Sub-theme:

Pest diagnostics in phytosanitary systems

BOTRYOSPHAERIA CANKER AND DIEBACK A THREAT TO DOMESTICATION OF BAOBAB (*ADANSONIA DIGITATA* L.) AND MARULA (*SCLEROCARYA BIRREA* A. RICH.) IN AGROFORESTRY SYSTEMS IN KENYA

Presented by:

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Introduction

- Food and nutrition insecurities are major challenges in ASALs
- Several strategies have been used to address food insecurities in drylands e.g., diversifications of diets using indigenous trees
- Agroforestry harness great potential in enhancing food security in ASALs.
- ICRAF developed a key strategy to promote domestication of high priority indigenous fruit trees to enhance food security, nutrition and economic needs for dryland communities.

Introduction cont’

- However, recently forest and agroforestry trees are increasingly targeted by highly invasive pathogens
- These proliferation is boosted by climate change and human activities.
- Observation of canker and dieback were reported on baobab and marula under domestication in Eastern Kenya
- Impact can threaten conservation of important tree species.

Problem Statement

- 10 million people live in drought prone ASALs and face food and nutrition insecurity
- Indigenous fruit trees play critical role in food security and poverty reduction which are key SDGs
- Canker and dieback diseases have threatened conservation efforts
- Plurivorous fungi need to be well managed for sustainable agroforestry in dry areas.
- Need to characterize canker and dieback associated pathogens to accurately develop mitigation strategies.

Justification

- Domestication of indigenous trees is key strategy in achieving food security for dryland communities in Kenya
- They provide key nutrients and due to its tolerance to drought are suitable for the ASALs.
- Widespread canker and dieback symptoms on farms and domestication trials threatened domestication efforts
- It is important to identify associated pathogens since knowledge of pest and disease management is important to ensure safe exchange of health germplasm

Objectives

Specific objectives:

- To determine incidence and severity of canker affecting *A. digitata* and *S. birrea* under domestication during seasonal variations
- To characterize Botryosphaeriaceae fungi isolated from asymptomatic and symptomatic tissues.
- To determine virulence of isolated pathogenic fungi on baobab, marula and other commonly associated tree sps; *A. xanthophloea* and *C. capense*
- Provide a way forward for management of identified pathogenic fungi to enhance sustainability of dryland agroforestry in Kenya

Methodology

Study site: The study was conducted in Makueni and Kitui counties

Sampling method : Sampling was done using random sampling method form 3 sites (Tiva, Ikanga and Mukange)

Disease incidence and severity assessment: Assessment was done during wet and dry seasons based on occurrence of 3 main disease symptoms; Dieback, canker, leaf spots & blights

-Disease incidence and severity per farm was calculated as percentage (PDI) as described by Njuguna et al.(2011).

Sampling and Isolation: Total of 150 symptomatic and 15 asymptomatic trees were sampled, and isolation done following standard procedure.

-Pieces was surface sterilized and plated aseptically on agar media amended with *streptomycin sulphate*

Methodology cont’

-Plates were incubated at 25 degrees under alternating light and dark cycles and monitored daily for fungal growth

Morphological and conidial characterization; grouping was done based on morphotypes and further grouping done based on conidial description

Molecular characterization: DNA was extracted using CTAB(10%)-Chloroform) method

- DNA was quantify using gel electrophoresis and Nanodrop

- DNA amplification done, gel electrophoresis

- Purification of PCR product and sequencing done using ITS primers

- Sequences were aligned using MEGAX and BLAST analysis showed 98-100% similarity

Methodology cont'

Pathogenicity: Most isolated species were selected and inoculated on baobab, marula and other agroforestry trees; *A. xanthophloea* and *C. capense*

