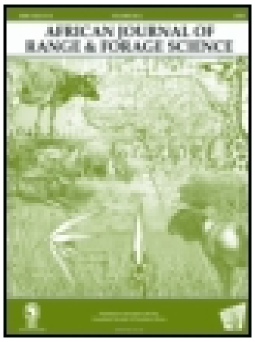

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Disease surveillance and farmers' knowledge of *Brachiaria* (Syn. *Urochloa)* grass diseases in Rwanda

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***Brachiaria* (syn. *Urochloa*) is one of the most important tropical forages grass of African origin. Its performance is affected by different constraints, including diseases. This study assessed the distribution, incidence and severity of *Brachiaria* diseases and documented farmers' knowledge on *Brachiaria* diseases in Rwanda. Surveys were conducted in five districts in the dry and wet seasons of 2018 and 2019. Fungi associated with major diseases were isolated and identified based on internal transcribed spacer sequences. The demographic information and farmers' knowledge of *Brachiaria* diseases and yield loss were collected using structured questionnaire. Surveys revealed widespread distribution of leaf blight, leaf rust and leaf spot diseases in Rwanda. Incidence and severity of these diseases differed significantly by districts, seasons and district × season interactions; the exception was the non-significant effect of season and district × season interactions on rust incidence in 2018. Molecular identification revealed *Phakopsora apoda* as a provisional leaf rust pathogen, and frequent association of fungi *Epicoccum* spp. and *Nigrospora* spp. with leaf blight, and *Bipolaris secalis* and *Fusarium* spp. with leaf spot symptoms. This study provides baseline information for future studies on *Brachiaria* diseases and recognises diseases as a major challenge to sustainable production of *Brachiaria* grass in Rwanda and East Africa.**

Keywords: Agro-ecological zone, leaf blight, leaf spot, management practices, rust

Introduction

Brachiaria is one of the most extensively cultivated tropical pasture with acreage of approximately 99 million ha in Brazil alone (Jank et al. 2014). All *Brachiaria* species with known forage values occur naturally in eastern Africa, which represent the centre of diversity of the genus (Keller-Grein et al. 1996). The genus *Brachiaria* (Trin.) Griseb. belongs to the tribe Paniceae in the subfamily Panicoideae of the family Poaceae (Jungmann et al. 2009). It consists of more than 100 documented species distributed in the tropics mainly in Africa (Renvoize et al. 1996). Of these, seven perennial species have been used for fodder production particularly in tropical America, Asia, the South Pacific and Australia. These species are *Brachiaria arrecta* (Hack. ex. Th. Dur & Schinz) Stent, *Brachiaria brizantha* (A. Rich.) Stapf, *Brachiaria decumbens* Stapf, *Brachiaria dictyoneura* (Fig. & De Not.) Stapf, *Brachiaria humidicola* (Rendle) Schweick, *Brachiaria mutica* (Forssk.) Stapf and *Brachiaria ruziziensis* Germain & Evrard (Keller-Grein et al. 1996). The use of *Brachiaria* grass for pasture production has been limited in Africa until recently, because other forages were more appropriate to the prevailing livestock production systems (Ndikumana and Leeuw 1996) and, as a result of a low priority given to forage research and development activities in Africa.

An increase in livestock production coupled with diminishing forage availability, as a result of overgrazing, rangeland degradation, dwindling natural pasture and frequent and/or prolonged droughts have inspired livestock farmers in the region to grow improved and nutritious forages, including *Brachiaria* grass. Institutions, such as the International Livestock Research Institute (ILRI), Kenya Agricultural and Livestock Research Organization (KALRO) and Rwanda Agriculture and Animal Resources Development Board (RAB), have dedicated research and development (R&D) programs on *Brachiaria* grass. The ILRI runs the *Brachiaria* R&D programme across sub-Saharan Africa. These programs have developed *Brachiaria* grass technologies for Africa, integrated them successfully into mixed crop-livestock production systems, documented benefits of *Brachiaria* grass on forage availability (especially in the dry season) and livestock productivity, and created new income generation opportunities through sale of *Brachiaria* hay and planting materials (Ghimire et al. 2015; Maass et al. 2015). In Rwanda, approximately 70% of the population own livestock and the success of national livestock programs, such as 'One Cow Per Poor Family' and 'Livestock Intensification Programme' rely on the sustainable production of quality feed. Studies have shown a good performance of improved *Brachiaria* grass cultivars across different agro-ecological zones of Rwanda and their significant contribution to alleviate livestock feed shortage in the country, including in the dry seasons (Mutimura and Everson 2012). These studies reported appreciation for improved *Brachiaria* grass by farmers in Bugesera and Nyamagabe districts of Rwanda.

