmnopq}	3.2^{lmnopqrst}	442^{jklmnop}	R
		Piata	18.3^{jklmnop}	4.3^{ghijklmno}	517^{hijklmn}	R
		Xaraes	8.1^{qrstuvwx}	1.6^{qrstuvwx}	242^{pqrstu}	
Rubona	First harvest	Basilisk	9.4^{pqrstuv}	2.3^{opqrstuvw}	175^{qrstuv}	R
		Cayman	10.8^{opqrstu}	3.2^{lmnopqrst}	263^{pqrst}	R
		Cobra	11.7^{nopqrstu}	4.1^{hijklmnop}	304^{mnopqrs}	R
		Humidicola	19.2^{jklmno}	5.3^{efghijk}	400^{lmnopq}	R
		Marandu	12.8^{mnopqrst}	3.4^{klmnopqrs}	267^{pqrst}	R

Site	Harvest	Cultivar	Incidence (%)	Severity (%)	AUDPC	Host response
		MG4	$13.9^{lmnopqrs}$	$4.1^{hijklmno}$	$317^{mnopqrs}$	R
		Mulato II	19.2^{jklmno}	5.7^{efghij}	$450^{ijklmnop}$	R
		Piata	$16.7^{klmnopq}$	$4.8^{eghijklm}$	$363^{lmnopqr}$	R
		Xaraes	19.2^{jklmno}	$4.8^{eghijklm}$	$363^{lmnopqr}$	
	Second harvest	Basilisk	21.4^{hijklm}	$5.4^{efghijk}$	$450^{ijklmnop}$	R
		Cayman	0.3^{wx}	0.0^{x}	0^{v}	R
		Cobra	$16.7^{klmnopq}$	$5.3^{efghijk}$	400^{lmnopq}	R
		Humidicola	3.3^{uvwx}	0.7^{vwx}	50^{tuv}	R
		Marandu	20.6^{ijklmn}	$5.4^{efghijk}$	417^{klmnop}	R
		MG4	$18.3^{jklmnop}$	$5.0^{fghijklm}$	$375^{lmnopqr}$	R
		Mulato II	$11.7^{nopqrstu}$	$3.7^{jklmnopq}$	275^{opqrst}	R
		Piata	20.0^{ijklmn}	6.0^{efghi}	$463^{ijklmnop}$	R
		Xaraes	20.6^{ijklmn}	6.2^{efgh}	$471^{ijklmnop}$	R
	Third harvest	Basilisk	30.8^{efg}	6.2^{efgh}	725^{efgh}	R
		Cayman	92.8^{a}	19.1^{a}	$2,317^{a}$	R
		Cobra	79.2^{b}	16.3^{b}	$2,029^{b}$	R
		Humidicola	3.3^{uwx}	0.7^{vwx}	50^{tuv}	R
		Marandu	45.0^{d}	9.0^{d}	$1,142^{d}$	R
		MG4	20.0^{ijklmn}	$4.0^{ijklmnop}$	$475^{ijklmnop}$	R
		Mulato II	64.2^{c}	13.0^{c}	$1,583^{c}$	R
		Piata	56.7^{c}	13.3^{c}	$1,517^{c}$	R
		Xaraes	28.6^{efghi}	5.7^{efghij}	804^{e}	R
Source of variation			*p values*			
Site			<.001	<.001	<.001	
Harvest			<.001	<.001	<.001	
Cultivar			<.001	<.001	<.001	
Site × harvest			<.001	<.001	<.001	
Site × cultivar			<.001	<.001	<.001	
Harvest × cultivar			<.001	<.001	<.001	
Site × harvest × cultivar			<.001	<.001	<.001	

AUDPC: area under disease progress curve, R: resistant. Values with different superscript letters within the columns are statistically different at $p \leq 0.05$. Mean separation was done using LSD.

6.4.3 Progress of leaf rust, spot and blight diseases on *Brachiaria* grass cultivars planted at Gashora and Rubona experimental sites in Rwanda

Results of the evolution of foliar diseases from the 4th week to the 20th week showed that incidence of leaf rust, leaf spot and leaf blight increased with the number of weeks after standardisation cut and/or harvest (Figures 6.3, 6.4 and 6.5). Higher incidence of leaf rust disease ranging from 80 – 100% was observed for moderately susceptible and susceptible cultivars at Rubona at the 4th week for all harvests considered together. Groups of *Brachiaria* cultivars were made according to their response to different diseases. It was as follows: For leaf rust: susceptible (Cayman and Cobra), moderately susceptible (Mulato II) and moderately resistant (Basilisk, Humidicola, Marandu, MG4, Piata and Xaraes). For leaf spot: susceptible (Humidicola) and resistant (Basilisk, Cayman, Cobra, Marandu, MG4, Mulato II, Piata and Xaraes) while for leaf blight all groups were in one group as resistant. The incidence of leaf rust reached 100% at eight weeks and 12 weeks for moderately susceptible and moderately resistant cultivars at Rubona respectively. Over time, leaf rust incidence was the lowest in moderately resistant cultivars at Gashora (Figure 6.3). The leaf spot showed high diversity of cultivar responses throughout the crop growth periods (Figure 6.4). Leaf blight incidence was low in all cultivars until 16 weeks, then spiked at 20 weeks. Except at 16 weeks, leaf blight incidence was consistently higher at Rubona than Gashora (Figure 6.5).

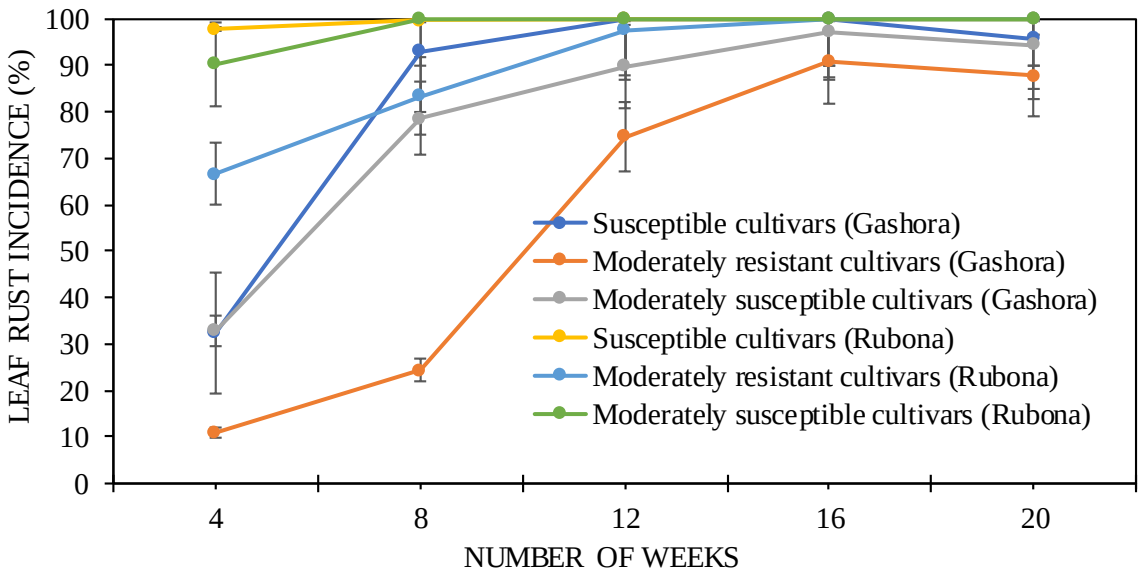

Figure 6.3: Leaf rust incidence over time at Gashora and Rubona experimental sites, in the years 2019 and 2020. The values presented are average for the group and error bars indicate standard errors of the mean.

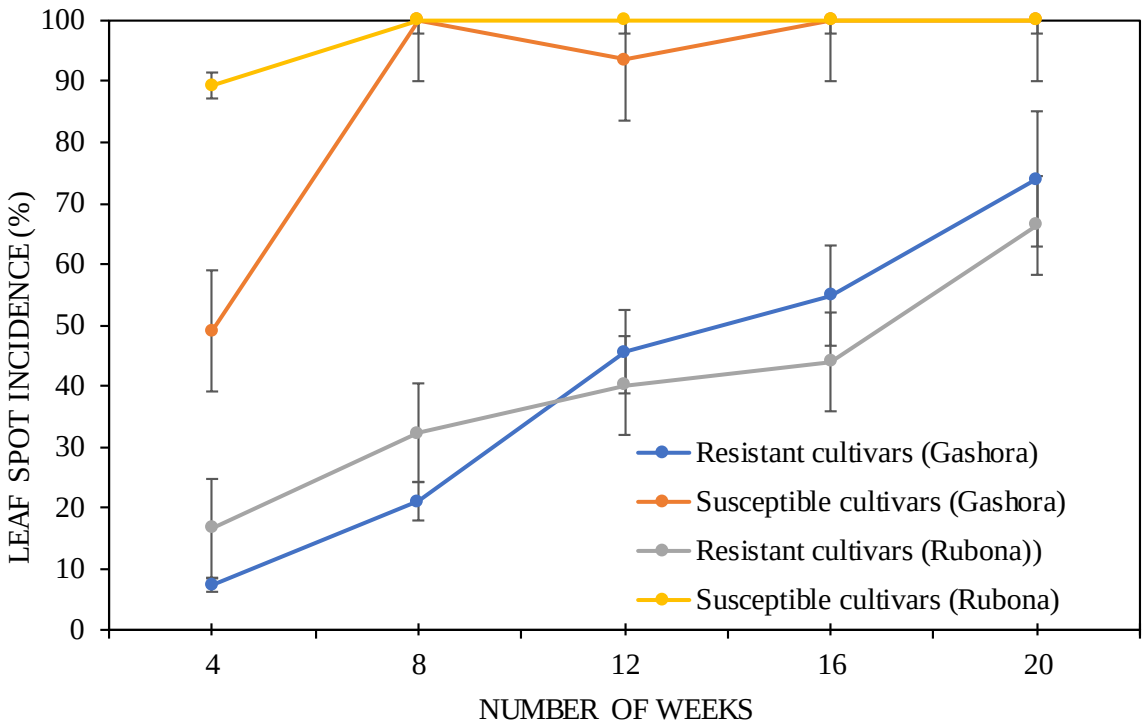

Figure 6.4: Leaf spot incidence over time at Gashora and Rubona experimental sites, in the years 2019 and 2020. The values presented are average for the group and error bars indicate standard errors of the mean.

