Phylogeny, morphology and growth characterization of *Lasiodiplodia theobromae* associated with dieback symptoms of cacao (*Theobroma cacao*) in the Philippines

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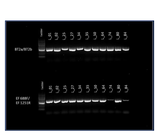
Background and Objectives

In recent years, fungal pathogens in the genus *Lasiodiplodia* has been recognized as significant threats to crops and woody plants in many parts of the world, including major cacao growing areas. A survey of dieback diseases conducted in cacao-growing agroecosystems in the Philippines showed that vascular streak dieback (VSD) is present in several provinces in Mindanao. This was confirmed through PCR-based detection using species-specific primers and isolation of the reported causal pathogen *Ceratobasidium theobromae.* However, the frequent isolation and detection of *Lasiodiplodia* spp. in collected samples exhibiting foliar necrosis, vascular tissue discoloration and twig dieback lead to the hypothesis of its possible association with the symptoms observed. This study aims to:

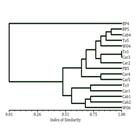
- Examine the diversity of *Lasiodiplodia* spp. associated with dieback symptoms in cacao
- Determine optimum conditions for growth and sporulation of fungal isolates selected based on their phylogenetic relationship
- Determine the effect of water activity and incubation temperature on the mycelial growth and spore germination of the selected isolates

Methods

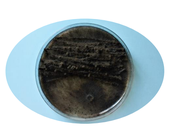
Sample collection and fungal isolation
Sample collection was conducted in 12 cacao growing provinces in the Philippines targeting twigs that are exhibiting dieback symptoms during the warm and dry season; isolation protocol for endophytic vascular fungi was followed



DNA Extraction, PCR amplification and Sanger sequencing
DNA extraction was done using the CTAB method and the target loci, ITS *and tef1-α* were amplified using high fidelity PCR kit followed by post-PCR treatment an sent out for sequencing



Phylogenetic analysis and selection of representative isolates
The diversity of selected *Lasiodiplodia* spp. cacao isolates along with three *L. theobromae* isolates and published sequences of selected tropical species were analyzed using maximum likelihood tree with RAxML set using the GTR model of molecular evolution, gamma-distributed rate heterogeneity among sites with rapid bootstrapping for 1000 iterations.



Morphological and growth characterization
Morphological characters of the pycnidia and conidia were examined for the selected isolates along with mycelial growth ang spore germination at different levels of temperature and water potential

Results

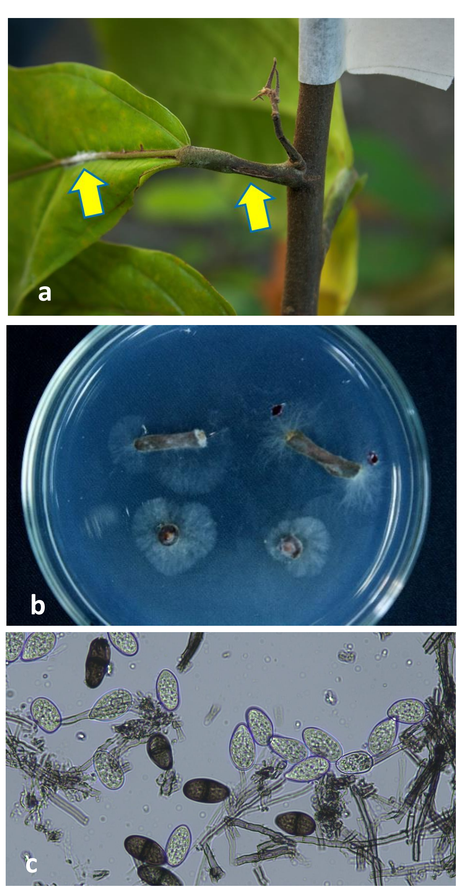


Figure 1. Symptomatic cacao twig (a) and isolation plate showing fungal outgrowths from plant tissues (b) and conidia (mature and immature) of *Lasiodiplodia theobromae* (c)