Most of the dairy farmers in East African countries rely on natural pastures and Napier grass (*Pennisetum purpureum*) for dairy production (Klapwijk et al. 2014). Although Napier grass has been the main animal feed in the region, the outbreak of Napier stunt and smut diseases has adversely affected this forage, posing serious challenge to livestock production (Farrell 1998; Lukuyu et al. 2009; Nyiransengimana et al. 2015; Umunezero et al. 2016). The high incidence and severity of smut disease in Napier grass fields were reported in Rwanda (Nyiransengimana et al. 2015). The recent introduction of improved *Brachiaria* grass cultivars has provided additional forage options to alleviate exiting problems of livestock feed shortage. Some of *Brachiaria* species have shown broad adaptation to multiple environments across Africa (Ndikumana and de Leeuw 1996; Njarui et al. 2016). However, the expansion of *Brachiaria* acreage in sub-Saharan Africa requires some cautions, because the center of crop origin is also seen as the centre of variability of pathogens and pests (Jennings and Cock 1977) and may result in exposure of *Brachiaria* grass cultivars to native pests and diseases in Africa. Since the introduction of improved *Brachiaria* cultivars by ILRI in 2013, there have been reports that these cultivars have been attacked by diseases, including leaf rust, leaf spot, leaf blight, and smut in Kenya (Nzioki et al. 2016). However, current information about *Brachiaria* grass diseases in Africa is inadequate and not available for Rwanda. Therefore, this study was carried out with the following objectives: (i) to determine prevalence, incidence and severity of *Brachiaria* grass diseases; (ii) to document farmers' knowledge on *Brachiaria* grass diseases; and (iii) to determine organisms associated with symptoms of major diseases affecting *Brachiaria* grass in Rwanda.

Materials and methods

Survey sites

Disease surveys were conducted in five districts of Rwanda (Figure 1), located in five different agro-ecological zones (Congo Nile Watershed Divide, Central Plateau and Granitic Ridges, Eastern Plateau, Eastern Savanna, Mayaga and Bugesera) (Table 1). Survey districts were selected based on the importance of livestock in the area and the number of dairy farmers who have planted improved *Brachiaria* grass. Disease surveys were conducted in both the dry season and the wet season of 2018 and 2019. The dry season covers the months of June, July and August where July is the driest month, whereas the wet season covers the months of September, October, November and December. The rainfall and temperature data during the dry and the wet seasons of 2018 and 2019 were collected (Table 1). A total of 15 farms with established *Brachiaria* grass plots were selected in each district for the first disease survey in 2018 and same farms were revisited in the subsequent surveys. GPS coordinates for each surveyed farm were recorded and plotted in the map of Rwanda using Quantum GIS software.

Understanding farmers' knowledge on Brachiaria grass diseases

Farmers with established *Brachiaria* grass plots in their farms were included in the survey. An open interview and a structured questionnaire were used to collect information on education level, age, acreage under *Brachiaria* grass, weed infestation level, cropping system, and farmers' knowledge of *Brachiaria* grass diseases and the yield loss, as a result of diseases. Farmers were asked to estimate the yield loss in *Brachiaria* grass in a four categorical scales: below 5%, between 5% and 25%, between 26% and 50% and >50%.

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

Field surveys were conducted for the assessment of disease prevalence, incidence and severity at farm level. Disease prevalence was determined in each district as the number of farms where a given disease was present, divided by the total number of farms surveyed and converted to a percentage (Nutter et al. 2006). Disease incidence and severity were assessed on 20 stools from four different quadrats (each quadrant of 1 m^{-2}) selected at random from each *Brachiaria* farm. Within one quadrat, observations were taken from five *Brachiaria* stools selected following an 'X' shape pattern. Disease incidence was determined as the number of stools that were diseased divided by the total number of all assessed stools and presented as a percentage (Agrios 2005; Nutter et al. 2006). Disease severity was recorded as the amount of the disease on individual stool following the established scoring system for each specific disease (Table 2).