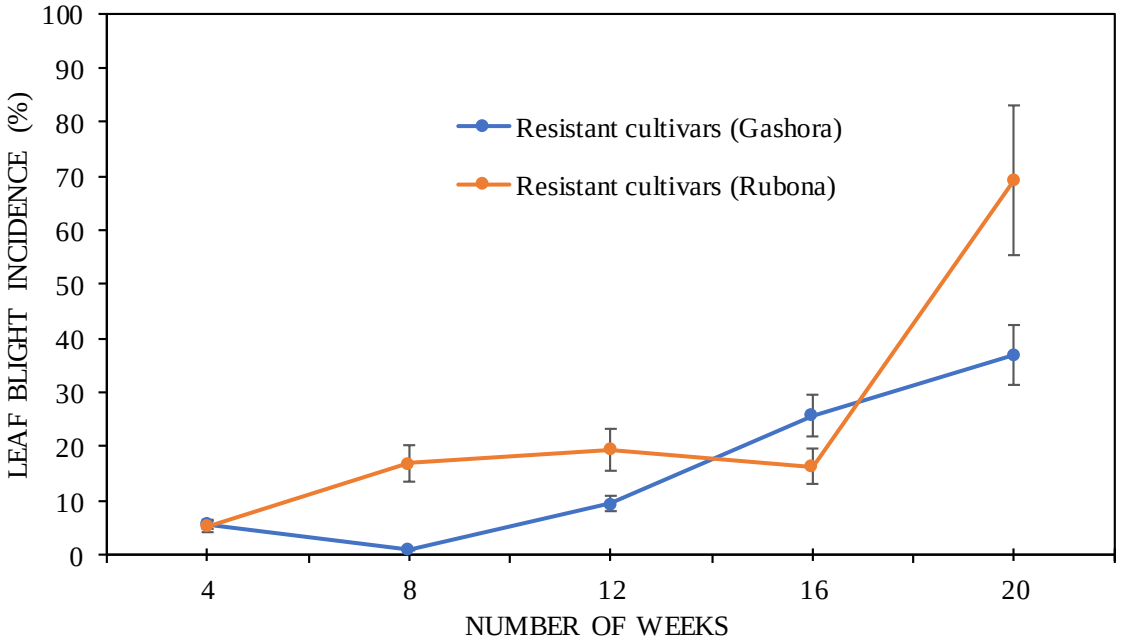

Figure 6.5: Leaf blight incidence over time at Gashora and Rubona experimental sites, in the years 2019 and 2020. The values presented are average for the group, and error bars indicate standard errors of the mean.

6.4.4 Agronomic performances of the *Brachiaria* cultivars under field conditions

The effects of cultivar, site, harvest, and their interactions were significant for plant height, number of tillers, dry biomass yield and dry matter content ($p \leq 0.001$; Table 6.5) except the effect of site on dry matter yield ($p = 0.415$). Cayman plants were shorter (34.2 cm) in the second harvest at Rubona than Humidicola, Xaraes and Piata plants (102.8 – 107.6 cm) in the first harvest at Rubona. Plants at Rubona (71 cm) were taller than those at Gashora (68 cm). Similarly, plants were taller (62.0 – 107.6 cm) in the first harvest than those in the second harvest (34.2 – 75.9 cm) and those in the third harvest (52.6 – 105.2 cm). The number of tillers per stool among cultivars ranged from 33 (Xaraes) in the first harvest at Gashora site to 299 (Humidicola) and 259 (Mulato II) in the third harvest at Rubona site. The dry biomass production ranged between 1.7 t ha^{-1} (Humidicola at Gashora in the second harvest) to 20.1 t ha^{-1} (Marandu in the first harvest at Rubona) and 20.2 t ha^{-1} (Xaraes in the third harvest at Gashora). Humidicola was the lowest biomass producer and Marandu, and Xaraes were the highest biomass producers. Rubona site gave higher biomass yield (14 t ha^{-1}) than Gashora site (11 t ha^{-1}), and the first and third harvests yielded more biomass (3.1 – 20.1 and 3.8 – 20.2 t ha^{-1} respectively) than the second harvest (1.7 – 20.1 t ha^{-1}). The percentage dry matter content among the tested cultivars ranged from 27.0% (Humidicola) to 42.6 – 47.0% (Cobra, Cayman and Piata). Cultivar Piata had the highest dry matter content in the third harvest at Rubona. Cultivar Humidicola had the lowest dry matter content in the third harvest at Gashora.

Table 6.5: Growth and dry matter yield of nine improved *Brachiaria* cultivars at Gashora and Rubona experimental sites, in the years 2019 and 2020

Site	Harvest	Cultivar	Plant height (cm)	Number tillers ($stool^{-1}$)	Dry matter yield (t ha^{-1})	Dry matter (%)
Gashora	First harvest (July 2019)	Basilisk	96.4^{bcd}	$125^{klmnopq}$	$14.6^{abcdefghi}$	41.3^{bcd}
		Cayman	76.3^{hijk}	9^{pqrst}	6.0^{nopqr}	40.5^{bcdef}
		Cobra	79.9^{fghij}	$164^{fghijkl}$	$11.7^{defghijklmn}$	40.7^{bcde}
		Humidicola	85.8^{efgh}	42^{uv}	3.1^{qr}	$37.6^{cdefghi}$
		Marandu	84.4^{efghi}	66^{tuv}	$15.1^{abcdefg}$	$38.3^{bcdefgh}$
		MG4	72.7^{jklm}	75^{rstuv}	$8.0^{ijklmnopqr}$	39.5^{bcdefg}
		Mulato II	$62.0^{nopqrstu}$	131^{klmnop}	$13.4^{bcdefghijkl}$	39.3^{bcdefg}
		Piata	68.4^{klmn}	42^{uv}	$7.1^{lmnopqr}$	38.0^{cdefgh}
		Xaraes	67.3^{klmnop}	33^{v}	6.4^{mnopqr}	$38.1^{bcdefgh}$

Site	Harvest	Cultivar	Plant height (cm)	Number tillers (stool^{-1})	Dry matter yield (t ha^{-1})	Dry matter (%)
	Second harvest (December 2019)	Basilisk	63.3^{mnopqrst}	155^{ghijkl}	10.8^{efghijklmno}	38.3^{bcdefgh}
		Cayman	40.9^{xy}	133^{jklmnop}	9.3^{fghijklmnopq}	42.6^{abc}
		Cobra	39.7^{xy}	194^{defg}	15.6^{abcdef}	42.9^{abc}
		Humidicola	53.0^{uvw}	54^{uv}	1.7^{r}	29.6^{mno}
		Marandu	65.4^{mnopqr}	144^{hijklmn}	15.6^{abcdef}	34.4^{ghijklm}
		MG4	56.0^{rstuvw}	112^{mnopqr}	8.7^{ghijklmnopq}	32.0^{jklmno}
		Mulato II	46.^{7wx}	217^{bcde}	18.7^{ab}	42.2^{abc}
		Piata	65.9^{lmnopq}	79^{rstu}	10.3^{fghijklmnop}	35.9^{efghij}
		Xaraes	68.7^{klmn}	97^{pqrst}	14.7^{abcdefgh}	34.5^{ghijklm}
	Third harvest (May 2020)	Basilisk	75.2^{ijkl}	144^{hijklmn}	7.1^{lmnopqr}	30.2^{lmno}
		Cayman	58.8^{opqrstuv}	132^{klmnop}	11.1^{efghijklmno}	30.5^{klmno}
		Cobra	58.4^{pqrstuv}	175^{efghij}	7.5^{klmnopqr}	31.4^{jklmno}
		Humidicola	63.9^{mnopqrs}	73^{rstuv}	3.8^{pqr}	27.0^{o}
		Marandu	88.2^{cdef}	141^{ijklmno}	17.2^{abcde}	30.0^{lmno}
		MG4	72.8^{jklm}	107^{mnopqrst}	15.2^{abcdefg}	31.7^{jklmno}
		Mulato II	57.0^{qrstuv}	197^{defg}	10.4^{fghijklmno}	31.2^{jklmno}
		Piata	91.9^{cde}	84^{qrstu}	14.2^{abcdefghij}	30.8^{jklmno}
		Xaraes	86.4^{efg}	98^{pqrst}	20.2^{a}	28.5^{no}
Rubona	First harvest (July 2019)	Basilisk	86.8^{ef}	108^{mnopqrst}	12.8^{bcdefghijklm}	36.1^{defghij}
		Cayman	80.1^{fghij}	97^{pqrst}	5.6^{nopqr}	37.6^{cdefghi}
		Cobra	68.1^{klmno}	134^{jklmop}	10.9^{efghijklmno}	39.4^{bcdefg}
		Humidicola	102.8^{ab}	109^{mnopqrs}	6.0^{nopqr}	37.5^{cdefghi}
		Marandu	97.3^{bc}	100^{opqrst}	20.1^{a}	33.1^{hijklmn}
		MG4	97.2^{bc}	98^{pqrst}	18.6^{abc}	37.7^{cdefghi}
		Mulato II	70.1^{klmn}	106^{nopqrst}	11.8^{defghijklmn}	35.8^{efghijk}
		Piata	107.6^{a}	73^{rstuv}	17.9^{abcd}	35.8^{efghijk}
		Xaraes	103.6^{ab}	68^{stuv}	18.2^{abcd}	34.6^{ghijklm}
	Second harvest (December 2019)	Basilisk	56.3^{rstuv}	183^{efghi}	14.0^{abcdefghijk}	35.8^{efghijk}
		Cayman	34.2^{y}	122^{lmnopq}	3.5^{qr}	38.2^{bcdefgh}
		Cobra	35.5^{y}	186^{efgh}	6.3^{mnopqr}	31.9^{jklmno}
		Humidicola	75.9^{ijk}	192^{defg}	7.8^{jklmnopqr}	40.4^{bcdef}

Site	Harvest	Cultivar	Plant height (cm)	Number tillers (stool^{-1})	Dry matter yield (t ha^{-1})	Dry matter (%)
		Marandu	55.0^{stuvw}	185^{efgh}	12.0^{defghijklmn}	30.9^{jklmno}
		MG4	51.7^{vw}	166^{fghijk}	8.6^{ghijklmnopq}	33.1^{hijklmn}
		Mulato II	36.3^{y}	203^{def}	8.4^{hijklmnopqr}	30.4^{lmno}
		Piata	64.4^{mnopqrs}	136^{jklmnop}	12.0^{cdefghijklmn}	38.4^{bcdefgh}
		Xaraes	67.5^{klmnop}	123^{klmnopq}	11.8^{defghijklmn}	32.5^{ijklmn}
	Third harvest (May 2020)	Basilisk	64.4^{mnopqrs}	229^{bc}	11.9^{defghijklmn}	41.8^{abc}
		Cayman	52.6^{uvw}	148^{hijklm}	4.7^{opqr}	43.4^{ab}
		Cobra	56.7^{qrstuv}	213^{cde}	7.4^{klmnopqr}	38.5^{bcdefg}
		Humidicola	105.2^{ab}	299^{a}	8.7^{ghijklmnopq}	35.3^{fghijkl}
		Marandu	66.1^{lmnopq}	251^{bc}	15.0^{abcdefg}	37.9^{cdefgh}
		MG4	64.5^{mnopqrs}	195^{defg}	10.6^{efghijklmno}	39.6^{bcdefg}
		Mulato II	54.0^{tuvw}	259^{ab}	10.4^{fghijklmnop}	39.0^{bcdefg}
		Piata	87.4^{def}	230^{bcd}	15.3^{abcdef}	47.0^{a}
		Xaraes	76.9^{ghijk}	203^{def}	14.4^{abcdefghij}	38.0^{cdefgh}
Source of variation			*p* values			
Site			<.001	<.001	0.415	<.001
Harvest			<.001	<.001	0.025	<.001
Cultivar			<.001	<.001	<.001	<.001
Site × harvest			<.001	<.001	<.001	<.001
Site × cultivar			<.001	<.001	<.001	<.001
Harvest × cultivar			<.001	<.001	<.001	<.001
Site × harvest × cultivar			<.001	<.001	<.001	<.001

Values with different superscript letters within the columns are statistically different at $p \leq 0.05$. Mean separation was done using LSD.

6.4.5 Correlation between area under disease curve and agronomic parameters

Pearson's correlation analysis revealed significant differences ($p \leq 0.05$; Table 6.6) between agronomic parameters (plant height, dry matter yield and dry matter content) and the AUDPC for leaf rust, leaf spot and leaf blight diseases. The analysis considered all cultivars, sites and harvests together. There was a positive significant correlation of plant height with dry matter yield. A significant- negative correlation was found between plant performance in terms of height and AUDPCs for leaf rust and leaf blight diseases.

