

DISTRIBUTION AND GENETIC DIVERSITY OF *PSEUDOCERCOSPORA* SPP. ASSOCIATED WITH BANANA SIGATOKA DISEASES IN EAST AFRICA



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Content

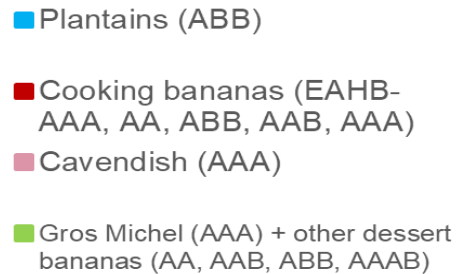
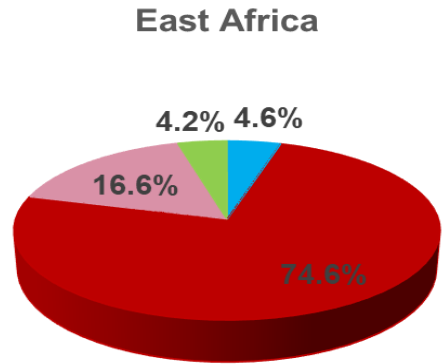
- Chapter 1. The diversity of *Pseudocercospora* pathogens, and their interaction with bananas
- Chapter 2. Distribution of *Pseudocercospora* species causing Sigatoka leaf diseases of banana in Uganda and Tanzania
- Chapter 3. Genetic diversity and mating type distribution of *Pseudocercospora fijiensis* on banana in Uganda and Tanzania
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- Chapter 6. Conclusion

Why Banana?



- Grown in tropical and sub-tropical regions
- Food and cash crop grown in more than 150 countries (Lescot, 2015).
- Consumed as table fruit, cooked, roasted, fried, and processed into juice and alcoholic drinks
- Residues used for decoration and animal feeds

Importance of bananas in ECA



- Africa produces 40.9 million tons (27.6% of global production)
- ECA 16.9 million tons of bananas (11.4% global production)
- Consumption per capita: 120-460 kg year⁻¹ (6-20 times the global average)
- Diversity of bananas in ECA:
 - EAHB bananas (more than 70%)

Source: Lescot, 2015

- Dessert bananas: 20.8%
- Other cooking bananas: Bluggoe, plantains (4.6%)
- Beer bananas: Pisang Awak, Kisubi, Km5

Banana production constraints

Social economic factors

- Declining arable land
- Labour unavailability
- Markets

Abiotic factors

- Declining soil fertility
- Water shortage/Drought

Musa spp

Biotic factors

Pest e.g Nematodes, Weevils

Disease e.g. BXW, Fusarium wilt, Sigatoka



Sigatoka leaf spots

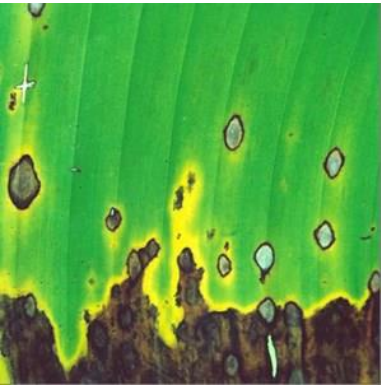


- Foliar diseases caused by a Hemibiotrophic ascomycete fungus in the genus *Pseudocercospora* (previously *Mycosphaerella*).
- Over 20 species have been isolated on bananas (Arzanlou, 2008)
- Three are economically important; *P. fijiensis* (Black Sigatoka), *P. musae* (Yellow sigatoka), and *P. eumusae* (Eumusae leaf spot).
- *P. musae* first reported in Java 1902; *P. fijiensis*, Fiji 1963; *P. eumusae* S.E Asia 1990s.

- Black sigatoka more damaging and globally distributed
- Can lead to complete defoliation
- Yield losses 20-80%