Collection of diseased Brachiaria grass samples and isolation of associated organisms

Symptomatic plant leaf and stem samples were collected from surveyed farms in paper envelops and transported in an icebox to the laboratory. The samples were washed in tap water, cut into 3–5 mm pieces, including both diseased and adjoining healthy tissue. The tissue samples were surface disinfected in 1% sodium hypochlorite (NaOCl) solution for 3 min, rinsed twice in sterile distilled water and blot dried. The tissue pieces were plated on Potato Dextrose Agar (PDA) supplemented with ampicillin (100 μg ml^{-1}) and incubated at 22 °C for 24–48 h (Narayanasamy 2011). Pure cultures were obtained by hyphal tip transfer to fresh PDA medium (El-Morsi and Abdel-Monaim 2015). Long-term preservation of the fungal isolates was done on Whatman FTA cards at −80 °C. For rust infected leaf samples, the infected leaves were collected in paper bags and allowed to dry at room temperature for two days. The rust spores were harvested from leaves using brushes, and the spores were stored in darkness at −4 °C (Guo et al. 2016).

Identification of the fungi associated with diseases symptoms

Preliminary identification of the isolated fungi was done through microscopic examination (40 × magnification) and confirmed by DNA analysis. Except for rust, the fungal isolates were grown on PDA at 22 °C for 1–3 weeks. The mycelium was then harvested using scalpel blade and transferred into 1.5 ml Eppendorf tube. The fresh fungal

Figure 1: Map of Rwanda showing the districts surveyed for *Brachiaria* grass diseases. Surveyed farms in each district are indicated in white dots using Quantum GIS Software

mycelium or rust spores were frozen in liquid nitrogen and ground to fine powder using mortar and pestle. Genomic DNA was extracted from 100 mg of the ground samples using QIAGEN DNeasy kit following the manufacturer's instructions. The concentration and integrity of DNA was checked using a NanoDrop spectrophotometer ND-1000 (NanoDrop Technologies) and agarose gel electrophoresis, respectively. The DNA was kept at −20 °C for later use.

All fungal DNA (except rust fungi) were used for targeted amplification of internal transcribed spacer region in ribosomal DNA of fungal isolates using universal fungal primers ITS1F (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-CCTCCGCTTATTGATATGC-3′) (White et al. 1990). The PCR reaction was set at 25 µl volume (3 µl of diluted genomic DNA containing 20–40 ng DNA, 12.5 µl premix, 0.5 µl each ITS1F and ITS4 and 8.5 µl sterile distilled water), along with the negative control reaction without DNA template. The PCR condition was 4 min of denaturation at 94 °C, followed by 35 cycles of 94 °C for 45 s, 56.7 °C for 45 s and 72 °C for 45 s, with final extension at 72 °C for 10 min and hold at 4 °C. For rust DNA, rust specific primers ITS1rustF10d (5′-TGAACCTGCAGAAGGATCATTA-3′) and rust1 (5′-GCTTACTGCCTTCCTCAATC-3′) were used (Barnes and Szabo 2007). The PCR reaction volume and conditions were same as above, except for the annealing temperature, which was set at 59.5 °C for 45 s. The presence of targeted products was confirmed by loading 3 µl of PCR product on 1.5% agarose gel for one hour at 70 Volt and GelRed staining. The PCR products were purified using QIAquick PCR Purification Kit (QIAGEN) following the manufacturer's instructions. The purified PCR products were sequenced at MACROGEN, Seoul, South Korea using the respective primer sets used for PCR amplifications. Raw DNA sequences were cleaned, and consensus sequences were determined by aligning nucleotide sequences generated by forward and reverse primers.

Table 1: *Brachiaria* grass diseases survey districts, corresponding agro-ecological zone, altitude, average temperature and total rainfall in the dry and the wet seasons of 2018 and 2019 in Rwanda. The dry season covers June, July, August, whereas the wet season covers September, October, November and December. Source: National Institute of Statistics of Rwanda 2019, Rwanda Meteorology Agency 2019. *Missing data for December 2019 (rainfall in Huye and Rwamagana, temperature in Huye) and missing data for October and December 2019 (temperature in Rwamagana) were replaced with the 30 years mean, while computing this value

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	1 440	20.9	22.3	22.8	22.3	67.4	239.0	74.7	602.3
Huye	Central Plateau and Granitic Ridges	1 700	19.5	19.8	19.8	20.7*	98.3	417.3	126.3	541.4*
Nyagatare	Eastern Savanna	1 575	20.8	19.7	21.4	19.6	105.2	353.3	210.5	542.6
Nyamagabe	Congo Nile Watershed Divide	2 400	18.8	20.0	19.2	20.1	107.8	434.2	222.4	929.0
Rwamagana	Eastern Plateau	1 300	21.0	21.9	21.2	22.6*	21.2	515.1	12.4	475.4*

Consensus sequences were submitted to the National Centre for Biotechnology Information (NCBI) for homology search and identification using the Basic Local Alignment Search Tools (BLAST) programme.