A significant-negative correlation was observed between AUDPCs for leaf rust and leaf spot diseases and dry matter yield (Table 6.6).

Table 6.6: Coefficients for Pearson's correlation between agronomic traits and AUDPC for three major foliar diseases infecting *Brachiaria* grass, in the years 2019 and 2020

	Plant height	AUDPC leaf rust	AUDPC leaf spot	AUDPC leaf blight	Dry matter yield	Dry matter (%)
Plant height	1.000					
AUDPC leaf rust	-0.295**	1.000				
AUDPC leaf spot	0.037*	-0.081**	1.000			
AUDPC leaf blight	-0.134**	0.311**	0.085**	1.000		
Dry matter yield	0.284**	-0.119**	-0.103**	-0.012ns	1.000	
Dry matter (%)	-0.118**	0.194**	0.137**	0.171**	0.067**	1.000

**: significant correlation at $p \leq 0.01$, *: significant correlation at $p \leq 0.05$, ns: non significant at $p \leq 0.05$, AUDPC: area under disease progress curve.

6.5 Discussion

Diseases have been reported to be among the major biotic factors limiting fodder production and qualities of *Brachiaria* grass that negatively affect forage availability and livestock production. Different studies reported the cases of fungi, bacteria and viruses as causal agents of diseases on *Brachiaria* grass (Lenné, 1990; Nzioki *et al.*, 2016; Valerio *et al.*, 1996; Uzayisenga *et al.*, 2020). Negative effects of some of these diseases can lead to complete deterioration of the crop. Therefore, the effective and economic management of these diseases are critical for the sustainability of *Brachiaria* grass production in the tropical and sub-tropical regions. Among various methods for disease management, using cultivars that are resistant have been proven to be an effective and least cost control measure that can be easily adopted by farmers including resource-limited smallholder livestock keepers from the developing countries. Under field conditions, nine improved *Brachiaria* grass cultivars were evaluated against three major foliar diseases - leaf rust, leaf spot and leaf blight - in two sites each representing different agro-ecological zones of Rwanda for three consecutive harvests, that correspond to three distinct growing seasons. In addition, each cultivar's agronomic performances in terms of plant height, number of tillers and biomass production were documented and relationships between diseases and selected agronomic traits were determined.

The *Brachiaria* grass cultivars reacted differently to leaf rust, leaf spot and leaf blight diseases. Moreover, the interaction effect between cultivar, site and harvest on the foliar disease development was obvious. These results could be combined effects of (i) difference in the genetic background of cultivars, (ii) variation in the virulence level of pathogens, and (iii) the biophysical characteristics of experimental sites that support or limit disease development (Agrios, 2005). The observation that genotypes including Cobra and Cayman expressed symptoms of all three diseases may indicate the lack of host gene against a specific fungus associated with each disease. The effect of *Brachiaria* cultivar, experimental site and harvest and interaction between cultivar, site and harvest on the foliar disease development were evident. Earlier studies reported the presence of susceptible and resistant traits in *Brachiaria* grass germplasms to leaf rust and foliar blight (Alvarez *et al.*, 2014; Torres and Trutmann, 1991), implying varying level of susceptibility to diseases among *Brachiaria* cultivars (Kamidi *et al.*, 2016).

The evaluation of *Brachiaria brizantha*, *Brachiaria decumbens*, *Brachiaria dictyoneura* and *Brachiaria humidicola* accessions against leaf rust in two regions of Colombia indicated susceptibility of all cultivars except *Brachiaria decumbens* in both regions (Torres and Trutmann, 1991).

Surveillance of *Brachiaria* grass diseases in Kenya and Rwanda revealed differences in the level of disease development in a cultivar between study sites and seasons (Nzioki *et al.*, 2016; Uzayisenga *et al.*, 2020), ratifying the influence of the biophysical characteristics of test locations on the disease development. Environmental factors played important roles in the initiation and development of diseases. Though the differences in mean temperature between experimental sites were minimal, there were noticeable differences between sites for rainfall. A significant variation in levels of both incidence and severity of *Brachiaria* foliar diseases in different districts of Rwanda with diverse agro-climatic conditions has been reported previously (Uzayisenga *et al.*, 2020). A high level of leaf rust development at Rubona in this study could be associated with moist conditions due to high and well distributed rainfall throughout the crop growing periods. Rubona received 1.65 times greater rainfall than Gashora during the wet to semi-dry season of 2019, as well as in the wet season of 2020. The effect of climatic variables such as temperature and humidity on wheat rust disease development has been reported by Barrera *et al.* (2012) and Sandhu *et al.* (2017). The high incidence and severity of leaf rust and blight at Rubona could have been favoured by a high rainfall regime which is associated with a moist environment. On the contrary, the relatively dry weather at Gashora might have supported leaf spot disease development. High incidence and severity were registered as the week advanced, and to some extent in the subsequent harvests. These findings suggest that continuous presence of a perennial crop like *Brachiaria* grass in the field favoured pathogen population build-up over seasons and occurrence of the disease early in the season affecting crop performance and yields. In most cultivars, leaf rust incidence declined after 16 weeks, which might be due to the removal of rust spores as a result of climatic conditions including rain. Another probable reason is adult plant resistance to rust disease. The *Brachiaria* cultivars Basilisk, Marandu, MG4 and Xaraes were moderately resistant to all three foliar diseases and they had higher biomass yields than other cultivars confirming their suitability for cultivation in wider geographical regions.

The low level of leaf blight disease development in all cultivars could be due to a low natural disease pressure in experimental sites and/or presence of intrinsic resistance in some cultivars (Alvarez *et al.*, 2014; Kelemu *et al.*, 1995) that merits further investigation. Alternative disease management options for susceptible cultivars that produced high dry matter content (e.g., Cayman and Cobra in this study) can be another area for further investigation, as this trait has a significant role in feed availability and improving livestock productivity.

Significant-negative correlation between AUDPC of foliage diseases and dry matter yield indicate negative impact of these diseases on herbage yield of improved *Brachiaria* cultivars. This might be attributed to the reduction of the photosynthetic area in the diseased leaf tissues as reported for wheat diseases (Kandel *et al.*, 2009; Lamsal *et al.*, 2017; Pandey *et al.*, 2018) as well as pathogen's dependence on host for nutrients and water. Significant and negative correlation between leaf rust and foliar blight and plant height was observed, conferring the negative effect of diseases on plant performance. Other studies have also reported negative correlation between wheat spot blotch disease and plant height (Joshi *et al.*, 2002; Neupane *et al.*, 2013; Rosyara *et al.*, 2009). The use of *Brachiaria* grass for pasture improvement and ruminant feeding in Africa started through the introduction of improved cultivars and hybrids mostly from South America (Maass *et al.*, 2015; Njarui *et al.*, 2016). Within a very short period, *Brachiaria* grass has become a forage of choice for many livestock farmers on the continent to address challenges including shortage of livestock feeds, poor nutritive value of local forages, extended and frequent drought, and declining productivity of extensively cultivated Napier grass (Ghimire *et al.*, 2015; Negawo *et al.*, 2017). The use of *Brachiaria* grass as an additional forage option in Sub-Saharan Africa to improve livestock productivity through improving supply of quality herbage have been established recently (Mutimura *et al.*, 2016, 2018; Njarui *et al.*, 2016). Different studies reported on attack of improved *Brachiaria* cultivars by multiple diseases with fungi, bacteria and viruses as the causal agents, affecting the yields and qualities of forage and seed crops (Kamidi *et al.*, 2016; Nzioki *et al.*, 2016; Uzayisenga *et al.*, 2020). This calls for a need to develop new cultivars with improved disease resistance qualities, exploring genetic diversity available in genebanks and natural populations, preferably from East Africa that is also the centre of diversity of the genus *Brachiaria* (Keller-Grein *et al.*, 1996).

6.6 Conclusions

Under field experiment, this study revealed a wide variation in nine improved *Brachiaria* grass cultivars to leaf rust, leaf blight and leaf spot diseases in Rwanda. Besides variable responses to diseases, cultivars were also different for important agronomic traits such as plant height, tiller numbers, biomass yield and dry biomass content. This study identified Basilisk, Marandu, MG4 and Xaraes as moderately resistant/resistant to all three foliar diseases and had high biomass yields. These cultivars are more appropriate for upscaling *Brachiaria* grass in wider geographical regions in Rwanda and neighbouring countries. There are reports available on the short life of disease-resistant cultivars of many important crops; thus, activities such as periodic disease surveys, breeding new disease-resistant cultivars and development of other disease management options should be given high priority for effective, economic and sustainable management of *Brachiaria* grass diseases. Farmers need to use certified planting materials from recognised sources and maintain their plots appropriately for sustainale production of *Brachiaria* grass.

CHAPTER SEVEN:
EFFICACY OF DISEASE MANAGEMENT OPTIONS ON LEAF RUST IN BRACHIARIA GRASS IN RWANDA

7.1 Abstract

Leaf rust is an emerging threat to the expansion of *Brachiaria* grass acreage, as it negatively affects herbage production and quality. Therefore, this study was conducted to determine the best options for managing leaf rust affecting *Brachiaria* grass in smallholder farms in Rwanda. In total, six disease management options (mineral fertiliser application, mancozeb application, manual weeding, no fungicide application, no weeding, and no fertiliser application) were evaluated for *Brachiaria* hybrid cv. Cayman at the Rubona Research Station of RAB from October 2019 to June 2020. The design of the experiment was a randomised complete block with four replicates. Leaf rust incidence, severity, and agronomic performance data were collected every eight weeks for four consecutive harvests. The results showed that leaf rust incidence and severity were significantly reduced ($p \leq 0.05$) as a result of mancozeb and mineral fertiliser treatments revealing simultaneous increases in plant growth, number of tillers, and biomass production. However, there was an increase in leaf rust incidence and severity in consecutive harvests, irrespective of the disease management option employed. Further, the no weeding treatment resulted in a low incidence and severity of the disease, in addition to a low biomass yield. These results demonstrate the effectiveness of mancozeb and mineral fertiliser application in managing leaf rust and increasing crop performance. The information provided in this study regarding the efficacy of mancozeb application for the control of leaf rust in *Brachiaria* grass should be explored and used by seed companies and forage seed producers to obtain planting material free from leaf rust disease.

Keywords: Forage, disease management, biomass yield, manual weeding, incidence, severity, agronomic parameters.

7.2 Introduction

Brachiaria (syn. *Urochloa*) grass belongs to the *Poaceae* family (Jungmann *et al.*, 2009) and is one of the most nutritious tropical forages originating from Africa. All *Brachiaria* species that are used for pasture production are naturally found in eastern Africa, which is considered a centre of diversity for this genus (Keller-Grein *et al.*, 1996). Moreover, *Brachiaria* grass is a preferred forage for farmers in Rwanda and other East African countries (Mutimura and Everson, 2012; Mutimura and Ghimire, 2021).

These cultivars were developed outside of Africa for specific purposes. For instance, *Brachiaria* hybrid cv. Cayman was developed for an increased tolerance to waterlogging (Pizarro *et al.*, 2013). Initiated research and development programmes on *Brachiaria* grass have been focusing on the introduction and evaluation of improved cultivars in different agroclimatic conditions and consider the cultivars' adaptation to drought and low fertility soils, agronomic performance, livestock productivity, and upscale feasibility for fodder production. However, the production of *Brachiaria* grass in Africa faces multiple challenges, mainly pests and diseases, including leaf rust disease.