Sigatoka leaf spots



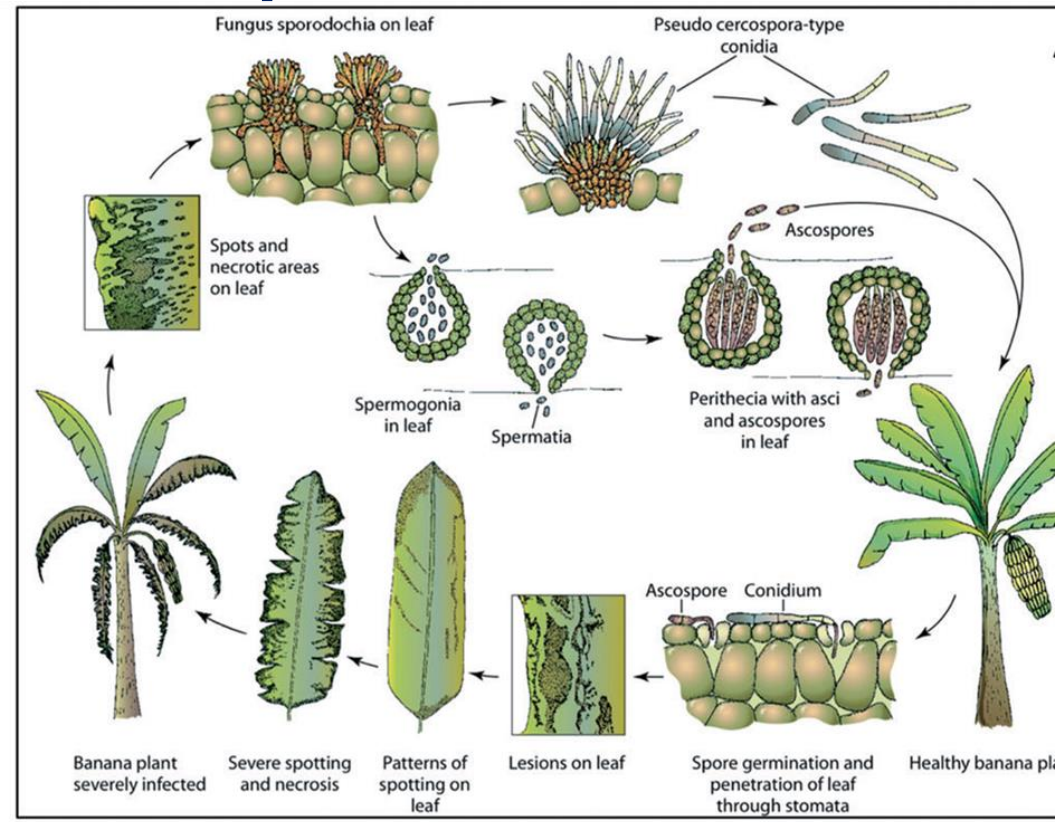
Yellow Sigatoka



Black Sigatoka



Eumusae leaf spots

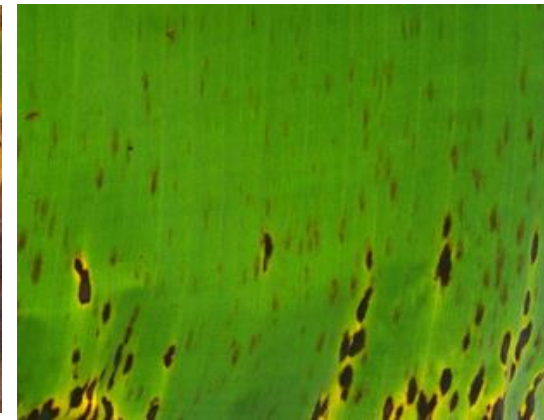
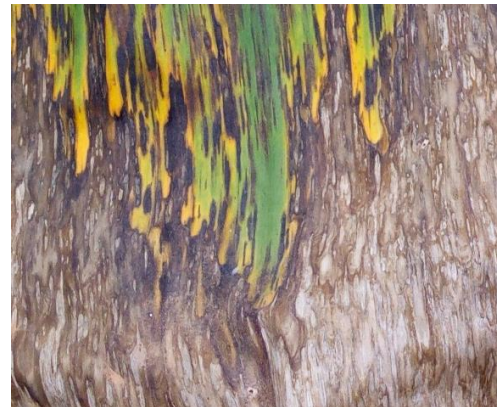
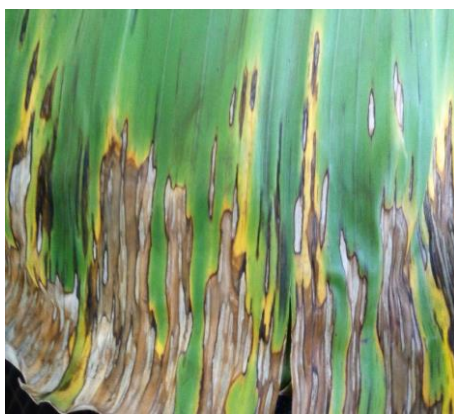


Source: Churchill, 2011

- These pathogens have similar lifecycle
- They produce infective conidia and ascospores- Dispersed by watersplash or are airborne.
- Symptoms are almost similar

Sigatoka in EA

- *P. musae* reported in 1938; restricted to high altitudes areas; mainly affecting exotic cultivars
- *P. fijiensis* first reported in 1987; Pemba islands
 - A low altitude disease- below 1350m; temperature above 14°C
 - Previously considered unimportant above 1500m
- All banana varieties in the region especially EAHB are susceptible to *P. fijiensis*.
- *P. eumusae* not reported in EA



Genetic diversity in Sigatoka pathogens

- Maintain high genetic diversity
- Sigatoka pathogens can reproduce clonally or sexually.
- Sexual recombination generates novel genotypes
- Genetically diverse pathogens are hard to control
 - Adapt to changing environments
 - Overcome fungicides and host resistance
- African populations reported to be less diverse

Management of Sigatoka leaf spots

- Management challenging due to airborne spores and perennial nature of banana
- **Cultural practises-** reduces the inoculum load and creates uncondusive conditions for pathogen
- **Chemical Control-** Effective but not affordable to smallholder farmers
 - Risk of developing resistance
 - Environment/health hazard
- **Host resistance-** Effective, sustainable and environment friendly
 - Time consuming

Objectives

1. Map distribution and severity of Sigatoka leafspots in Uganda and Tanzania.
2. Determine mating type distribution, genetic diversity and population structure of *P. fijiensis*.
3. Evaluate resistance of NARITA hybrids to *P. fijiensis* under different environments.
4. Identify potential sources of resistance to *P. fijiensis*.