Data analyses

Farmer interview data were analysed using SPSS 22.0 software (Statistical Package for Social Sciences). Disease incidence and severity data were subjected to analysis of variance to determine the effects of main factors (district and season) on each variable and the interaction between district × season. The means of incidence and severity of diseases were compared by Least Significant Difference (LSD) mean separation test at 0.05 probability level using GenStat for Windows 20th Edition (VSN International 2019). DNA sequences data from Sanger sequencing were processed using CLC Genomics Workbench Version 8.0.3 software (https//digitalinsights.qiagen.com).

Results

Characteristics of Brachiaria farms and farmers

The acreage of *Brachiaria* grass planted by interviewed farmers was less than 0.5 ha. The most grown cultivar was Mulato II, which was planted by 62% of the farmers, followed by Basilisk (16%), Piata (9.5%), Xaraes (7%), MG4 (4%) and Cayman (1.5%). *Brachiaria* was grown as a monoculture in 82.7% of the farms and the conditions of the fields varied from well maintained to high weed infestation irrespective of survey districts and seasons. Approximately 40% of the respondent farmers were women and 67.1% of the interviewed farmers had a primary school level of education (Table 3).

Farmers' knowledge about Brachiaria diseases

Across the surveyed districts, 28% of the farmers reported the presence of diseases on their *Brachiaria* grass. Farmers at Bugesera reported the highest prevalence of the diseases (60%) than the farmers from other districts (Table 4). Some farmers at Bugesera estimated disease associated losses of up to 50%, but most farmers reported less than 5% loss. Yellowing of leaves was the most common symptoms reported by 17.3% farmers. Approximately 73% of the farmers had no knowledge about *Brachiaria* grass diseases (Table 4).

Prevalence, incidence and severity of Brachiaria diseases

Disease surveys revealed widespread evidence of leaf blight, leaf rust, leaf spot and virus-like diseases in *Brachiaria* grass in Rwanda, whereas ergot disease was recorded at Nyagatare district in the dry season of 2018 (Table 5). Evidence of leaf blight, leaf rust and leaf spot were found on *Brachiaria* grass across all districts and growing seasons, except for the absence of leaf spot disease at Nyagatare during the dry season of 2018 (Table 5). The prevalence of leaf blight was greater at Huye, Nyagatare and Rwamagana than at other districts in the 2018 dry season. Similarly, leaf rust prevalence was consistently greatest (87%) at Rwamagana in the wet season of 2018 and 2019.The prevalence of leaf spot was the highest at Huye in the 2019 dry season. Virus like diseases had a low prevalence in both years and season,

Table 2: Disease rating scale used to record severity of different diseases affecting *Brachiaria* grass in Rwanda

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

Table 3: Demographic information of farmers interviewed during *Brachiaria* grass diseases survey in different districts of Rwanda. The total number of farmers interviewed in each district was 15.

District	Average age (years)	Gender (%)		Education level (%)		
		Male	Female	Primary school	Secondary school	University
Huye	41.7	40.0	60.0	40.0	53.3	6.7
Nyamagabe	50.2	80.0	20.0	40.0	53.3	6.7
Bugesera	45.5	60.0	40.0	93.3	6.7	0.0
Rwamagana	48.0	60.0	40.0	93.3	6.7	0.0
Nyagatare	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

and ergot disease was recorded at Nyagatare in the dry season of 2018 and prevalence level was minimal (Table 5).

Symptoms of leaf blight disease consisted of necrotic lesions on the leaves, often drying from the tip (Figure 2a). Symptoms of leaf rust consisted of presence of yellowish or brownish pustules mainly on the adaxial surface of leaves (Figure 2b). Leaf spot disease was characterised by black spots or necrotic purple spots with whitish centre on the adaxial surface of leaves (Figure 2c1–2). Ergot disease was characterised by the presence of honeydew on the inflorescence (Figure 2d), whereas the virus-like disease (Figure 2e1–3) was characterised by chlorosis, reduced leaf size and stunting of the whole plant.

The incidence of leaf blight, leaf rust and leaf spot diseases varied significantly ($p \leq 0.001$) among the surveyed districts in 2018 and 2019. Except for leaf rust during 2018, the effects of season and interaction of district × season were evident for incidence of all three diseases in both years ($p \leq 0.001$) (Figures 3 and 4). The incidence of leaf blight was significantly greater at Huye district than other districts for 2018, whereas Rwamagana had the greater leaf blight incidence than other districts in 2019. The leaf blight incidence was greater in 2018 than 2019 irrespective of district and season. Leaf rust incidence was greater at the Nyamagabe district than other districts in both seasons of 2018. The rust incidence was the highest in the dry season of 2019 irrespective of the survey districts. The Bugesera district had the highest incidence of leaf spot disease in 2018 and in the dry season of 2019.