Leaf rust is one of the most devastating diseases affecting *Brachiaria* grass worldwide. In particular, the susceptibility of several *Brachiaria* species, including *Brachiaria brizantha*, *Brachiaria decumbens*, *Brachiaria dictyoneura*, and *Brachiaria humidicola* has been reported (Lenné and Trutmann, 1994; Miles *et al.*, 1996; Rao and Ghimire, 2016; Uzayisenga *et al.*, 2020). Leaf rust is an endemic *Brachiaria* grass disease that occurs in Kenya and Rwanda (Nzioki *et al.*, 2016; Uzayisenga *et al.*, 2020). It reduces both the quantity and quality of *Brachiaria* biomass. Leaf rust is caused by various obligate pathogens with more than 8,000 documented species (Aime *et al.*, 2014) among which *Phakospora apoda* has been proposed to cause leaf rust disease in Rwandan *Brachiaria* grass (Uzayisenga *et al.*, 2020). In Brazil, *Brachiaria* leaf rust is widely distributed and is caused by *Uromyces setariae-italicae* (Lenné and Trutmann, 1994). Symptoms of *Brachiaria* leaf rust are the presence of yellowish or brownish pustules on the adaxial leaf surface (Uzayisenga *et al.*, 2020). Biomass reduction caused by leaf rust can reach up to 100% in *Brachiaria* grass, and the reduction in crude proteins is estimated to be between 49% and 53%. The availability of other nutrients is also highly affected, even when the affected leaf area is below 5% (Lenné and Trutmann, 1994).

Leaf rust disease management options include establishing hedges, accelerating *Brachiaria* growth via nitrogen fertiliser application, using rust-free planting materials, planting at an appropriate time because rust is favoured by rainfall, using diverse *Brachiaria* genotypes, avoiding burning, and harvesting *Brachiaria g*rass early (Alvarez *et al.*, 2014; CIAT, 2004).

Nevertheless, the acreage of *Brachiaria* grass has increased in Africa despite the challenges of various pests and diseases, including leaf rust (Njarui *et al.*, 2016; Nzioki *et al.*, 2016; Mutimura and Ghimire, 2021; Uzayisenga *et al.*, 2020). Leaf rust is widely distributed in Rwanda, and while most cultivars currently grown therein are susceptible, there are no specific recommendations for leaf rust management. The planting of susceptible cultivars coupled with environmental conditions that favour the development of diseases necessitates the use of fungicides or other management options to avoid qualitative and quantitative biomass yield losses. Therefore, this study was conducted to evaluate the efficacy of fertilization, fungicide application and weeding in managing leaf rust on *Brachaiaria* grasses under field conditions.

7.3 Materials and Methods

7.3.1 Description of experimental site

An open field experiment was conducted at the Rubona Research Station of the RAB in October 2019. The station is located in the Huye District, Southern Province of Rwanda, in the Central Plateau, and has granitic ridges at 1,673 m a.s.l, a latitude' coordinates of 02°29'S, and a longitude' coordinates of 029°46"E. Rubona has sandy and clay soil types and distinct seasons, including dry and rainy seasons. The long and short dry seasons occur from June to August and from mid-January to mid-March, respectively. Conversely, the short and long rain seasons occur from middle of March - May and from September – December, respectively. The region has 298 mm as an average annual rainfall and has an average temperature of 19.0 °C (RMA, 2021, Figure 7.1).

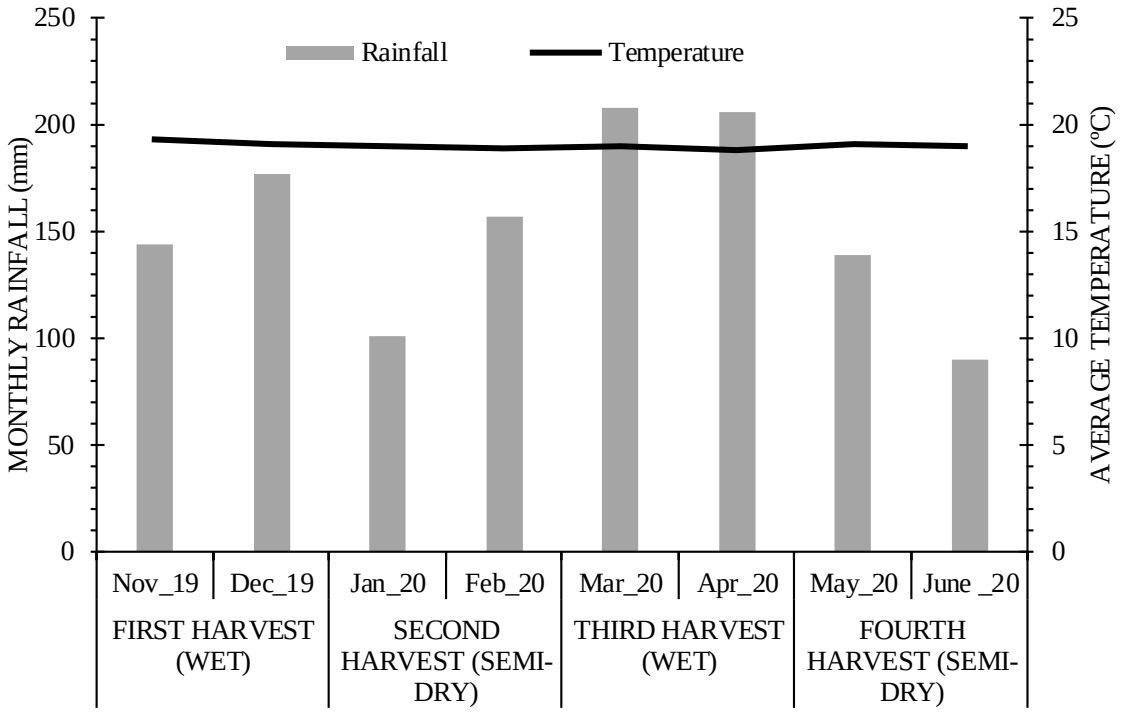

Figure 7.1: Monthly average rainfall and temperature at the Rubona Research Station during experimental periods.

7.3.2 Experimental design and crop management

Ploughing and harrowing were performed manually, during which all weeds were removed from the field. For the initial soil characterisation, composite soil samples were taken from designed experimental plots prior to the experiment. These soil samples were subjected to a complete analysis, including pH, organic matter content, macronutrients, and soil texture at the Soil Science Laboratory at the RAB. Rooted tillers of *Brachiaria* hybrid cv. Cayman, a leaf rust susceptible cultivar (Uzayisenga *et al.*, 2021) obtained from the RAB germplasm collection, was used in the experiment. Planting was conducted in October 2019 using one-month-old rooted vegetative tillers in 2 m × 1.8 m plots with 1 m spacing between experimental plots and replicates were spaced at 1.5 m. The crop spacing was 30 cm between seedlings and 50 cm between rows. The randomised complete block design was used with six treatments, namely (i) mineral fertiliser application, (ii) fungicide (mancozeb) application, (ii) manual weeding, (iv) control 1 (no fungicide application), (v) control 2 (no weeding) and (vi) control 3 (no fertiliser application), each of which had four replicates. During the preparation of experimental plots, organic manure (10 t ha^{-1}) was applied, except for the no-fertiliser control treatment. For the mineral fertiliser treatment, a dose of 100 kg NPK17-17-17 ha^{-1} was applied.

Except the no-weeding control plots, weeds were removed manually using hand hoes to maintain a weed free plot. No control measures were applied to any other pest or disease. A standardisation cut was performed three weeks after planting to stimulate the uniformity of plant growth for all seedlings. The first harvest was conducted eight weeks after the standardisation cut, and subsequent harvests were performed every eighth week. Thus, the first harvest was performed in December 2019, the second harvest was in February 2020, the third harvest was conducted in April 2020 and the fourth harvest was performed in June 2020 (Figure 7.1).

7.3.3 Fungicide selection and application

The fungicide used in this study, mancozeb, was purchased from a local agrochemical dealer in Rwanda. It is a non-systemic fungicide, broad-spectrum fungicide with protective action. This selection was based on availability, affordability, efficacy, safety to humans, plants, and the environment, and applicability. Mancozeb is a common fungicide that is available, registered, and popular among farmers in Rwanda. It is used to control rust disease in different crops, including the vegetable crop French beans (Gullino *et al.*, 2010; Vuyyuru *et al.*, 2018). Mancozeb has several desirable attributes. Specifically, it has a short waiting period, which helps to avoid potential harm to animals, humans, the environment, and an oral lethal dose (LD50) value of greater than 8,000 mg per kg (Gullino *et al.*, 2010; Vuyyuru *et al.*, 2018). Mancozeb was applied three times before biomass harvesting: one day after planting or one day after harvesting, and two and four weeks after the application of the previous spray. Mancozeb is known under different trade names, including penncozeb, trimanoc, vondozeb, dithane, manzeb, nemispot, manzane, and dithane M-45 (Vuyyuru *et al.*, 2018). A mixture of 40 g of mancozeb in 10 L of water was applied to the *Brachiaria* stools using knapsack sprayers. Plots that received mancozeb treatment were surrounded by plastic sheets during spraying to prevent drifting to neighbouring plots. Control plots were sprayed with water without a mancozeb suspension.

7.3.4 Assessment of incidence and severity of leaf rust

Leaf rust incidence and severity were recorded on tagged stools and six stools appearing uniform in their growth were selected and tagged for each treatment per replication. Data were collected every two weeks after the standardisation cut until the last date of data collection when the experiment ended. For the whole experiment, four recordings were collected for each of the four consecutive harvests. Disease incidence was assessed on the six tagged stools for each replication and was determined by first identifying the number of stools showing disease symptoms and then converting this value to the percentage of all assessed stools. For each stool, the severity of the leaf rust disease was assessed using a 0 – 6 scale, where 0 = no infection, 1 = 5% infection, 2 = 10% infection, 3 = 20% infection, 4 = 40% infection, 5 = 60% infection, and 6 = 100% infection (CIMMYT, 1985; Peterson *et al.*, 1948). The AUDPC was calculated using the severity data collected over four different time points for each harvest (Shaner and Finney, 1973).

7.3.5 Evaluation of agronomic parameters

Agronomic parameters including plant height and number of tillers per stools were measured at an interval of two weeks following standardisation cut until harvest and the dry matter (DM) yield and percent DM content were recorded at harvest for all four harvests. Plant height was recorded from the ground as the base of the stool up to the longest leaf of each tagged stool using a graduated rod (Rayburn *et al.*, 2007). Six stools appearing uniform in their growth were selected and tagged in each treatment for each replicate to evaluate the disease and agronomic parameters. The experiment performed four consecutive harvests from November 2019 to June 2020 as described above. In order to determine the dry matter (DM) content, 200 g of each sub-sample from fresh biomass was placed in a paper bag and dried in the oven at 105°C for a time life of 24 hours. Using these results, the total DM yield (kg ha^{-1} DM) and the percentage of DM were calculated (Oliveira *et al.*, 2019; Wassie *et al.*, 2018).

7.3.6 Statistical data analysis

The analysis of variance using GenStat for Windows 20th Edition (VSN International, 2019) was performed for data on disease parameters, mainly incidence, severity, and AUDPC, as well as for agronomic traits, including plant height, number of tillers, DM yield, and the percentage of DM.

General linear model predictions using repeated measurements (Littell *et al*., 1991) was used to account for the overall trends of disease incidence and severity, and agronomic traits. In particular, the data were analysed for all harvest × management options/treatments interactions, and the significance of interactions was used to present results. Further, Spearman correlations were calculated to examine the relationships between plant height, number of tillers, DM yield, and the AUDPC.