Chapter 2

Distribution of *Pseudocercospora* species causing Sigatoka leaf diseases of banana in Uganda and Tanzania

1. Map the distribution and severity of Sigatoka leaf spots
2. Identify pathogens associated with sigatoka leaf spots

Materials and methods

Uganda

Mbarara

Luwero



43 farms

Tanzania

Kagera

Mbeya

Kilimanjaro



81 farms

Disease severity-

Visual estimation **15 plants/farm**

Leaf samples collected

DNA extraction

PCR

Sequencing- **26 samples**

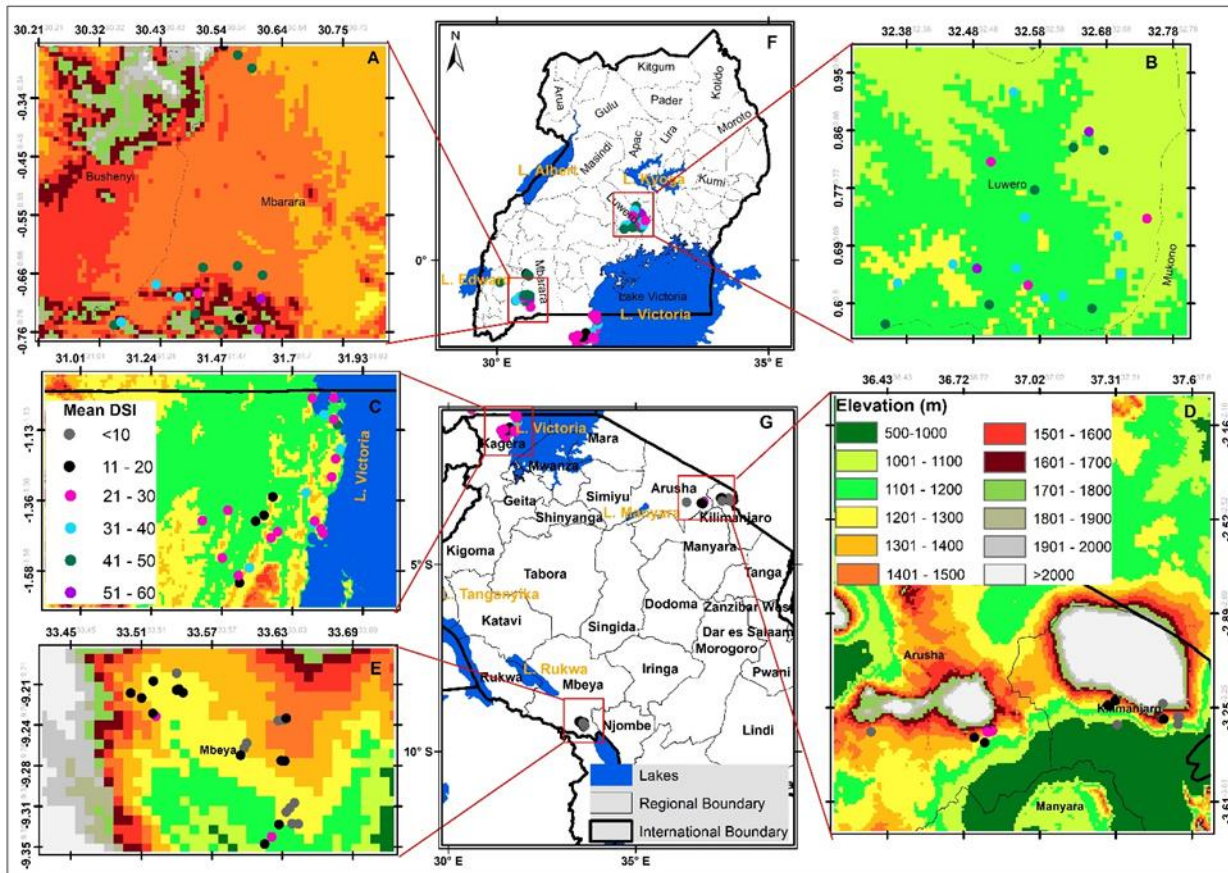


869 Samples

Historical weather data analysis

1980-2016

Disease incidence and severity



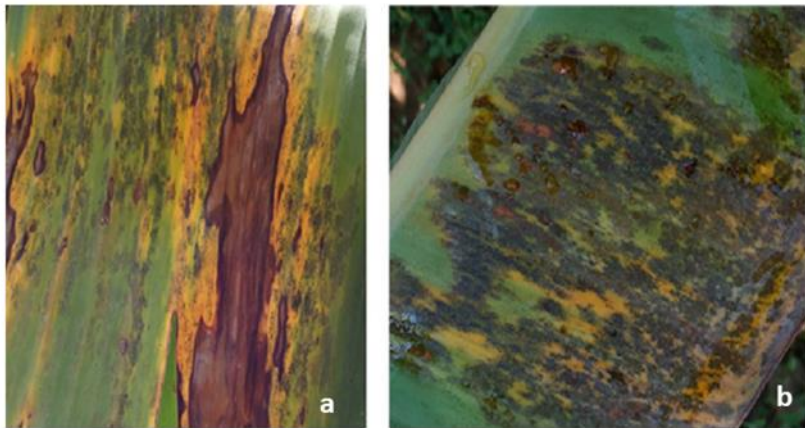
- Sigatoka like symptoms present at all sites, altitudes and cultivars.
- Disease severity higher in Uganda (39.3%) than Tanzania (20.1%).
- Higher severity on EAHB (Matooke and Mchare) and FHIA hybrids
- All banana cultivars including Yangambi KM5 were susceptible to Sigatoka leaf spots.