The severity of leaf blight, leaf rust and leaf spot diseases varied significantly by survey district, season and interaction of district × season ($p < 0.001$) in Rwanda (Table 6).

Table 4: Farmers' knowledge of *Brachiaria* disease symptoms, prevalence of disease and estimated yield loss in different districts of Rwanda

District	Farmers' knowledge of disease symptoms (%)						Disease prevalence (%)	Yield loss (%)	
	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

Table 5: Prevalence of different *Brachiaria* grass diseases during the dry and wet seasons of 2018 and 2019 in surveyed districts of Rwanda

Season	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

Leaf blight severity was the highest at Rwamagana in the dry season of both years; leaf rust severity was the highest at Nyamagabe and Huye in the dry seasons of 2018 and 2019, respectively, and leaf spot severity was the highest at Bugesera in the dry season of both years and in the wet season of 2018.

Fungi associated with Brachiaria grass diseases

Molecular identification confirmed the association of *Phakopsora apoda* with rust symptom. The sequence identity was 96% with e-value of zero (Table 7). Despite some variations in nucleotide sequences among the rust isolates analysed, all had top match to fungus *Phakopsora apoda,* but the query sequence coverage was low (60%). Multiple fungi were isolated from leaf blight and leaf spot diseases symptoms. Sixty-nine fungi belonging to 12 genera were isolated from leaf blight infected *Brachiaria* leaves. The most frequent genera were *Epicoccum* (33.2%), *Nigrospora* (21.9%) and *Pestalotiopsis* (14.4%). The fungi genera occurred in the low frequency were *Arthrinium, Didymella, Leptosphaeria, Curvularia, Alternaria, Lasiodiplodia, Rhizopus, Fusarium and Cochliobolous*. A total of 23 isolates from nine genera were recovered from leaf spot symptoms. These fungi were *Alternaria arborescens, Bipolaris secalis, Chaetomium globosum, Curvularia trifolii, Didymella* sp. *Epicoccum nigrum, Epicoccum* spp. *Fusarium equiseti, Fusarium verticillioides, Pestalotiopsis microspora, Nigrospora sphaerica,* and *Nigrospora* spp. The *B. secalis* was found to be a frequently isolated fungus (Table 7).

Discussion

This is the first study that documents the distribution, incidence and severity of *Brachiaria* grass diseases in major *Brachiaria* growing districts of Rwanda. It also documents for the first time the knowledge of farmers on *Brachiaria* grass diseases and their assessment on production loss caused by the diseases. The results of this study provide baseline information for all future studies on *Brachiaria* diseases in the country. The study showed the widespread occurrence of leaf blight, leaf rust, leaf spot diseases on *Brachiaria* grass, and occasional and more seasonal occurrences of ergot and virus diseases. All diseases recorded in this study have been reported in previous studies in other countries, including Kenya (Lenné and Trutman 1994; Valério et al. 1996; Cook et al. 2005; Nzioki et al. 2016). It is interesting to note that leaf blight, leaf rust and leaf spot diseases were consistently present in all five survey districts (except leaf spot disease at Nyagatare in the dry season of 2018) at variable incidence and severity levels suggesting their endemic nature and a wider distribution in Rwanda.

Figure 2: Symptoms of the major diseases of *Brachiaria* grass observed during surveys in Rwanda: a) Leaf blight, b) leaf rust, c1–c2) leaf spots, d) ergot, and e) virus-like disease affected stool in the field e1), diseased uprooted stool e2), and stool showing many small and stunted leaves, a common symptom of virus-like disease e3)

Most farmers interviewed in this study did not recognise *Brachiaria* grass diseases. They were also not informed about disease symptoms and had no knowledge on losses caused by diseases. Though surveillance confirmed the presence of diseases in all five surveyed districts in Rwanda, only 28% of the farmers knew about the presence of the diseases on their farms. This result agreed with a previous study that report ability of a few farmers to recognise the crop diseases (Kiros-Meles and Abang 2007). On the contrary, most farmers in Kenya were aware of Napier stunt disease (Khan et al. 2014). This could be because of the popularity of Napier grass among the livestock farmers in Kenya and a visible decline in Napier grass productivity, as a result of stunt diseases. Because the diseases and pests have been ranked as one of the major challenges to crop production in sub-Saharan Africa (Kiros-Meles and Abang 2007), it is essential to educate farmers on *Brachiaria* grass diseases, potential yield losses from the diseases and disease management methods when we introduce and promote this new forage. It is important to take an inventory of indigenous