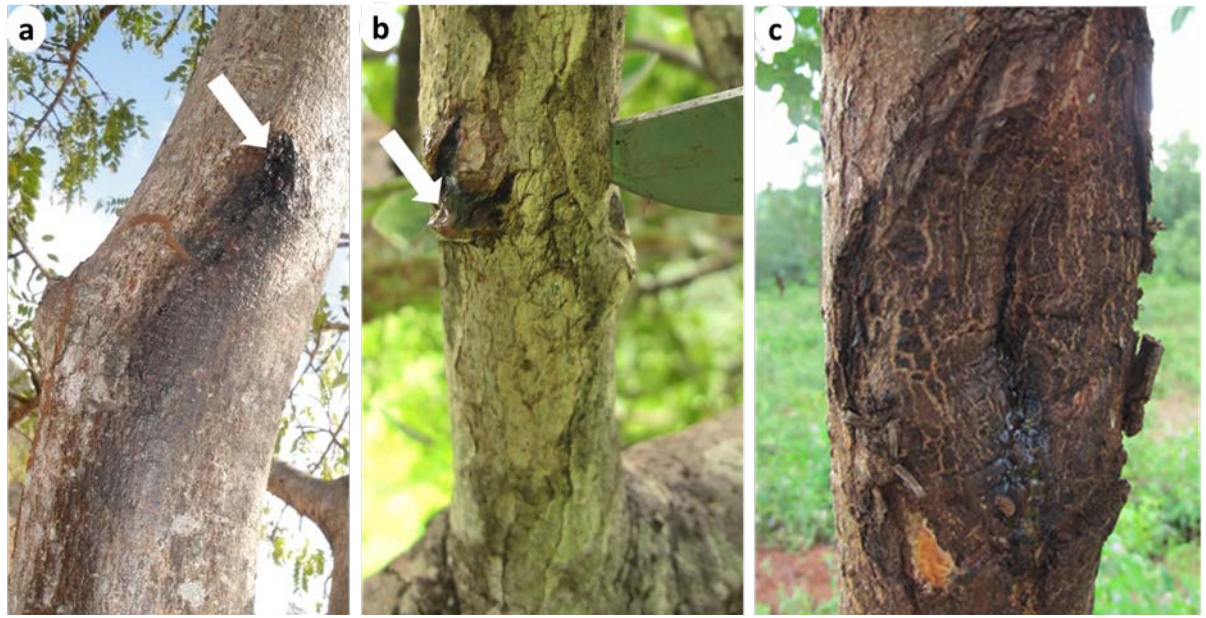
- Inoculation was done by vertical incision on stems of healthy seedlings under glasshouse conditions and monitored daily for development of Canker, dieback, leaf blight and spot symptoms

-Virulence were estimated based on 2 variables; Average days to show early canker symptoms and mean internal lesions categorized in 1-6 category scale.

Data analysis: Analysis done using Gen stat version 19:1, One-way ANOVA

Results

Fig 1: ***Field disease symptoms observed***



- Canker and dieback symptoms were widespread across the two sites
- Canker varied in sizes and characterized with lesion and resin.

Disease incidence and severity under seasonal variation: Disease index was high during dry than wet seasons and across the sites

		Incidence[1] (%)		Severity[2] (%)	
Location	**Tree species**	**wet season**	**dry season**	**wet season**	**dry season**
Tiva	*Adansonia digitata*	20	30	6.0	7.0
	Sclerocarya birrea	26	70	6.7	21.3
Ikanga	*Adansonia digitata*	42	54	12.3	21.8
Mukange	*Adansonia digitata*	40	57	10.8	23.0
	Sclerocarya birrea	47	69	12.5	25.2

Table 1: Incidence and severity of canker and dieback under seasonal variations

Results cont’

Morphological grouping and characterization: Seven morphological groupings were characterized in 3 main morphotypes corresponding to *Lasiodiplodia*, *Neofusicoccum* and Dothiorella