Species identification

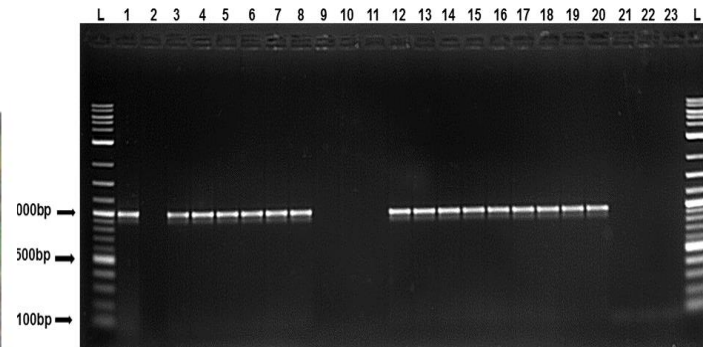
- *P. fijiensis* most predominant (80%) except in Kilimanjaro
- Detected up to 1877masl
- *Mycosphaerella musae* pathogen in Kilimanjaro
- *P. musae* and *P. eumusae* not detected



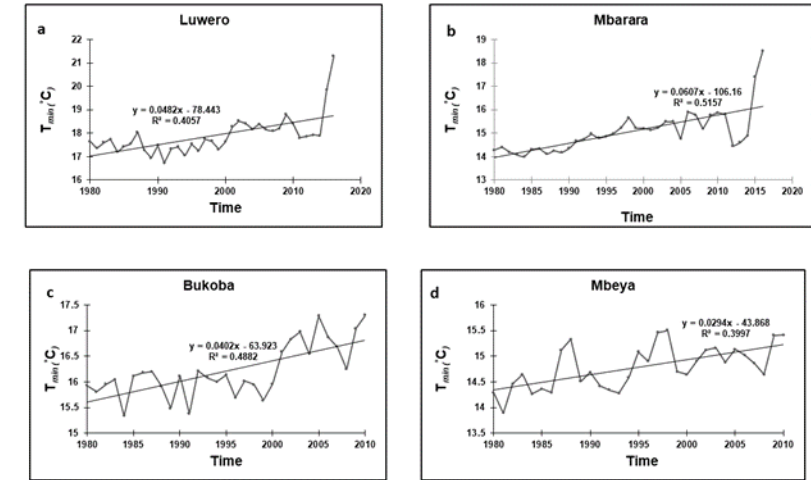
Black Sigatoka symptoms



Mycosphaerella speckle symptoms



Detection of *P. fijiensis* from infected leaves



- Minimum temperature has been rising
- Highest increase a Mbarara 0.06°C/year
- Lowest Mbeya 0.03°C/year

Findings

- Sigatoka leafspots are widespread in Uganda and Tanzania and pose a challenge to banana production.
- All bananas grown in EA are susceptible to Sigatoka leaf spots.
- *P. fijiensis* the predominant pathogen in the region.
- Adaptation range expansion of *P. fijiensis* has expanded from 1350m (Johanson et al., 2000) to 1877 in this study.
 - Historical weather data revealed gradual temperature increase.
- *P. musae* (Yellow sigatoka) was not detected in this study.
- *Mycosphaerella musae* (speckle) predominant in Kilimanjaro. Need for studies to understand economic importance.
- Natural resistance in Yangambi Km5 has been broken down. This could be a result of emergence of new strains of *P. fijiensis*.

Chapter 3

Genetic diversity and mating type distribution of *Pseudocercospora fijiensis* on banana in Uganda and Tanzania

1. Characterise mating type distribution of *P. fijiensis*.
2. Determine the genetic diversity and population structure of *P. fijiensis* populations.