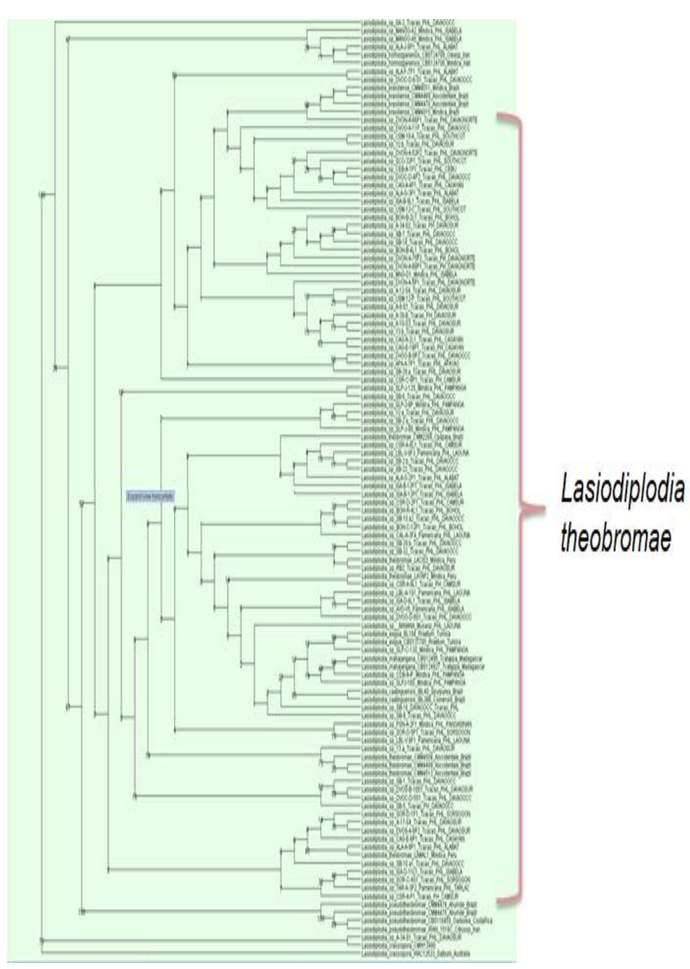


Figure 2. Maximum likelihood tree of *Lasiodiplodia theobromae* isolates from cacao using along with identified *Lasiodiplodia* species using ITS *and tef1-α* loci. Control isolates for *Lasiodiplodia theobromae* and sequences from closely related tropical species from Rodríguez-Gálvez et al., 2016 were included.

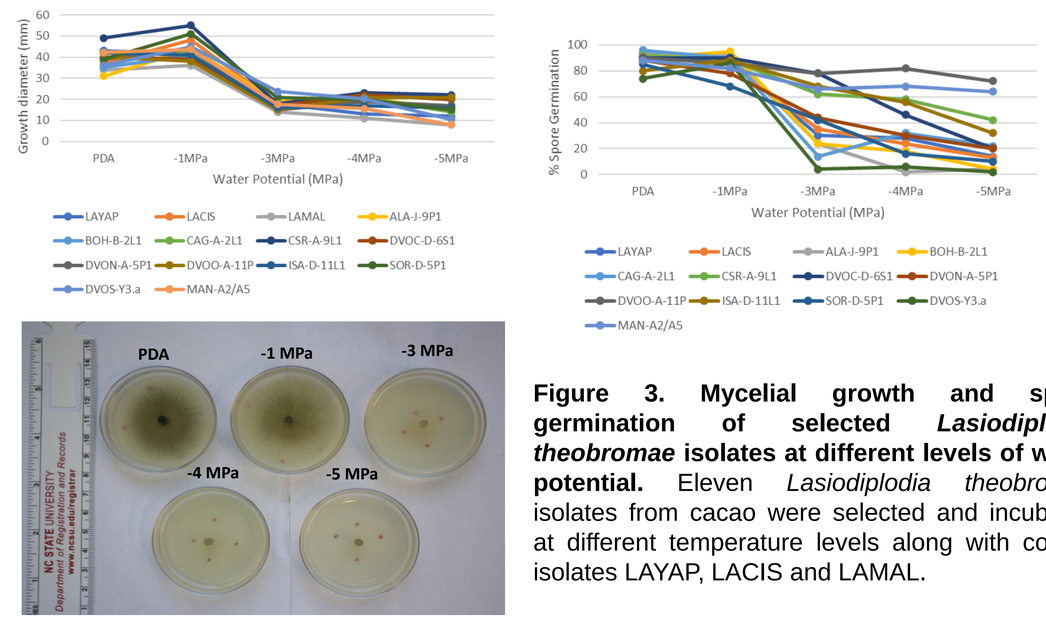


Figure 3. Mycelial growth and spore germination of selected *Lasiodiplodia theobromae* isolates at different levels of water potential. Eleven *Lasiodiplodia theobromae* isolates from cacao were selected and incubated at different temperature levels along with control isolates LAYAP, LACIS and LAMAL.