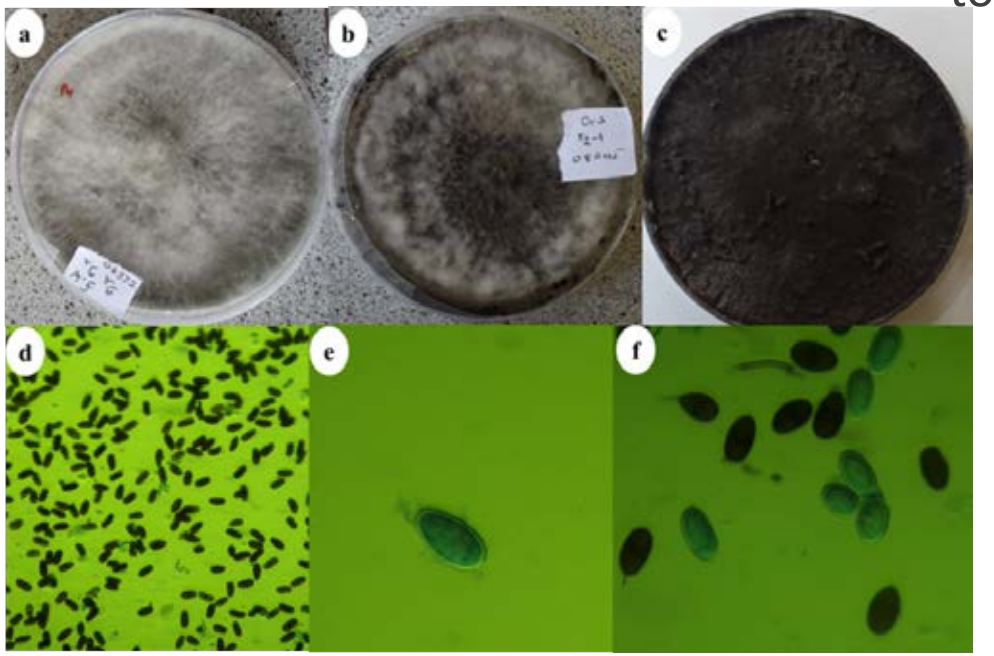


Fig 2: *Macro and microscopic cultural and conidial characteristics of Lasiodiplodia morphotypes (a) 5-day old colony of Lasiodiplodia theobromae on MEA, (b) one-week old colony of L. theobromae, (c) colony of L. mahajangana (e & f) immature and old conidia of L. mahajangana*

Results cont’

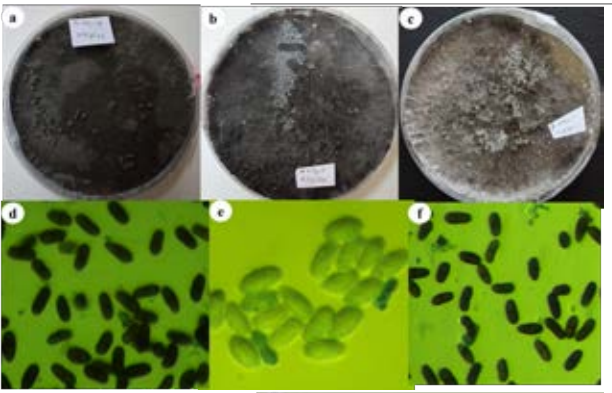


Fig 3: Macro and microscopic cultural and conidial characteristics of Dothiorella longicollis and Lasiodiplodia crassispora. (a) Colony and spores of L. crassispora respectively, (c & d) colony and spores of D. longicollis

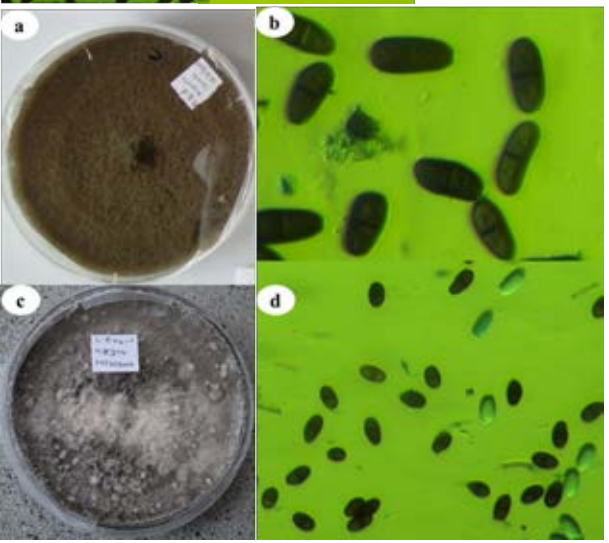


Fig 4: *Macro and microscopic cultural and conidial characteristics of Dothiorella longicollis and Lasiodiplodia crassispora. (a) Colony and spores of L. crassispora respectively, (c & d) colony and spores of D. longicollis*