7.4 Results

7.4.1 Chemical and physical properties of soils at the study site

Table 7.1 summarises the results of the soil parameters at the experimental site before and after the experiment. The soil of Rubona has both good organic carbon (2.5%) and nitrogen (0.14%) contents, and its texture is light with sand, silt, and clay contents of 73%, 15%, and 12%, respectively. The results from the soil test, when the experiment ended, showed no changes in organic carbon and total nitrogen levels. However, there was an increase in pH, and available phosphorus, and a decrease in the cation exchange capacity (Table 7.1).

Table 7.1: Chemical and physical properties of soil before and after experiment at Rubona, Rwanda

Soil properties		Prior to the experiment	After experiment					
			Disease management options					
			Mineral fertiliser	Fungicide application	Manual weeding	Control 1-No fungicide	Control 2-No weeding	Control 3-No mineral fertiliser
pH		4.5	5.5	5.5	5.5	5.5	5.6	5.6
Total N	(%)	0.1	0.2	0.2	0.2	0.2	0.1	0.2
Organic C	(%)	2.5	2.9	2.6	2.9	2.8	2.6	2.7
Available P	(ppm)	108.8	148.8	164.8	158.9	165.9	159.3	165.9
Ca	(meq/ 100 g soil)	2.1	2.6	2.4	1.8	2.2	2.2	2.2
Mg		0.6	0.7	0.5	0.3	0.4	0.5	0.3
K		0.3	0.2	0.2	0.2	0.2	0.4	0.2
Cation exchange capacity		24.4	18.2	15.2	13.2	17.6	13.3	12.4
Sand	(%)	73.0	77.0	77.0	79.0	76.0	77.0	81.0
Silt		15.0	11.0	9.0	7.0	10.0	9.0	7.0
Clay		12.0	12.0	14.0	14.0	14.0	14.0	12.0

Soil collected from topsoil (0 to 30 cm) was used in analysis.

7.4.2 Effect of management options on leaf rust incidence and severity

The incidence and severity of leaf rust varied significantly between the management options ($p < 0.001$; Table 7.2). Specifically, mancozeb application showed a lower incidence (19.8%) and severity (3.3%), as well as a lower AUDPC (150) than the other treatments. The control treatments (no mineral fertiliser and no pesticide) and manual weeding treatments exhibited a high leaf rust incidence and severity, in which the severity was the highest (55.4%) in the manual weeding treatment during the fourth harvest. Overall, the disease incidence and severity were the highest in the fourth harvest, as compared with the other harvests, and a similar trend was observed for the AUDPC (Table 7.2).

Regardless of the management strategy, the leaf rust incidence and severity result over time showed that both incidence and severity increased from the first to the fourth harvest and the magnitude consistently increased with harvest time (Figure 7.2). Leaf rust incidence ranged from 90% to 100% for all treatments in the sixth week, except for mancozeb application, which had a lower incidence than the other treatments. The leaf rust incidence and severity were consistently low under mancozeb application and consistently high for the no pesticide application and no weeding treatments for all harvests.

Table 7.2: Incidence and severity of leaf rust disease in *Brachiaria* hybrid cv. Cayman under different disease management options in Rubona, Rwanda during 2019 – 2020

Harvest	Management options	Incidence (%)	Severity (%)	AUDPC
First harvest	Mineral fertiliser application	58.3^{ij}	27.2^{ghi}	1,099^{ghij}
	Fungicide application	19.8^{l}	3.3^{l}	150^{l}
	Manual weeding	69.8^{gh}	32.2^{defghi}	1,385^{defghi}
	Control 1-No fungicide	66.7^{hi}	28.6^{fghi}	1,245^{fghi}
	Control 2-No weeding	66.7^{hi}	23.7^{hij}	1,048^{ghij}
	Control 3-No mineral fertiliser	78.1^{fg}	34.3^{defgh}	1,501^{defghi}
Second harvest	Mineral fertiliser application	70.8^{gh}	23.8^{hij}	1,089^{ghij}
	Fungicide application	55.2^{j}	11.5^{jkl}	526^{jkl}
	Manual weeding	87.5^{cdef}	41.8^{bcde}	1,932^{abcde}
	Control 1-No fungicide	90.6^{abcd}	43.2^{abcd}	1,990^{abcd}
	Control 2-No weeding	69.8^{gh}	20.3^{ijk}	943^{ijk}
	Control 3-No mineral fertiliser	88.5^{bcde}	35.1^{defgh}	1,594^{cdefgh}
Third harvest	Mineral fertiliser application	81.3^{def}	40.5^{bcdef}	1,786^{bcdef}
	Fungicide application	39.6^{k}	7.6^{kl}	328^{kl}
	Manual weeding	86.5^{cdef}	37.2^{cdefg}	1,656^{cdefg}
	Control 1-No pesticide	90.6^{abcd}	43.1^{abcde}	1,958^{abcde}

Harvest	Management options	Incidence (%)	Severity (%)	AUDPC
Fourth harvest	Control 2-No weeding	64.6^{hij}	22.1^{hij}	984^{hij}
	Control 3-No mineral fertiliser	79.2^{efg}	30.0^{efghi}	1,344^{efghi}
	Mineral fertiliser application	97.9^{ab}	50.2^{abc}	2,203^{abc}
	Fungicide application	91.7^{abc}	30.2^{defghi}	1,302^{fghi}
	Manual weeding	99.0^{a}	55.4^{a}	2,464^{a}
	Control 1-No fungicide	99.0^{a}	53.1^{ab}	2,333^{ab}
	Control 2-No weeding	92.7^{abc}	41.8^{bcde}	1,854^{abcdef}
	Control 3-No mineral fertiliser	100.0^{a}	51.9^{ab}	2,307^{ab}
Source of variation		*p* values		
Harvest		< 0.001	< 0.001	< 0.001
Management options		< 0.001	< 0.001	< 0.001
Harvest × management options		< 0.001	< 0.001	< 0.001

AUDPC: Area under disease progress curve. Values with different superscript letters within columns are statistically different at $p \leq 0.05$. Mean separation was done using LSD.

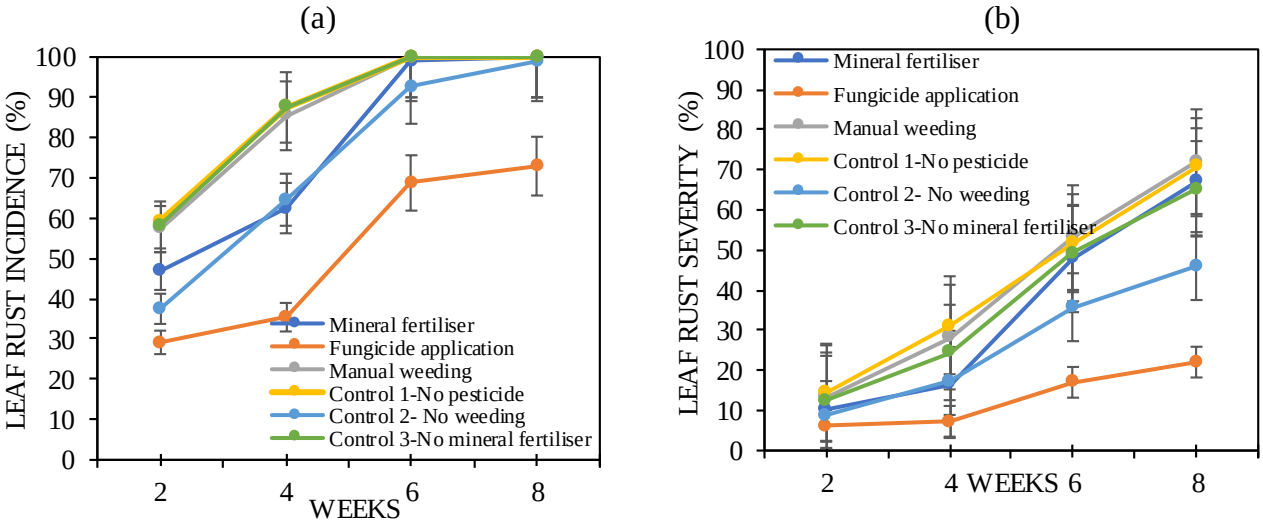

Figure 7.2: Leaf rust incidence and severity results over time under different disease management options in *Brachiaria* hybrid cv. Cayman in Rubona, Rwanda during 2019 – 2020. (a) and (b): All harvests are considered together. Bars indicate standard errors.

7.4.3 Agronomic performances under different disease management options

The effects of harvest time and management strategy were significantly different ($p \leq 0.002$) for plant height, number of tillers, DM yield, and percentage of DM content, whereas the interaction between management strategy and harvest time was not significant ($p > 0.05$) with respect to the percentage of DM content (Table 7.3). Plants were taller in the first harvest, followed by the second, third, and fourth (Table 7.3; Figure 7.3). Specifically, in the first harvest, where plant height was the highest, plant height declined as follows: Mineral fertiliser application > mancozeb application > manual weeding > no pesticide > no weeding > no mineral fertiliser (Table 7.3; Figure 7.3). Further, the number of tillers differed significantly between harvests, in which the first harvest had fewer tillers (for no manual weeding) and there was a gradual increase in tiller numbers in the subsequent harvests (the highest of which occurred in the fourth harvest for no pesticide and no mineral fertiliser) (Table 7.3). The DM yield was significantly higher under the mancozeb and mineral fertiliser management options in the second and first harvests, respectively, and was consistently lower in the no manual weeding treatment (Table 7.3).

Table 7.3: Agronomic performances of *Brachiaria* hybrid cv. Cayman under different leaf rust disease management strategies in Rubona, Rwanda during 2019 –2020

Harvest number	Diseases management Options	Plant height (cm)	Number of tillers (stool^{-1})	Dry matter (DM) yield (t ha^{-1})	DM content (%)
First harvest	Mineral fertiliser application	46.3^{a}	53^{g}	6.0^{ab}	18.1^{a}
	Fungicide application	43.8$^{ab`}$	48^{g}	4.9^{abc}	17.8^{a}
	Manual weeding	42.9^{ab}	46^{g}	4.3^{cd}	20.0^{a}
	Control 1-No fungicide	42.6^{ab}	46^{g}	4.2^{cd}	18.4^{a}
	Control 2-No weeding	42.3^{ab}	12^{i}	0.4^{j}	15.5^{a}
	Control 3-No mineral fertiliser	41.6^{abc}	43^{gh}	4.3^{cd}	19.2^{a}
Second harvest	Mineral fertiliser application	36.7^{cd}	85^{f}	4.6^{bc}	23.7^{a}
	Fungicide application	38.9^{bcd}	101^{f}	6.4^{a}	26.8^{a}
	Manual weeding	36.4^{d}	84^{f}	5.0^{abc}	25.5^{a}
	Control 1-No fungicide	36.5^{cd}	92^{f}	4.6^{bc}	24.7^{a}
	Control 2-No weeding	28.6^{ef}	22^{hi}	1.0^{ij}	25.0^{a}
	Control 3-No mineral fertiliser	36.9^{cd}	91^{f}	5.0^{abc}	25.4^{a}
Third harvest	Mineral fertiliser application	30.5^{e}	158de	4.7^{bc}	22.3^{a}
	Fungicide application	26.7^{efghi}	154de	4.3^{cd}	25.6^{a}
	Manual weeding	26.3^{efghij}	143^{e}	4.0^{cde}	27.3^{a}
	Control 1-No fungicide	28.0^{efg}	165^{cd}	4.0^{cdef}	24.4^{a}
	Control 2-No weeding	24.6^{fghij}	52^{g}	1.4^{hij}	22.6^{a}
	Control 3-No mineral fertiliser	27.0^{efgh}	157de	3.8^{cdefg}	24.6^{a}
Fourth harvest	Mineral fertiliser application	25.5^{efghij}	202^{ab}	2.8^{defgh}	32.2^{a}
	Fungicide application	21.7^{ij}	198^{ab}	2.4^{fghi}	28.6^{a}
	Manual weeding	22.5^{hij}	181^{bc}	2.1^{ghi}	31.5^{a}
	Control 1-No fungicide	23.2^{ghij}	207^{a}	2.5^{efghi}	30.6^{a}
	Control 2-No weeding	21.3^{ij}	80^{f}	1.2^{hij}	28.4^{a}
	Control 3-No mineral fertiliser	22.8^{hij}	204^{a}	2.3^{ghi}	30.6^{a}
Source of variation		*p* values			
Harvest		<0.001	<0.001	<0.001	<0.001
Management options		<0.001	<0.001	<0.001	0.002
Harvest × management options		<0.001	<0.001	<0.001	0.140

Values with different superscript letters within columns are statistically different at $p \leq 0.05$. Mean separation was done using LSD.