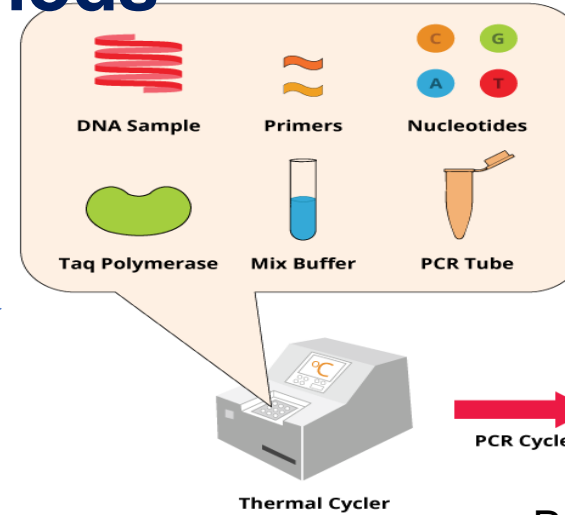
Materials and methods



Sample collection from infected banana fields



Single spore isolation and DNA extraction



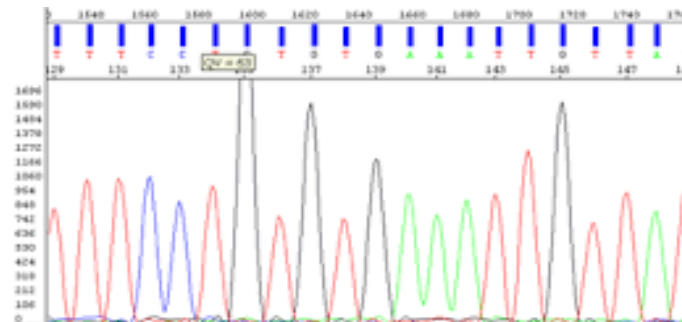
PCR analysis for species detection and mating type analysis



Gene mapper sequence analysis and allele scoring

MAT distribution-Binomial exact test

Genetic diversity and population structure-GenIAEx, Poppr, DAPC, MSN

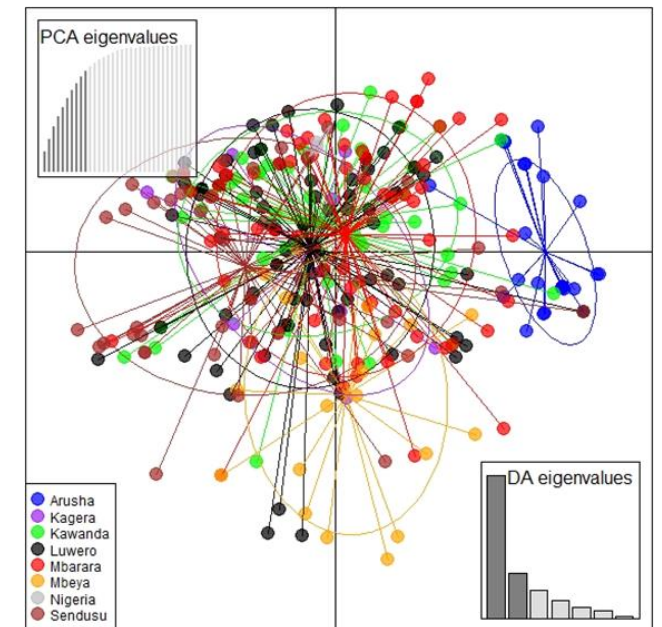


Capillary electrophoresis using 16 *P. fijiensis* labelled SSR markers

Diversity and population structure

Population (Country)	Subpopulation (region)	N	MLG	C.F(%)	eMLG	H	G	Lambda	E5	H _{exp}
Tanzania	Arusha	31	23	26	16.2	2.91	13.2	0.92	0.7	0.12
	Kagera	8	8	0	-	-	-	-	-	-
	Mbeya	21	21	0	21	3.04	21	0.95	1	0.26
Sub-total		60	52	13	9.5	3.82	35.3	0.97	0.77	0.25
Uganda	Kawanda	58	56	4	20.7	4.01	54.3	0.98	0.98	0.24
	Luwero	80	76	5	20.7	4.31	72.7	0.99	0.97	0.31
	Mbarara	70	65	7	20.3	4.11	53.3	0.98	0.87	0.27
	Sendusu	46	43	7	20.4	3.74	40.7	0.98	0.97	0.31
Sub-total		254	221	13	9.9	5.32	177.2	0.99	0.86	0.29
Nigeria	Ibadan	5	4	2	-	-	-	-	-	-
Total		319	296	7	20.6	5.5	205	0.99	0.84	0.29

- 296 MLGs-High genotypic diversity
- Low clonal fraction
- *P. fijiensis* in Uganda more diverse than Tanzania



- Poor regional differentiation-90% variation occur within populations

Mating type distribution

Population (Country)	Sub-population (Region)	Non-clone corrected				Clone corrected		
		N	MAT 1-1: MAT1-2 (%) ^a	MAT1-1 /MAT1-2 (%) ^c	p-value ^b	N	MAT 1-1: MAT1-2 ^a	p-value ^b
Uganda	Kawanda	69	51:43	6	0.53	62	55:45	0.23
	Sendusu	58	69:31	0	0.003	53	68:32	0.002
	Luwero	89	38:51	11	0.21	68	47:53	0.47
	Mbarara	77	45:51	4	0.64	64	48:52	0.76
	Sub-total	293	49:51	6	0.52	247	54:46	0.18
Tanzania	Arusha	35	68:23	9	0.004	28	71:29	0.06
	Kagera	8	0:75	25	_d	6	0:100	_
	Mbeya	32	100:0	0	<0.001	31	100:0	0.001
	Sub-total	75	75:19	6	0.001	65	78:22	0.003
Total		368	54:40	6	-0.003	312	59:41	0.01

- Both mating types present except at Kagera and Mbeya
- MAT 1 is overrepresented at Sendusu, Arusha and Mbeya.
- In Uganda, distribution is 1:1 while in Tanzania 4:1
- Populations in linkage equilibrium-supports frequent random mating

^aThe proportion (%) of isolates belonging to each of the mating types in a population and sub-population.