Results cont'

Molecular characterization:

- ITS sequence showed >98% identity with sequence in NCBI confirming identity of isolates
- Good bootstrap support of > 98%
- Isolates confirmed were; *L. parva L. pseudotheobromae*, *L. theobromae*, *L. mahajangana*, *L. crassispora*, *Neofusicoccum parvum and Dothiorella longicollis*

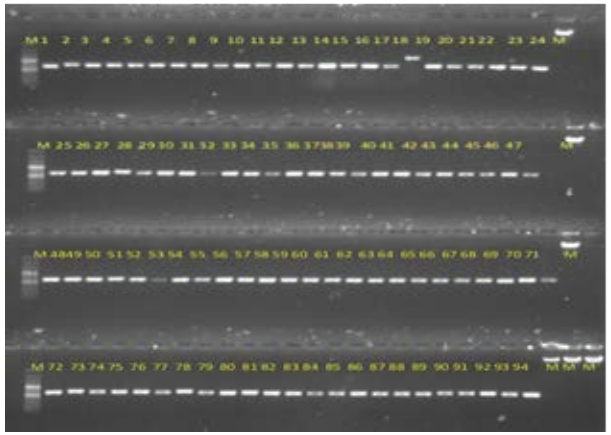


Fig 5: *Gel electrophoresis of ITS fungal isolates*

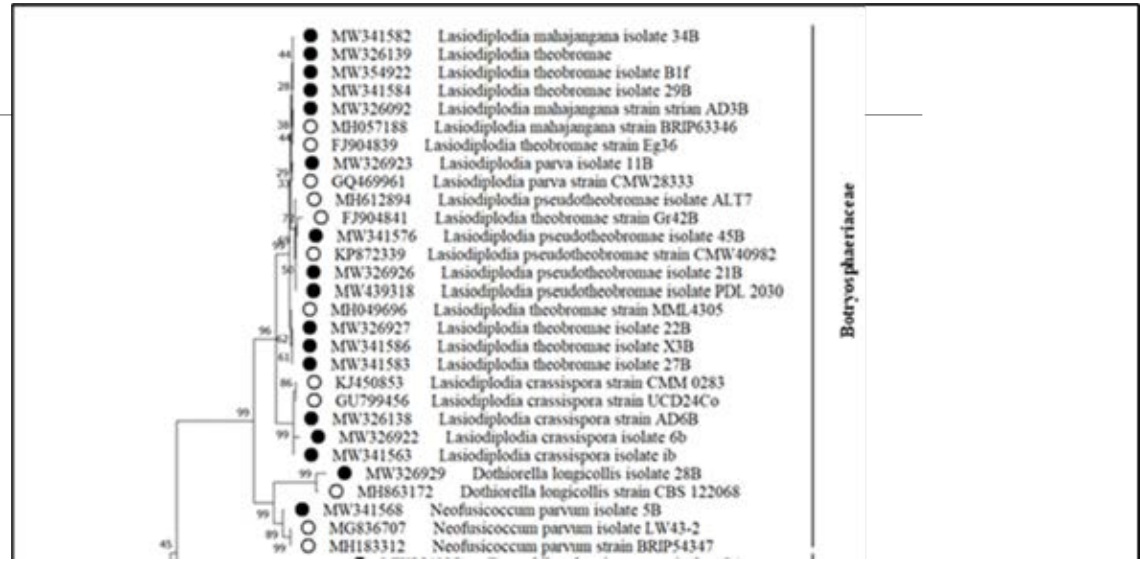


Fig 6: *Neighbor joining bootstrap consensus tree of ITS sequence data*

Results cont'