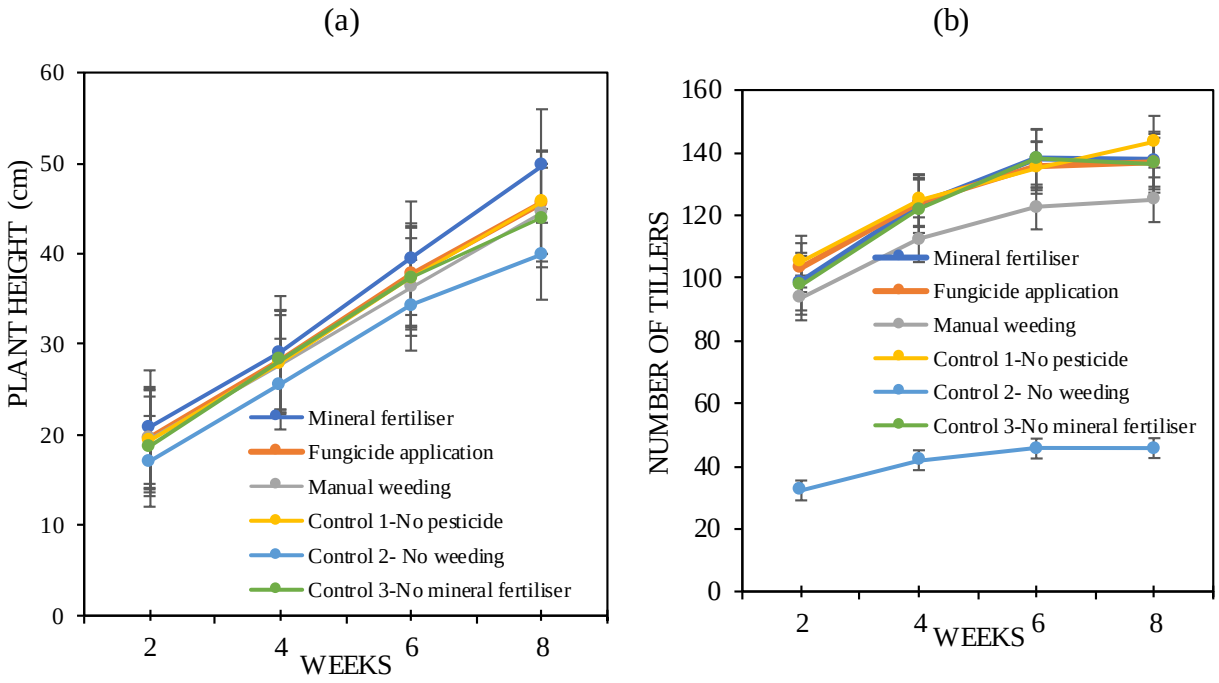

Figure 7.3: Plant height and number of tillers/stools over time in Rubona, Rwanda during 2019 – 2020. (a) and (b): All harvests are considered together. Bars indicate standard errors.

7.4.4 Correlation between disease intensity and agronomic parameters

Overall, a significant-negative correlation was found between plant height and the AUDPC for leaf rust disease. A signficant positive correlation was found between plant heigh and DM yield (Table 7.4). A negative-significant correlation was observed between the AUDPC for leaf rust disease and DM yield.

Table 7.4: Spearman's correlation coefficients between agronomic parameters and the AUDPC

Agronomic parameter	Plant height at harvest	DM yield (t ha^{-1})	AUDPC
Plant height at harvest	1.000		
DM yield (t ha^{-1})	0.483**	1.000	
AUDPC	-0.319**	-0.120**	1.000

**Correlation is significant at $p \leq 0.01$ probability level, ns = Not significant at $p \leq 0.05$ level. AUDPC = The area under the disease progress curve; DM= Dry matter. All harvests and management options are considered together.

The results of the stepwise correlation revealed that the number of tillers was positively and consistently correlated with DM yield from the first to the fourth harvest (Table 7.5). Further, there was a significant but negative correlation between manual weeding and the AUDPC in the fourth harvest, implying a high severity of leaf rust under manual weeding treatment.

Table 7.5: Spearman's correlation coefficients between agronomic parameters and AUDPC under different harvest and management scenarios

Harvest	Management option		Plant height at harvest	Number of tillers at harvest	AUDPC	DM yield	DM (%)
First harvest	Fertiliser application	Plant height at harvest	1.000				
		Number of tillers at harvest	0.288^{ns}	1.000			
		AUDPC	0.017^{ns}	-0.031^{ns}	1.000		
		DM yield	0.437^{*}	0.490^{*}	-0.106^{ns}	1.000	
		DM (%)	0.076^{ns}	0.099^{ns}	-0.153^{ns}	0.690^{**}	1.000
	Fungicide application	Plant height at harvest	1.000				
		Number of tillers at harvest	0.330^{ns}	1.000			
		AUDPC	-0.061^{ns}	0.152^{ns}	1.000		
		DM yield	0.266^{ns}	0.547^{**}	-0.023^{ns}	1.000	
		DM (%)	-0.253^{ns}	-0.141^{ns}	-0.024^{ns}	0.518^{**}	1.000
	Manual weeding	Plant height at harvest	1.000				
		Number of tillers at harvest	0.587^{**}	1.000			
		AUDPC	-0.403^{ns}	-0.458^{*}	1.000		
		DM yield	0.539^{**}	0.260^{ns}	-0.332^{ns}	1.000	
		DM (%)	-0.196^{ns}	-0.454^{*}	0.334^{ns}	0.304^{ns}	1.000
	Control 1-no pesticide	Plant height at harvest	1.000				
		Number of tillers at harvest	0.177^{ns}	1.000			
		AUDPC	-0.126^{ns}	-0.061^{ns}	1.000		
		DM yield	0.240^{ns}	0.406^{*}	-0.243^{ns}	1.000	
		DM (%)	0.052^{ns}	0.039^{ns}	-0.139^{ns}	0.678^{**}	1.000
	Control 2-no weeding	Plant height at harvest	1.000				
		Number of tillers at harvest	-0.062^{ns}	1.000			
		AUDPC	0.408^{*}	0.266^{ns}	1.000		
		DM yield	0.619^{**}	0.124^{ns}	0.316^{ns}	1.000	
		DM (%)	-0.220^{ns}	-0.058^{ns}	-0.208^{ns}	0.194^{ns}	1.000
	Control 3-no fertiliser	Plant height at harvest	1.000				
		Number of tillers at harvest	0.183^{ns}	1.000			
		AUDPC	0.009^{ns}	-0.556^{**}	1.000		
		DM yield	0.457^{*}	0.275^{ns}	-0.135^{ns}	1.000	

Harvest	Management option		Plant height at harvest	Number of tillers at harvest	AUDPC	DM yield	DM (%)
		DM (%)	0.085^{ns}	-0.422*	0.279^{ns}	0.592**	1.000
Second harvest	Fertiliser application	Plant height at harvest	1.000				
		Number of tillers at harvest	0.555**	1.000			
		AUDPC	0.182^{ns}	0.102^{ns}	1.000		
		DM yield	0.515*	0.754**	0.034^{ns}	1.000	
		DM (%)	0.161^{ns}	0.056^{ns}	-0.155^{ns}	0.411*	1.000
	Fungicide application	Plant height at harvest	1.000				
		Number of tillers at harvest	0.200^{ns}	1.000			
		AUDPC	0.417*	0.050^{ns}	1.000		
		DM yield	0.375^{ns}	0.411*	0.254^{ns}	1.000	
		DM (%)	-0.287^{ns}	-0.132^{ns}	-0.233^{ns}	0.292^{ns}	1.000
	Manual weeding	Plant height at harvest	1.000				
		Number of tillers at harvest	0.508*	1.000			
		AUDPC	0.076^{ns}	-0.377^{ns}	1.000		
		DM yield	0.508*	0.801**	-0.252^{ns}	1.000	
		DM (%)	-0.254^{ns}	-0.382^{ns}	0.166^{ns}	-0.066^{ns}	1.000
	Control 1-no pesticide	Plant height at harvest	1.000				
		Number of tillers at harvest	-0.242^{ns}	1.000			
		AUDPC	0.409*	-0.351^{ns}	1.000		
		DM yield	0.514*	0.282^{ns}	0.375^{ns}	1.000	
		DM (%)	-0.162^{ns}	-0.293^{ns}	0.233^{ns}	0.170^{ns}	1.000
	Control 2-no weeding	Plant height at harvest	1.000				
		Number of tillers at harvest	0.371^{ns}	1.000			
		AUDPC	0.298^{ns}	0.068^{ns}	1.000		
		DM yield	0.566**	0.382^{ns}	0.359^{ns}	1.000	
		DM (%)	0.070^{ns}	-0.316^{ns}	0.029^{ns}	0.099^{ns}	1.000
	Control 3-no fertiliser	Plant height at harvest	1.000				
		Number of tillers at harvest	0.131^{ns}	1.000			
		AUDPC	0.093^{ns}	0.395^{ns}	1.000		
		DM yield	0.018^{ns}	0.604**	0.156^{ns}	1.000	
		DM (%)	-0.184^{ns}	0.247^{ns}	0.003^{ns}	0.608**	1.000
Third harvest	Fertiliser application	Plant height at harvest	1.000				
		Number of tillers at harvest	0.114^{ns}	1.000			
		AUDPC	-0.501*	-0.160^{ns}	1.000		
		DM yield	0.289^{ns}	0.875**	-0.060^{ns}	1.000	
		DM (%)	-0.314^{ns}	-0.058^{ns}	0.479*	0.113^{ns}	1.000
	Fungicide application	Plant height at harvest	1.000				