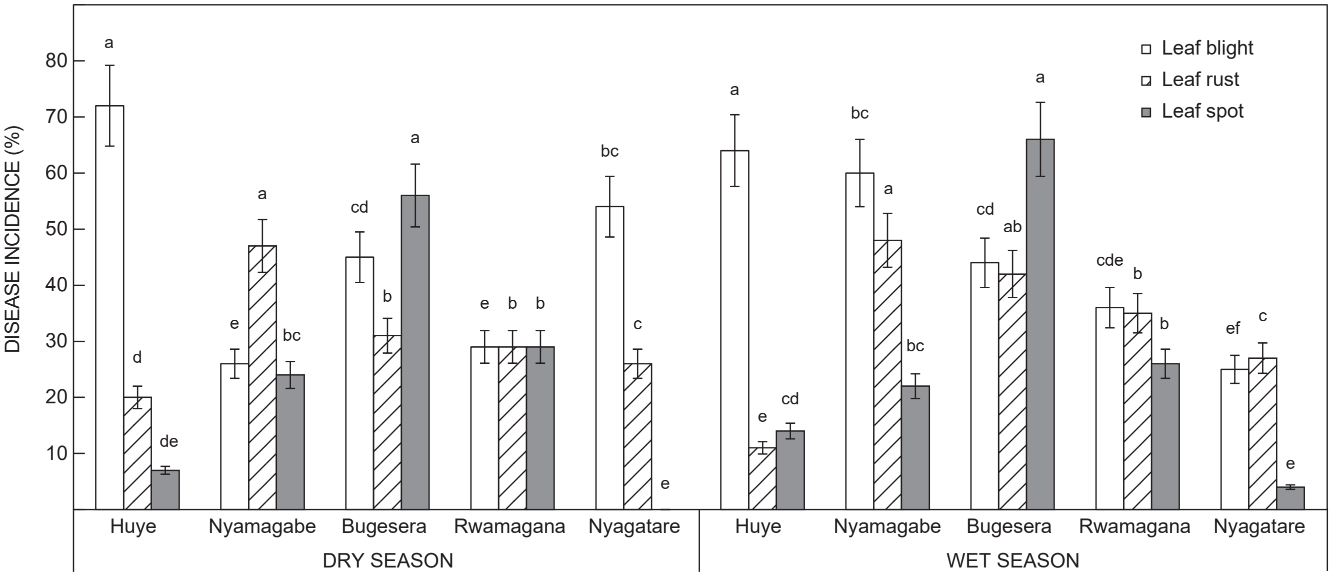

Figure 3: Incidence of leaf blight, leaf rust and leaf spot diseases infecting *Brachiaria* grass in different districts of Rwanda during the dry and the wet seasons in 2018. Bars with different letters for each disease are statistically different ($p \leq 0.05$). Error bars indicate the standard errors