^bProbability from an exact binomial analysis (two-tailed) to test whether mating type frequencies deviate significantly from a 1:1 ratio.

^cProportion of isolates with both mating types.

^dAnalysis not performed due to low number of isolates.

Findings

- Low clonal fraction, both mating types supports a mixed reproduction mode
 - High evolutionary potential
 - Creates novel genotypes (Milgroom, 2015)
 - Has implication in screening and deployment of resistant materials
- Moderate level of gene diversity
 - More recent introduction
- Little evidence for regional differentiation
 - Common origin
 - Frequent exchange of genetic material geneflow or infected plant materials

Chapter 4

Multi-environment evaluation of Matooke hybrids for resistance to black Sigatoka

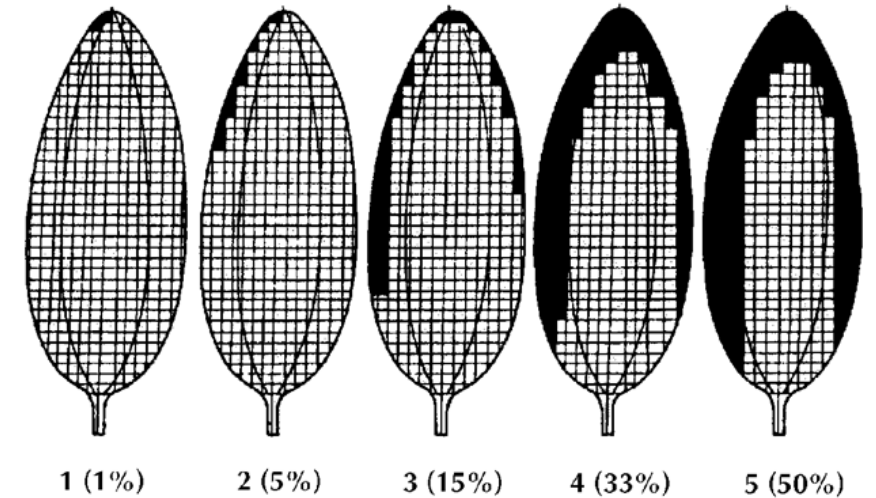
1. Evaluate resistance of Matooke hybrids to *Pseudocercospora fijiensis*
2. Determine stability and adaptability of Matooke hybrids
3. Identify suitable sites for Sigatoka screening



Materials and methods

- Plant materials- Matooke hybrids 21
- Trial locations: Kawanda, Mbarara(Uganda), Maruku, Mitarula and TACRI (Tanzania)
- Design: RCBD with 4 replications (12 plants per rep)

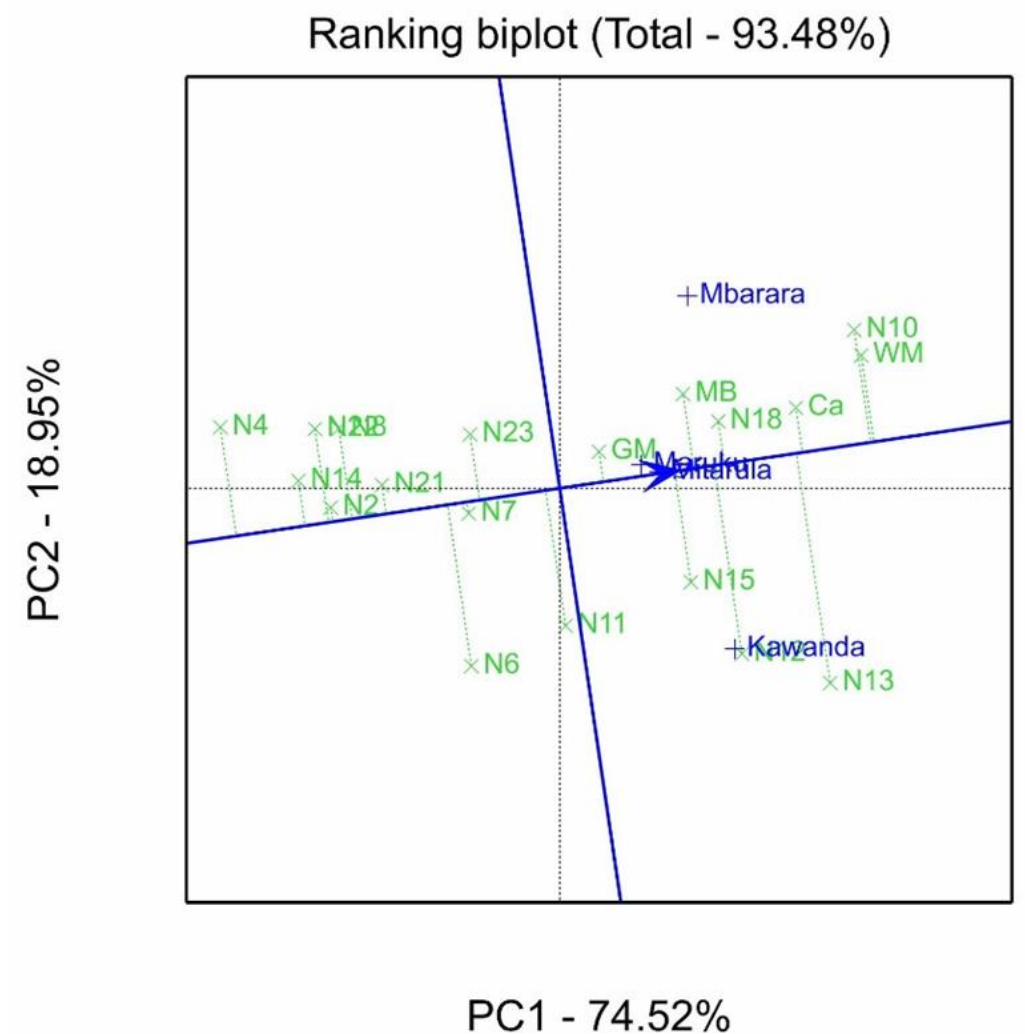
- Evaluations 6 months after planting
- Done on a quarterly basis for three crop cycles
- Disease parameters- Visual estimation of severity (0-6 scale)
- YLS, INSL, DSI, AUDPC
- Species confirmation- Species specific primers
- Analysis- AMMI and GGE biplots