Pathogenicity test: Visible symptoms observed were lesions with resin, dieback and wound healing

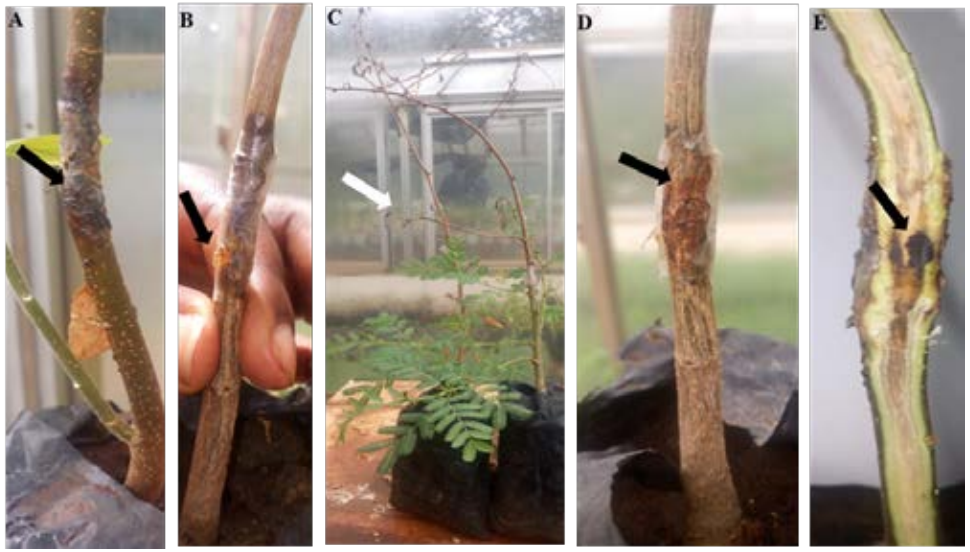


Fig 7: *Observed symptoms after inoculations*

Table2: Summary of analysis of Variance (ANOVA), Variate: Number of days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Fungal sps	3	189.222	63.074	7.56	<.001
Tree species	4	584.554	146.138	17.52	<.001
fungal sps. Tree species	5	393.818	78.764	9.44	<.001
Residual	67	558.988	8.343		
Total	**79**	**1726.582**			

Table 3: Summary of analysis of variance (ANOVA) at 95% confidence interval

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Fungal pathogen	3	16.62418	5.541392	999.71	<.001
Tree species	4	0.732276	0.183069	33.03	<.001
Fungal pathogen. Tree sps	5	0.422677	0.084535	15.25	<.001
Residual	67	0.371383	0.005543		
Total	**79**	**18.15051**			

Variate: lesion size (cm)

Conclusion

- Botryosphaeria canker is widespread, Domestication and conservation of indigenous fruit trees is under threat
- Botryosphaeria canker is disease complex since canker and dieback symptoms were indistinguishable.
- The two species could be regarded as potential risk to other plant species in agroforestry system because it could act as a source or reservoir of fungal inoculums.
- Wound healing and low susceptibility of baobab and marula and remain suitable species for ASALs
- Plurivorous nature of fungi expand their host range

Recommendations

- Need to establish if disease was **native or introduced**, therefore further mycological studies of the native vegetation surrounding small-scale farms are needed to ascertain whether the pathogens are native or introduced

- Studying such relationships would shed light on the **host-pathogen dynamics** for successful disease management programs.

- Finally, many studies have linked Botryosphaeriaceae diseases to stressful environmental factors, such as drought and extreme of temperature, but few have attempted to link these diseases with soil nutritional status. Studies of this aspect would give a view of the **influencing role of the environment on these pathogens.**

Acknowledgements

Theme: *"Enhancing Phytosanitary Systems for Healthy Plants, Safe & Sustainable Trade"*
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Thank you!

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