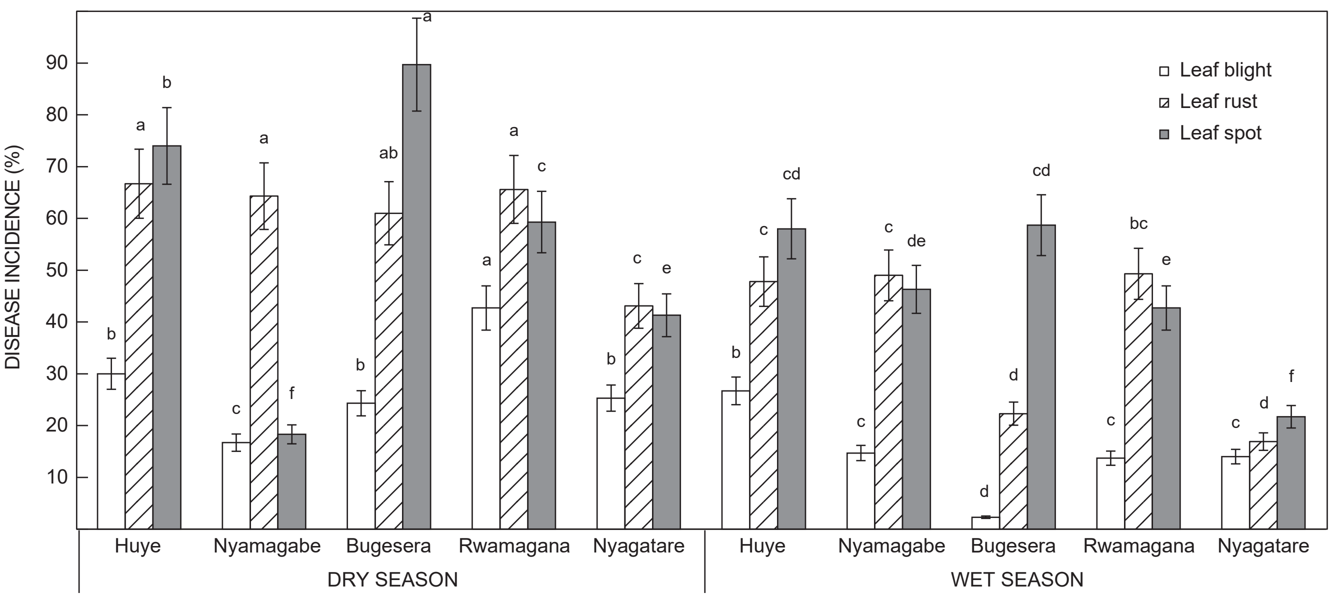

Figure 4: Incidence of leaf blight, leaf rust and leaf spot diseases infecting *Brachiaria* grass in different districts of Rwanda during the dry and the wet seasons in 2019. Bars with different letters for each disease are statistically different ($p \leq 0.05$). Error bars indicate the standard errors

disease management practices (Mahapatro and Sreedevi 2014) and integrate them while developing a new crop protection measures as appropriate.

This study reports a widespread distribution of leaf blight, leaf rust and leaf spot diseases in Rwanda. It may be attributed to several factors, including favourable climatic conditions for the development and spread of these diseases, and the presence of local *Brachiaria* grass and wild relatives that serve as alternate and/or collateral hosts to the causal agents. We also observed differences in prevalence, incidence and severity of diseases among survey districts. These variations could be as a result of agroclimatic differences among the survey districts, differences in host and pathogen genotypes, agronomic practices adopted by farmers, and other abiotic and biotic factors. For examples, the rainfall amount in Rwanda varied considerably between 2018 and 2019. The annual rainfall of the surveyed districts (Bugesera, Huye, Nyamagabe and

Table 6: Percentage severity of the major diseases affecting *Brachiaria* grass in various districts in Rwanda in 2018 and 2019 during the dry and the wet seasons

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

Values with the same superscript letters within the columns are not statistically different at $p \leq 0.05$

Nyagatare, Rwamagana) ranged from 966 to 1 833 mm in 2018, whereas it ranged from 1 232 mm to 2 009 mm in 2019 (National Institute of Statistics of Rwanda 2019, Rwanda Meteorology Agency 2019). Moreover, there were seasonal differences in rainfall between the districts, which ranged from 65 to 108 mm and 54 to 222 mm for the dry season of 2018 and 2019, respectively. Similarly, rainfall ranged from 239 to 515 mm and 466 to 929 mm for the wet season of 2018 and 2019, respectively (Table 1). Alhough the difference between districts and seasons for daily temperature were minimal, the difference in rainfall is likely to have had an effect on relative humidity and other environmental factors in favour of/against a given disease. Under higher rainfall regimes, the moist condition might have played a role in the dispersion and growth of some fungi. In general leaf spot and rust disease incidences were greater in 2019 than 2018, whereas leaf blight incidence was greater in 2018 than that observed in 2019 in most surveyed districts. Temperature plays a significant role in the susceptibility of host plants to rust diseases. Function of stem rust resistance genes (*Pg3* and *Pg4*) fails at temperature beyond 20 °C, whereas wheat leaf rust resistance gene (*Lr2a*) confers resistance when the temperature exceeds 25 °C (Martens et al. 1967, Das et al. 2017). The optimal temperature for teliospore germination and basidiospore formation in the Asian grapevine leaf rust pathogen (*Phakopsora euvitis*) has been reported in between 15 and 25 °C (Edwards 2015). The average daily temperature of 18.8 to 22.8 °C in all surveyed district during this study period (Table 1) might have favoured the development of the *Brachiaria* rust pathogen, *Phakopsora apoda* in all districts.

Molecular identification of rust pathogen isolates collected from *Brachiaria* grass had top match to *Phakopsora apoda* sequence at NCBI database, but the percentage query cover was only 60%. A low percentage query cover in our study was, because of uniqueness of these rust fungi sequences from sequences available in GenBank. It warrants additional investigation on these isolates for authentic identification and their proper taxonomic placement. *Phakopsora apoda* has been reported as the causal agent of rust disease on Kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov.) (Adendolff 2014). Previous studies have reported *Puccinia levis* var. *panici-sanguinalis* and *Uromyces setariae-italicae* as pathogens of *Brachiaria* grass rust disease (Lenné 1990; Marchi et al. 2007).