Sigatoka severity scoring (Gauhl. 1994)

Results

- Variable disease severity-Highest at Kawanda.
- Environment contributed most to disease severity (39.1%) while GEI was 23.4%.
- NARITA 2, 4, 6, 7, 8, 11, 14, 21, 22 and 23 lower severity than susceptible check Mbwazirume.
- NARITA 10, 12, 13, 15 and 18 susceptible.
- Hybrids exhibited broad adaptability
- Most stable across test locations NARITA 2,7,8,21 and 23
- Mitarula most discriminative and representative site
- *P. fijiensis* not detected at TACRI



Findings

- Environment the highest contributor to black Sigatoka severity
 - Need to conduct MET for selection
- Five stable hybrids (NARITA 2, 7, 8, 21 and 23) are candidates for deployment to farmers in the region.
- Hybrid selection recommended at Mitarula to reduce screening costs.
- TACRI should not be used as a Sigatoka screening site in future because presence of *P. fijiensis* could not be confirmed.

Chapter 5

Sources of resistance to *Pseudocercospora fijiensis*, the cause of black Sigatoka in banana

1. Identify additional sources of resistance to *P. fijiensis*

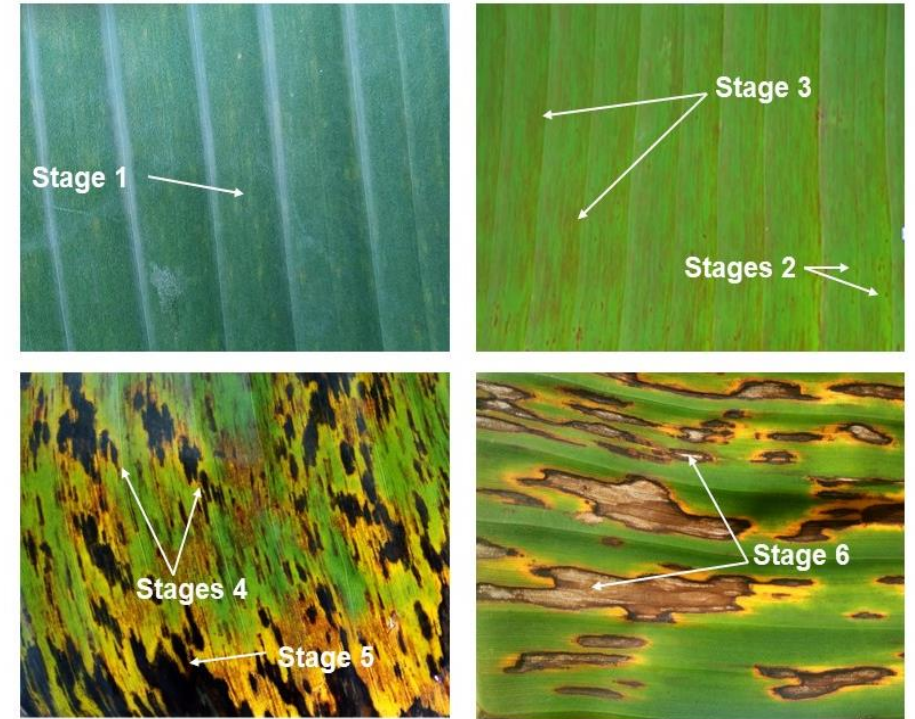
Materials and methods

Trials	Plant materials	Field evaluation	Disease parameters
Trial 1	79 banana cultivars from different origins	Evaluated after every 3 months.	<ul style="list-style-type: none"> • Youngest leaf spotted • Disease severity index • Area under disease progress curve
Trial 2	22 cultivars- breeding diploids, Improved diploids & tetraploids, resistant and susceptible checks	Evaluated quarterly starting 6 months for three crop cycles	<ul style="list-style-type: none"> • Index of non spotted leaves • Most advanced symptoms
Analysis	Correlation of disease parameters Genetic clustering based on SSR markers (Nakato <i>et al.</i> , 2018) Cultivar clustering based on disease parameters		