Harvest	Management option		Plant height at harvest	Number of tillers at harvest	AUDPC	DM yield	DM (%)
		Number of tillers at harvest	0.053^{ns}	1.000			
		AUDPC	-0.004^{ns}	0.432*	1.000		
		DM yield	0.352^{ns}	0.358^{ns}	-0.053^{ns}	1.000	
		DM (%)	-0.115^{ns}	-0.313^{ns}	0.141^{ns}	-0.199^{ns}	1.000
	Manual weeding	Plant height at harvest	1.000				
		Number of tillers at harvest	0.318^{ns}	1.000			
		AUDPC	-0.110^{ns}	-0.402^{ns}	1.000		
		DM yield	0.470*	0.452*	-0.086^{ns}	1.000	
		DM (%)	-0.142^{ns}	-0.482*	0.346^{ns}	0.169^{ns}	1.000
	Control 1-no pesticide	Plant height at harvest	1.000				
		Number of tillers at harvest	-0.591**	1.000			
		AUDPC	0.301^{ns}	-0.254^{ns}	1.000		
		DM yield	0.421*	-0.147^{ns}	0.201^{ns}	1.000	
		DM (%)	0.325^{ns}	-0.394^{ns}	0.415*	0.262^{ns}	1.000
	Control 2-no weeding	Plant height at harvest	1.000				
		Number of tillers at harvest	0.369^{ns}	1.000			
		AUDPC	0.152^{ns}	0.215^{ns}	1.000		
		DM yield	0.459*	0.926**	0.131^{ns}	1.000	
		DM (%)	-0.024^{ns}	0.090^{ns}	0.266^{ns}	0.252^{ns}	1.000
	Control 3-no fertiliser	Plant height at harvest	1.000				
		Number of tillers at harvest	0.529**	1.000			
		AUDPC	0.021^{ns}	-0.265^{ns}	1.000		
		DM yield	0.467*	0.603**	0.099^{ns}	1.000	
		DM (%)	-0.520**	-0.490*	-0.052^{ns}	-0.245^{ns}	1.000
Fourth harvest	Fertiliser application	Plant height at harvest	1.000				
		Number of tillers at harvest	0.002^{ns}	1.000			
		AUDPC	0.218^{ns}	-0.244^{ns}	1.000		
		DM yield	0.331^{ns}	0.811**	-0.012^{ns}	1.000	
		DM (%)	-0.059^{ns}	-0.160^{ns}	0.306^{ns}	-0.223^{ns}	1.000
	Fungicide application	Plant height at harvest	1.000				
		Number of tillers at harvest	0.238^{ns}	1.000			
		AUDPC	0.196^{ns}	-0.041^{ns}	1.000		
		DM yield	0.612**	0.440*	0.318^{ns}	1.000	
		DM (%)	-0.466*	-0.215^{ns}	0.129^{ns}	-0.074^{ns}	1.000
	Manual weeding	Plant height at harvest	1.000				
		Number of tillers at harvest	0.476*	1.000			
		AUDPC	-0.267^{ns}	-0.176^{ns}	1.000		
		DM yield	0.431*	0.749**	-0.490*	1.000	

Harvest	Management option		Plant height at harvest	Number of tillers at harvest	AUDPC	DM yield	DM (%)
		DM (%)	-0.566**	-0.399^{ns}	0.387^{ns}	-0.294^{ns}	1.000
	Control 1-no pesticide	Plant height at harvest	1.000				
		Number of tillers at harvest	0.003^{ns}	1.000			
		AUDPC	0.137^{ns}	0.014^{ns}	1.000		
		DM yield	0.318^{ns}	0.450*	-0.385^{ns}	1.000	
		DM (%)	0.037^{ns}	-0.023^{ns}	0.028^{ns}	0.324^{ns}	1.000
	Control 2-no weeding	Plant height at harvest	1.000				
		Number of tillers at harvest	0.353^{ns}	1.000			
		AUDPC	0.353^{ns}	0.042^{ns}	1.000		
		DM yield	0.561**	0.828**	0.242^{ns}	1.000	
		DM (%)	0.073^{ns}	0.003^{ns}	0.420*	0.049^{ns}	1.000
	Control 3-no fertiliser	Plant height at harvest	1.000				
		Number of tillers at harvest	0.152^{ns}	1.000			
		AUDPC	0.059^{ns}	-0.182^{ns}	1.000		
		DM yield	0.597**	0.705**	-0.122^{ns}	1.000	
		DM (%)	0.223^{ns}	-0.596**	0.132^{ns}	-0.208^{ns}	1.000

* Means significant correlation at the $p \leq 0.05$. ** means significant correlation at $p \leq 0.01$. ns = Not significant. DM (%) = Dry matter content.

7.5 Discussion

This study observed an increase in the incidence and severity of leaf rust disease in *Brachiaria* grass with the progression of time and harvests. The presence of leaf rust disease in all harvests, and gradual increase in disease in subsequent harvests may be due to various factors, of which the major roles could be environmental factors favouring the disease, a gradual build-up of inoculum over time, and the use of a leaf rust susceptible cultivar. High relative humidity, high temperature, and high rainfall have been shown to increase stem rust incidence and severity in wheat under natural and artificial conditions (Helfer, 2014). The effect of temperature on the susceptibility of host plants to rust disease has also been reported by several authors, who identified the ineffectiveness of stem rust resistance at temperatures greater than 25 °C, while the success of the wheat leaf rust resistance gene has been recorded at temperatures above 25 °C (Das *et al.*, 2017; Martens *et al.*, 1967).

A temperature range of 15 – 25 °C has been reported for the germination of teliospores and formation of basidiospores of the Asian grapevine leaf rust pathogen (Edwards, 2015), while the common rust found in maize is favoured by temperatures between 16 °C and 24 °C (Wright *et al.*, 2014).

In this study, the temperature was approximately 19 °C, and the rainfall varied between 90 mm and 207 mm. The variable temperature and rainfall amount during the study period might have led to the difference in leaf rust incidence and severity in *Brachiaria* grass. Nevertheless, the leaf rust incidence ranged from 90% to 100% for all treatments in the sixth week, except in the plots receiving mancozeb application, which registered the lowest incidence, and the severity remained high at the eighth week for all treatments other than mancozeb application. These findings suggest that leaf rust incidence and severity reach a maximum when the *Brachiaria* grass reaches its full growth stage. A study concerning wheat showed that there was higher rust severity in old plants than in young plants (Farber and Mundt, 2017). The low leaf rust incidence and severity at the beginning of this experiment may have been due to the low inoculum level. However, there was a gradual increase in disease occurrence, peaking in the fourth harvest, which may be the result of the continuous build-up of inoculum over time.

The results revealed that mancozeb is an effective control of leaf rust disease in *Brachiaria* grass and that the mancozeb-treated plots had better performance than the plots not receiving mancozeb treatment. It has been reported that mancozeb can decrease culturable bacterial and fungal populations (Pankhurst *et al.*, 2005).

Further, the application of mancozeb was found to improve the growth performance of sugarcane in Australia (Pankhurst *et al.*, 2003; Peterson *et al.*, 1948.

Leaf rust incidence and severity were consistently low in plots with mancozeb application compared to all the other plots. Mancozeb is a broad-spectrum fungicide used to control plant diseases worldwide (Yang *et al.*, 2019). It was reported that the yield loss due to corn rust was reduced by the application of mancozeb when the pesticide was applied at the beginning in an early stage for three consecutive applications (Wegulo *et al.*, 1998). Furthermore, several authors have reported that the application of a fungicide to a plant infected with common rust led to the eradication of pustules and the termination of new infections (Berger *et al.*, 1997). This corroborates the results of this study, in which the fungicide was applied three times (one day after harvest, followed by two applications at intervals of two-weeks), and the rust

incidence and severity were consistently reduced. Thus, research regarding the efficacy of mancozeb application for the control of leaf rust of *Brachiaria* grass must be explored and used by farmers, seed companies and forage seed producers to achieve planting materials free from diseases and for the sustainable management of leaf rust disease in *Brachiaria* grass.

Meanwhile, the manual weeding method resulted in a high severity of leaf rust disease, whereas the no weeding method resulted in low incidence and severity, but low biomass yield. The high quantity of biomass production in the plots receiving weed control treatments could be attributed to reduced competition between *Brachiaria* plants and weeds for light, space, water, and nutrients. The low leaf rust incidence and severity observed in the no-weeding plots may be explained by the presence of plant species other than *Brachiaria* grass, which may have acted as barriers and buffers against the initiation and spread of rust spores. These results agree with the findings of Ihejirika (2007) and Boudreau (2013), who observed decreases in disease incidence and severity in intercropping systems. Furthermore, monoculture has been reported to favour rust disease development (Helfer, 2014).

The results of this study showed that there was a negative correlation between DM yield and the AUDPC. Turner *et al.* (2017) reported a similar observation in which rust pathogens negatively affected biomass production in different economically important crops. The reduction in biomass observed in this study might originate from a reduction in the photosynthesis rate combined with the use of the photosynthate by the rust pathogen. Apart from the reduction in photosynthesis, the pathogens might have triggered physiological changes, including water use efficiency and Carbon dioxide (CO_2) uptake reductions.

Moreover, the results showed that incidence and severity of rust disease were consistently low under mancozeb application and consistently high in the no pesticide application and no weeding treatments, indicating the effectiveness of mancozeb in controlling *Brachiaria* leaf rust. Furthermore, the DM yield was significantly higher under the mancozeb and fertiliser application plots in the second and the first harvest, respectively, and was consistently lower in the no manual weeding treatment. These findings imply that mancozeb application, as well as fertiliser application, significantly reduced leaf rust incidence and increased crop performance (high plant height, number of tillers, and high biomass). Note that this trend was consistently observed for all harvests.

7.6 Conclusions

This study documented the effectiveness of different disease management options against leaf rust disease in *Brachiaria* hybrid cv. Cayman. The incidence and severity of leaf rust disease were significantly reduced by the applications of a fungicide, mancozeb, and mineral fertiliser, thereby crop performance was enhanced, including plant growth, number of tillers, and DM yields. The results indicated that manual weeding resulted in a higher severity of leaf rust disease than the no-weeding option, which registered low incidence and severity. The association of low disease pressure in no-weeding treatment required further investigation to examine the effect of cultivar mixtures on leaf rust disease management in Rwanda, as well as in East Africa as a whole. The information provided in this study regarding the efficacy of mancozeb application for the control of leaf rust in *Brachiaria* grass should be explored as an integrated pest management option to be used by seed companies and forage seed producers to obtain planting material free from leaf rust disease.

CHAPTER EIGHT:
GENERAL DISCUSSION, CONCLUSIONS AND ECOMMENDATIONS

8.1 General discussion

The genus *Brachiaria* is ranked among the high-quality nutritious forages that originate from Africa and its importance in animal feeding continued to be highlighted worldwide. This study is the first one to sought out the comprehensive information on *Brachiaria* grass diseases in Rwanda and aimed to determine the distribution, disease incidence and severity of *Brachiaria* grass diseases in Rwanda; to isolate and confirm causative relationship between *Bipolaris secalis*/ *Phakopsora apoda* and leaf spot/leaf rust; to determine the whole genome sequence and genomic characterization of *Bipolaris secalis*; to evaluate the reaction of different *Brachiaria* species to foliar diseases under different environments and to evaluate management options for leaf rust affecting *Brachiaria* grass in Rwanda.

The results revealed that several diseases including leaf rust, leaf spot, leaf blight, ergot and viral diseases were distributed in *Brachiaria* grass fields in different districts of Rwanda. The three diseases including leaf rust, leaf blight and leaf spot were found to be the major diseases of *Brachiaria* grass in Rwanda. This implies the endemic nature of these diseases and requires specific attention for disease surveillance and management. A wide range of diseases including viral, fungal and phytoplasma were reported affecting *Brachiari*a grass in different countries. (Lenné and Trutmann, 1994). The survey results indicated that farmers had limited knowledge of diseases affecting *Brachiaria* grass and most of them were not aware of disease symptoms, yield loss associated with diseases and the presence of the diseases in their farms. This finding is consistent with the report of previous authors (Kiros-Meles and Abang, 2007) and it indicates that it is very important to organise regular training of farmers on *Brachiaria* disease identification for sustainable production.