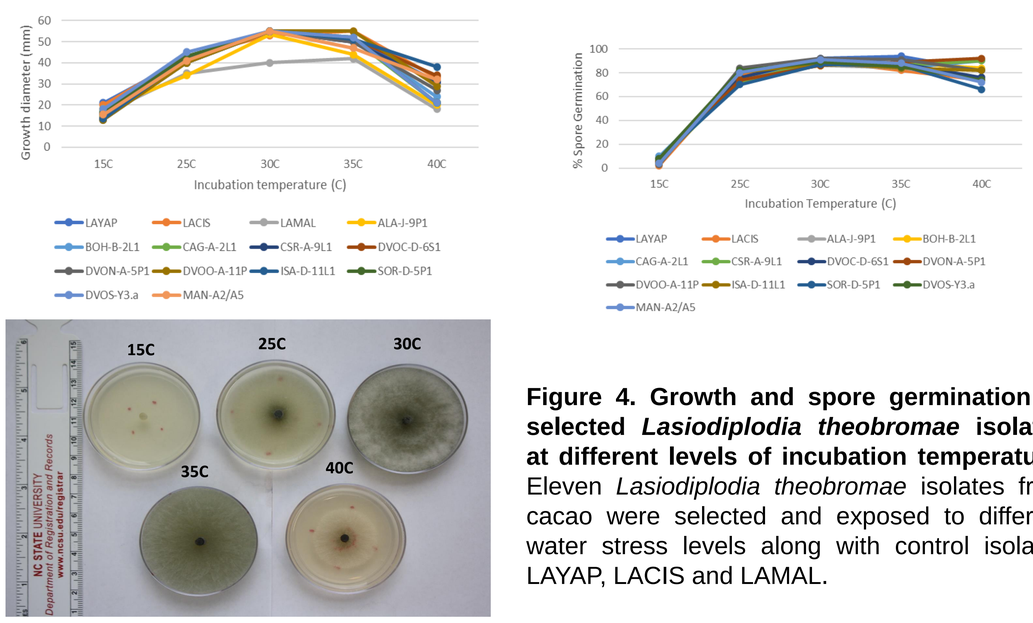


Figure 4. Growth and spore germination of selected *Lasiodiplodia theobromae* isolates at different levels of incubation temperature. Eleven *Lasiodiplodia theobromae* isolates from cacao were selected and exposed to different water stress levels along with control isolates LAYAP, LACIS and LAMAL.

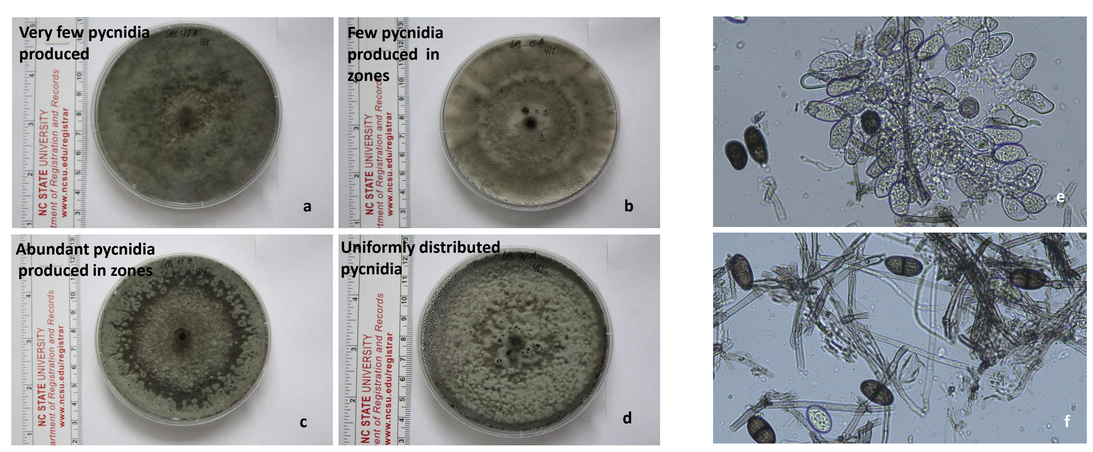


Figure 5. Differences in pycnidia production and incubation period for sporulation among selected *Lasiodiplodia theobromae* isolates in oatmeal agar. Sporulating plates of selected isolates showing differences in the number of pycnidia produced, SOR-D-5P1 (a), ALA-J-9P1 (b), BOH-B-2L1 (c), ISA-D-L1 (d). Differences in the maturation period of conidia of isolates which produced pycnidia late (e) and early (f) in the incubation period.

Summary and Future Research

- Optimum temperature for mycelial growth was 30°C with minimum growth at 15°C and reduced growth at 35°C.
- Spore gemination was greater than 85% at 40°C and less than 10% at 15°C across all the isolates.
- Water activity at -3 MPa reduced mycelial growth and spore germination across all isolates with the lowest values observed at -5 MPa.
- Our results show wide genetic variation but similar morphological and growth characters for cacao-associated *L. theobromae* and we are currently evaluating their disease-causing ability on cacao.

References

Alvindia, DG and Gallema, FLM., 2017. *Lasiodiplodia theobromae* causes vascular streak dieback (VSD)–like symptoms of cacao in Davao Region, Philippines. Austral. Plant Dis. 579 Notes, 12: 54.
Rodríguez-Gálvez, E., Guerrero, P., Barradas, C., Crous, P. W., & Alves, A. 2017. Phylogeny and pathogenicity of *Lasiodiplodia* species associated with dieback of mango in Peru. Fungal Biology, 121(4), 452–465. https://doi.org/10.1016/j.funbio.2016.06.004.

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