Results

- Calcutta 4 and Long Tavoy the most resistant
- Symptom progression stopped at different stages.
 - Stage 2- Calcutta 4, Pahang, Tani, Balbisiana
 - Stage 3-Borneo and Pisang Lilin, Cameroun, Zebrina,
 - Stage 4- Monyet ,

Variables	<u>Trial 1</u>						Coefficients of determination (R ²)
	DSI	INSL	YLS	YLst	SSD	AUDPC	
DSI	1						0.26
INSL	-0.84	1					0.46
YLS	-0.63	0.75	1				0.44
YLst	-0.33	0.64	0.85	1			0.22
SSD	0.63	-0.87	-0.68	-0.62	1		0.77
AUDPC	0.82	-0.77	-0.55	-0.54	0.71	1	0.69
	<u>Trial 2</u>						
DSI	1						0.36
INSL	-0.95	1					0.44
YLS	-0.82	0.89	1				0.35
YLst	-0.48	0.67	0.77	1			0.17
SSD	0.78	-0.81	-0.80	-0.69	1		0.89
AUDPC	0.97	-0.93	-0.68	-0.67	0.75	1	0.64



- Disease parameters were highly correlated
- Most advanced stage of symptom development (SSD) followed by AUDPC had the highest coefficient of determination (R²)
- Hierarchical clustering revealed three reactions types Resistant, intermediate and susceptible.

Results

Species	Sub-species	Genetic Cluster	Number of accessions in each response		
			^a Resistant	^b Intermediate	^c Susceptible
<i>Musa acuminata</i>	<i>burmannica</i>	I	4	^d -	-
	<i>malaccensis</i>	III	6	2	-
	<i>zebrina</i>	X	5	-	2
	<i>microcarpa</i>	X	1	-	-
	^e Unknown		4	1	8
	ISEA 1	III	1	-	-
	ISEA 2	IX	-	1	1
	Banksii	XI	-	1	3
	Ibota	III	-	1	-
	Cavendish	IX	-	-	4
	Pisang Jari	I	-	1	-
	IndonTriNG	IX	-	-	3
	Indon TriPri	X	-	1	-
	AA cv. African	IX	-	-	14
Lujugira/Mutik	X	-	1	14	
<i>Musa balbisiana</i>	<i>M. balbisiana</i>	VII	3	-	1
	Ney Poovan	VIII	1	-	-
	Mysore	VIII	1	-	-
	^f Pome	VIII	1	-	1
	Pisang Awak	VIII	3	-	-
	Blugoe/Mont	XII	1	1	1
	Silk	VIII	-	-	2

- Assessed cultivars distributed across 22 sub-species and nine genetic clusters.
- 31 (33%) cultivars resistant to *P. fijiensis*.
- Most resistant accessions were found in subspecies *Malaccensis*, *zebrina*, *burmannica*, *M. balbisiana* and Pisang Awak.
- 52% of susceptible accessions belong to Lujugira/Mutika and AA cv African.

Discussion

- Identified 8 resistant diploids similar to Calcutta 4.
 - These can be incorporated in breeding programs
- EAHB(Matooke and Mchare) the most susceptible to Sigatoka
 - Underscores the need to improve through breeding
- A good correlation of the different parameters used in assessing Sigatoka resistance
 - Most advanced stage of symptoms used for the first time.
 - It is fast and reliable selection criterion especially when selecting potential breeding materials

General conclusion

- *P. fijiensis* is the dominant leafspot in EA.
- *P. musae* may have been displaced in EA by *P. fijiensis* or conditions are not favourable.
- *P. fijiensis* in the region has high evolutionary potential and there is need to investigate if genotypic diversity is biologically significant.
- Convectional breeding has been successful in developing Sigatoka resistant hybrids that can be deployed to farmers.
- Additional resistance sources are available that can be incorporated in breeding programs to broaden the resistance gene base.

Future work

- Study and quantify the economic importance of other pathogens found on banana.
- Investigate presence of pathogenic groups among isolates from different regions and characterize their pathogenicity profiles.
- Screen more accessions from subspecies with resistant cultivars to broaden resistance gene pool.
- Determine role of pathogen adaptation on host resistance breakdown.

Research outputs



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Distribution of *Pseudocercospora* species causing Sigatoka leaf diseases of banana in Uganda and Tanzania

J. N. Kimunye, E. Were, F. Mussa, A. Tazuba, K. Jomanga, A. Viljoen, R. Swennen, F. K. Muthoni, G. Mahuku ✉

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SECTIONS

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Abstract

Sigatoka leaf diseases are a major constraint to banana production. A survey was



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Genetic Diversity and Mating Type Distribution of *Pseudocercospora fijiensis* on Banana in Uganda and Tanzania

Janet Njeri Kimunye, Norman Muzhinji, Diane Mostert, Altus Viljoen, Aletta E. van der Merwe, and George Mahuku ✉

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Abstract

Black Sigatoka, caused by *Pseudocercospora fijiensis*, is a major foliar disease of banana and plantain worldwide. Little information on the genetic diversity and

Details Figures Literature Cited



First Look

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