Among the fungi isolated from leaf tissues with leaf blight symptoms, *Nigrospora* has been reported to cause Nigrospora patch disease in Kentucky Blue Grass (Brown and Vargas 1982). Studies in Colombia have reported *Rhizoctonia solani* as pathogen of foliar blight disease in *Brachiaria* grass (Kelemu et al. 1995; Alvarez et al. 2013). Interestingly, none of the isolates in our study belonged to the genus *Rhizoctonia*. Analysis of fungal community on tissue with leaf spot symptom showed association of 12 different fungi with frequent occurrence of *Bipolaris secalis*. *Bipolaris secalis* has been reported as pathogen of rye (*Secale cereal*) and a native Mexican tree, Jangada Brava (Sisterna 1989). Many fungi isolated from leaf blight and leaf spot symptoms in this study were reported as endophytes and saprobes in several hosts (Barnes and Szabo 2007; Sánchez Márquez et al. 2007; White and Backhouse 2007; Ghimire et al. 2011; Adendorff 2014; Bernardi et al. 2018).

The widespread distribution of leaf blight, leaf rust and leaf spot diseases throughout major *Brachiaria* growing districts highlight their importance on sustainable production of *Bracharia* grass in Rwanda and East Africa region. The expansion of *Brachiaria* acreage in a wider geographical region should consider both current and emerging diseases challenges. Disease like ergot, though reported only in the Nyagatare district, has high potential to spread in larger geographic regions through planting materials, which may cause loss in quality and quantity of herbage and affect animal health and productivity (Young et al. 1983, Vermeulen et al. 2012). Similarly, the virus like symptoms found in this study may have significant negative impact on plant growth and forage quality and productivity (Valerio et al. 1996).

Table 7: Fungi associated with different diseases of *Brachiaria* grass in Rwanda

Disease	Total isolates	Fungi species isolated	Frequency (%)	Relationship with the host	Source
Leaf blight	69	*Nigrospora sphaerica*	6.0	Pathogenic	Liu et al. 2016
		Nigrospora oryzae	8.7	Endophyte	Sánchez Márquez et al. 2008; Ghimire et al. 2011
		Nigrospora sp.	7.2	Endophyte	Sánchez Márquez et al. 2008
		Pestalotiopsis microspora	10.1	Pathogenic, endophyte	Jeon et al. 2007; Lazarotto et al. 2012 Tejesvi et al. 2007
		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	Sánchez Márquez et al. 2008
		Rhizopus stolonifer	1.4	Endophyte	El-Nagerabi et al. 2013
Rust	9	*Phakopsora apoda*	100.0	Pathogenic	Gardner 1984; Adendorff and Rijkenberg 1995; McKenzie 1998; Starr 2004
Leaf spot	23	*Bipolaris secalis*	22.0	Pathogenic	Sisterna 1989; Bernardi et al. 2018
		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; Sánchez Márquez et al. 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	Sánchez Márquez et al. 2007; Ghimire et al. 2011
		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	Sánchez Márquez et al. 2010; Favaro et al. 2012
		Epicoccum sp.	8.7	Endophyte	Sánchez Márquez et al. 2008

Therefore, research to develop effective disease management methods targeting to the African smallholder farmers should be initiated to prevent likely outbreaks and associated economic losses. The diseases management efforts should focus on cultural methods, host resistance, field sanitations, soil fertility management and novel methods for improving host resilience to abiotic and biotic stresses.

Conclusion

This is the first study that documents distribution, incidence and severity of *Brachiaria* grass diseases in Rwanda. The acreage under *Brachiaria* grass has grown in Rwanda and other countries across sub-Saharan Africa. This study shows the widespread distribution of multiple diseases of *Brachiaria* grass in Rwanda. Diseases like leaf blight, leaf rust and leaf spot have high potential to cause severe yield loss. These endemic diseases have the potential to cause epidemic under favourable environmental conditions. Moreover, Rwanda lies within the centre of diversity of *Brachiaria* grass that corresponds to high pathogen diversity/specialisation, keeping currently grown *Brachiaria* cultivars at maximum vulnerability. Therefore, it is important to have a routine system of disease surveillance, and effective disease management practices and advisory systems in place. Identification of disease-causing organisms and farmer education on disease management are crucial for the sustainable production of *Brachiaria* grass in Rwanda. Organisms isolated in this study, particularly those of endophytic nature, can be explored further for potential agricultural applications as biofertilizers, biocontrol of pests and diseases and bioyield enhancement. Additional research is required to provide good understanding of pathogen associated with *Brachiaria* grass and their management options.

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