The association of microorganisms with three major diseases indicated that apart from leaf rust which was associated with only one microorganism, *Phakopsora apoda*, leaf spot and leaf blight were associated with multiple microorganisms suggesting the presence of a mixture of pathogens and endophytes.

The number of isolates recovered from symptoms were 23 and 62 for leaf spot and leaf blight respectively and they were classified into 12 taxa for leaf spot and 14 taxa for leaf blight.

Many fungi isolated from symptoms caused by leaf blight and symptoms caused by leaf spot were reported as endophytes in several hosts (Sánchez Márquez *et al*., 2007, 2008, 2010). Based on morphological, molecular techniques, and pathogenicity tests, *Bipolaris secalis* and *Phakopsora apoda* were confirmed to cause leaf spot and leaf rust diseases respectively. This confirms the findings of previous authors who reported fungal diseases as one of the major challenges of *Brachiaria* grass production worldwide (Lenné, 1990; Lenné and Trutmnn,1994; Njarui *et al*., 20216). *Bipolaris secalis* was found causing foliar disease on several crops (Ramesh, 2021; Sivanesan, 1987; Sun, 2020; Wang *et al*., 2012). *Phakopsora apoda* causing rust on Kikuyu grass was reported (Adendolf, 2014). The whole genome sequencing of *Bipolaris secalis*, BS7 isolate, isolated from *Brachiaria* grass showed the estimated genome size of 34,813,291 bp with an average GC content of 50.01%, organised into 108 contigs with the longest contig of 2,265,317 bp, the N50 of 1,032,497 bp and the L50 of 12. Genome characteristics found in this study are in the range of values of several reported *Bipolaris* species. The reported genome size of several *Bipolaris* species including *Bipolaris sorokiniana*, *Bipolaris victoriae*, *Bipolaris cookie*, *Bipolaris maydis*, *Bipolaris zeicola* and *Bipolaris oryzae* is between 31 MB and 37 MB (Aggarwal *et al*., 2019; Codon *et al*., 2013; Zaccaron and Bluhm, 2017). Genetic structure of *Bipolaris oryzae* was reported to be influenced by geographic locations, soil and varieties (Burgos *et al*., 2013). The study revealed that the reaction of cultivars to major diseases was diverse. This difference may be due to different factors including difference in the level of virulence of pathogen, experimental site characteristics and genetic characteristics of cultivars (Agrios, 2005). In this study, the application of mancozeb effectively controlled *Brachiaria* leaf rust. The disease incidence and severity reduced considerably in plots treated with the fungicide and the biomass production was high. The findings of this study are in agreement with the findings of different authors stating the use of mancozeb in fungal disease management of several corps including sugarcane, cereal grains, cotton, sorghum, tomatoes, corn, and peanuts (Ahmad and Khan, 2011; Hayes and Laws, 1991; Pankhurst *et al*., 2005).

8.2 Conclusions

This study revealed that multiple diseases of *Brachiaria* grass are widely spread and distributed in different agro-ecological zones of Rwanda. Several diseases mainly caused by fungi were recorded in Rwanda. These include leaf spot, leaf rust and leaf blight. If not controlled, there is a high risk of epidemic caused by these endemic diseases, especially under favourable environmental conditions. Prevalence, incidence and severity of major *Bracharia* diseases depend on districts, years and seasons. Regular disease surveillance, effective disease management practices and advisory systems are very important as Rwanda lies within the centre of diversity of *Brachiaria* grass that corresponds to high pathogen diversity, keeping currently grown *Brachiaria* cultivars at maximum vulnerability. Multiple microorganisms were associated with leaf blight and leaf spot symptoms, with frequent association of *Epicoccum* sp. and *Nigrospora* spp. with leaf blight symptoms and *Bipolaris secalis* with leaf spot symptoms. *Phakopsora apoda* was found to be associated with leaf rust. Morphological characteristics, molecular techniques and pathogenicity tests confirmed *Bipolaris secalis* and *Phakopsora apoda* as causal agents of leaf spot and leaf rust respectively. Illumina sequencing results of BS7 produced the estimated genome size of 34,813,291 bp with an average GC content of 50.01%, organised into 108 contigs with the longest contig of 2,265,317 bp, the N50 of 1,032,497 bp and L50 of 12. The self-mapping of BS7 was 97.69%. The results obtained when mapping the dataset of 11 isolates to BS7 indicated that the final mapping ratio was in the range of 80 – 95%, consisting of 28,950,637 – 15,611,348 total mapped reads. This study indicated a wide variation in the response of nine improved *Brachiaria* grass cultivars to leaf rust, leaf blight and leaf spot diseases in Rwanda. Besides variable responses to diseases, cultivars were also different for important agronomic traits such as plant height, tiller numbers, biomass yield and percent dry biomass content. This study showed that Basilisk, Marandu, MG4 and Xaraes had moderately resistant to resistant response to all three foliar diseases and had high biomass yields. These cultivars are more appropriate for upscaling *Brachiaria* grass in wider geographical regions in Rwanda and neighbouring countries. Likewise, the application of mancozeb and mineral fertiliser significantly reduced the incidence and severity of leaf rust disease, therefore enhanced crop performance including plant growth, number of tillers and dry matter yields of a leaf rust susceptible cultivar.

8.3 Recommendations

Based on key findings obtained in this study, the following recommendations need to be taken into consideration:

i. It is paramount to build capacity of extensionists and farmers on disease identification and organise a regular disease surveillance to guide application of effective disease management options for sustainable production of *Brachiaria* grass in Rwanda.
ii. Genome data of *Bipolaris secalis* should be explored for identification of novel sources of genetic resistance for improvement of disease management and other new strategies for effectiveness and sustainability of management of *Brachiaria* grass diseases.
iii. *Brachiaria* cultivars which showed moderate to resistance response to major diseases with increased biomass yield including Xaraes, MG4, Marandu and Basilisk are recommended to be used in upscaling and promoting *Brachiaria* grass in various areas of Rwanda and neighbouring countries
iv. The use of mancozeb for the control of leaf rust of *Brachiaria* grass can be explored and used by seed companies and forage seed producers to avail planting material free from leaf rust.
v. Association of low disease pressure in no weeding plots requires further comprehensive investigation to examine effect of cultivar mixture in leaf rust disease management in Rwanda, as well as in East African countries.

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APPENDICES

Appendix I. Questionnaire used during the survey on *Brachiaria* grass diseases in Rwanda

1.GENERAL INFORMATION

Date of interview	……/……/……..
Province	
District	
Sector	
Cell	
Village	
Farmer name	
Farmer age	
Farmer sex	
Farmer marital status	1=Single, 2= married, 3=Widowed, 4=Separated, 5=Divorced, 6=Other (Specify)
Education level of the farmer	1: No formal education
	2: Primary level
	3: Secondary level
	4: University level
Farmer telephone number	
Name of the scientist	
Telephone number	
GPS coordinates	Longitude:
	Latitude:
	Altitude:

2. CHARACTERISTIC OF THE FIELD

Filed ID	
Size of the surveyed field	1: Less than 1 ha 2: 1 -5 ha 3: More than 5 ha
Soil	Soil type: Slope:
Cultivar name	1 : Basilisk 2 : Cayman 3 : Cobra 4: Humidicola 5: Marandu 6: MG 4; 7: Mulato 8: Mulato II 9: Piata; 10: Toledo 11: Xaraes 12: Do not know 13: Other:……..
Planting date	
Planting materials used and its source	1: Seeds…… Source…… 2: Root splits…… Source……
Crop type	1: No cut 2: One cut (ratoon) 3: Multiple cuts (ratoon)
Growth stage	1: Pre-tillering 2: Tillering 3: Stem elongation 4: Booting 5: Flowering 6: Grain filling 7: Maturity 8: Senescence
Number of harvests per year	
Weed infestation level in the field	1: No weeds 2: Low 3: Medium 4: High:
Previous crop	
Applied agriculture practices	1: Organic manure (OM) at planting
	2: Mineral fertiliser (F) at planting, which one? A: NPK, B: DAP C: Urea D: Other Specify……..
	3: Mineral fertiliser at weeding, which one? A: NPK, B: DAP, C: Urea,

	D: Other Specify……..…..…
	4: OM + F together at planting
	5: Weeding
	6: Watering
	7: Other: ……
	8: Nothing was done since planting
Cropping system	1: Mixed with crops, specify which one…….
	2: Monoculture
	3: Mixed with trees (Agroforestry)……
	4: *Brachiaria* planted on fences for erosion control
Location of the field	1: Marshland
	2: Hillside
	3: Others (specify) ……
Above all agriculture practices you mentioned, which one did you find most useful for good health and good production of *Brachiaria* grass	1: Application of all practices in integrated manner 2: Organic manure at planting 3: Mineral fertiliser at planting 4: Organic manure and mineral fertiliser at the same time 5: Weeding 6: On fences for erosion control 7: It is waste of time and resources; it is not necessary to apply any agriculture practices 8: Other (specify) ……

3. Farmer's perception on *Brachiaria* diseases and pests

3.1 Have you ever seen any *Brachiaria* disease in your farm? Yes/ No

3.2 If yes, when did you find it in your farm for the very first time?

a. Before the first cutting
b. After the first cutting
c. After more than one cutting
d. Dry season
e. Rainy season
f. Other…….

3.3 At which level the disease reduced your expected production?

a. Below 5%
b. Between 5% and 25%
c. Between 25 to 50%
d. More than 50%
e. No impact

3.4 What did you do to manage the disease?

a. Nothing
b. Up rooting
c. Other……

3.5 Describe the disease symptoms you saw in your *Brachiaria* field. Show the farmer the photo-sheet to choose the corresponding disease

……..

3.6 Describe any insect pest you have seen

……

4. Recording sheet for disease incidence and severity

Disease…….

Quadrat	Stool Number	Incidence (Yes: 100/ No:0)	Severity (5 tillers (T1 – T5) per stool, all leaves per tiller)	Sample code if taken
1	1		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	2		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7……L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	3		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8…..	

Quadrat	Stool Number	Incidence (Yes: 100/ No:0)	Severity (5 tillers (T1 – T5) per stool, all leaves per tiller)	Sample code if taken
			T5: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	4		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	5		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7……L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
2	1		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8…..	

Quadrat	Stool Number	Incidence (Yes: 100/ No:0)	Severity (5 tillers (T1 – T5) per stool, all leaves per tiller)	Sample code if taken
			Average:	
	2		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	3		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	4		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	

Quadrat	Stool Number	Incidence (Yes: 100/ No:0)	Severity (5 tillers (T1 – T5) per stool, all leaves per tiller)	Sample code if taken
	5		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
3	1		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	2		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	3		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8…..	

Quadrat	Stool Number	Incidence (Yes: 100/ No:0)	Severity (5 tillers (T1 – T5) per stool, all leaves per tiller)	Sample code if taken
			T2: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	4		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	5		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
4	1		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8…..	

Quadrat	Stool Number	Incidence (Yes: 100/ No:0)	Severity (5 tillers (T1 – T5) per stool, all leaves per tiller)	Sample code if taken
			T3: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	2		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	3		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	4		**T1**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8…..	

Quadrat	Stool Number	Incidence (Yes: 100/ No:0)	Severity (5 tillers (T1 – T5) per stool, all leaves per tiller)	Sample code if taken
			T4: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	
	5		**T2**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T3**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T4**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **T5**: L1…...L2…. .L3….. L4……L5… .L6…… L7…… L8….. **Average:**	