Moniliophthora roreri: Sample collection and culturing

M. Catherine Aime & Jorge Díaz-Valderrama, unpublished. May 2022.

Pure culture isolation of *M. roreri* has traditionally been the first step to study this pathogen in the laboratory. It allows the microscopic confirmation of diagnosis, as well as the ability to extract good quality DNA from fresh mycelia for molecular analyses and diagnoses. However, the process of isolation, especially if sampling occurs in remote areas, can be a limiting factor because of potential contamination during isolation and transport. To mitigate this issue we collected necrotic internal tissue where possible from FPR-diseased cacao pods directly into DNA extraction buffer as well as onto culture plates. DNA extraction from pure culture isolates tends to yield less contaminated DNA but in most cases yields much less DNA than extractions from internal necrotic tissue and white stroma directly collected. Therefore, we recommend both approaches. NOTE: most mummified pods are so desiccated that performing pure culture isolation is impossible from interior stroma; in these cases the collection of white stroma from the pod surface directly into DNA extraction buffer may be the only way to sample.

<u>Collection</u>: Diseased cacao pods should be collected in paper bags. GPS coordinates, date, host or cultivar and other relevant information about the sample, tree and plantation should be recorded on the bag. Isolations should occur the same day of harvest and performed under the most aseptic conditions possible.

<u>Sampling of pods</u>: Carefully crack the pod open (do not cut with a knife as this will force exterior contaminants into the sterile tissue inside. NOTE: if a pod is infected with *Moniliophthora*, it will have the odor of mushrooms when you crack it open. This is not present in pods infected with other pathogens such as *Phytophthora*. Use sterile forceps to remove a small portion of the necrotic tissue from pulp and beans.

1. Culture method: disinfect the internal tissue in a 2.5% sodium hypochlorite solution for three minutes, followed by three consecutive washes in sterile water for 1 minute each. Place the tissue directly onto Potato Dextrose Agar (PDA) plates, augmented with an antibiotic if possible, using aseptic technique. Incubate at room temperature. Check daily for signs of growth.
2. DNA method: place the pulp into DNA buffer. We use the Wizard® Genomic Purification Kit (Promega, Madison, WI), but other extraction methods will also work. (Go to molecular handout for further instructions).
3. Mummified pods: For mummified pods, interior stroma will be too desiccated to culture, and exterior stroma has too many other microorganisms present to pure culture. For these pods we recommend using sterile forceps to remove a good portion of the exterior white stroma from pod surfaces and placing this directly into DNA buffer. (Go to molecular handout for further instructions).

<u>Cultures</u>: Once hyphae are evident growing out from the point of inoculation, these should be excised and sub cultured onto individual PDA plates. Allow to incubate at room

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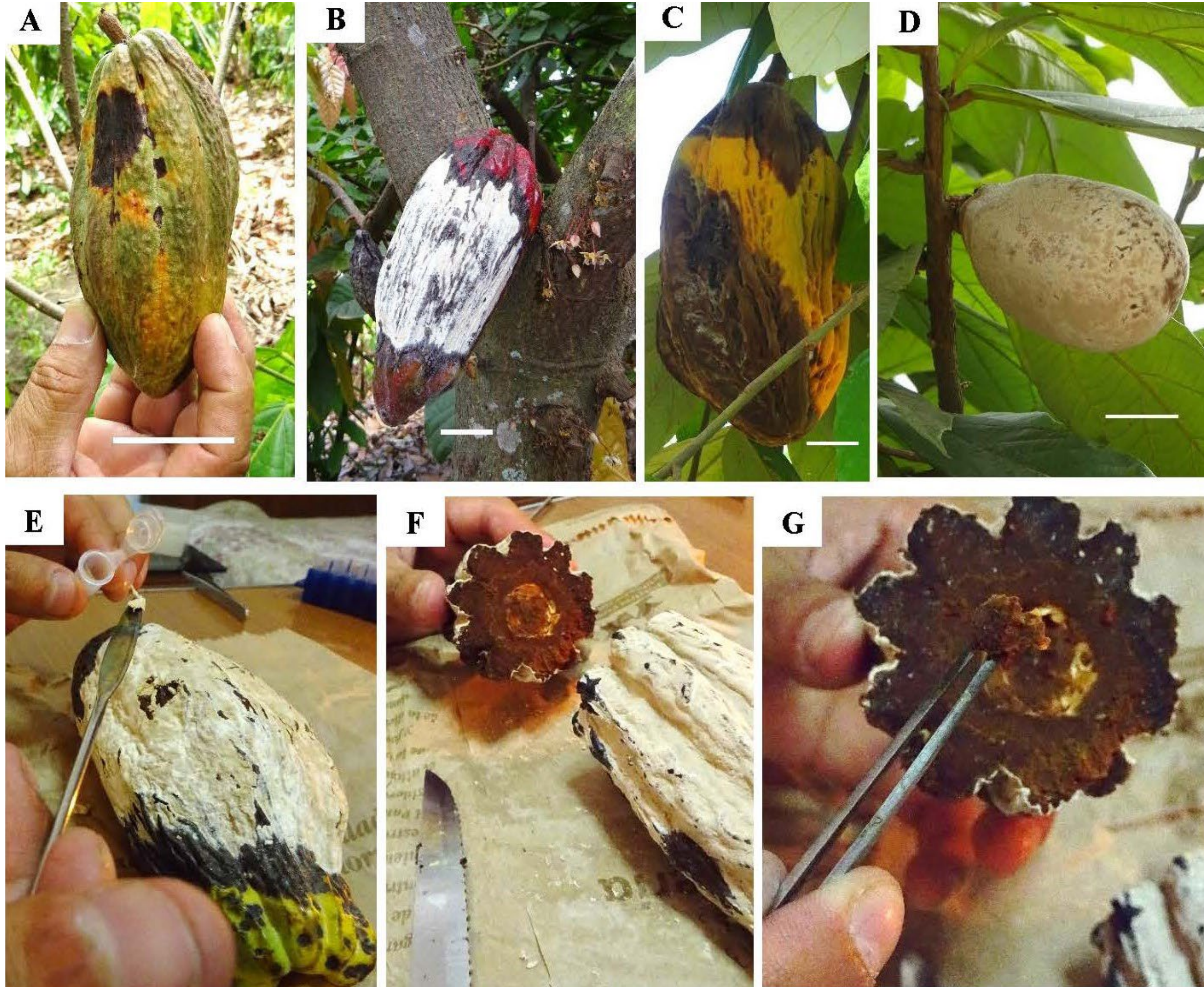
temperature for ca. 1 week. Pure cultures will be cream in color; conidia will begin developing after 1-2 weeks; these will be darker in color.

Moniliophthora roreri: Sample collection



Moniliophthora roreri signs, symptoms, and tissue collection. **A,** Identification of brown spots on cacao pods, an early symptom of frosty pod rot (FPR). **B,** Identification of mummified cacao pods with white stroma on the surface, symptom and sign in an advanced stage of the disease. **C,** Symptoms (brown spots) and signs (white stroma) of FPR on *T. bicolor* infected pod. **D,** Symptoms (mummification) and signs (white stroma) of FPR on *T. grandiflorum* infected pod. **E,** Use of Eppendorf tube containing Nuclei Lysis solution (See methods) to collect the white stroma on surface of mummified pod. **F,** Dissected pod. **G,** Collection of necrotic tissue to be place in Eppendorf tube with Nuclei Lysis solution, or onto PDA plates. Scale bare = 5 cm. *From: Díaz-Valderrama JR, et al. 2022. Diversity in the invasive cacao pathogen*

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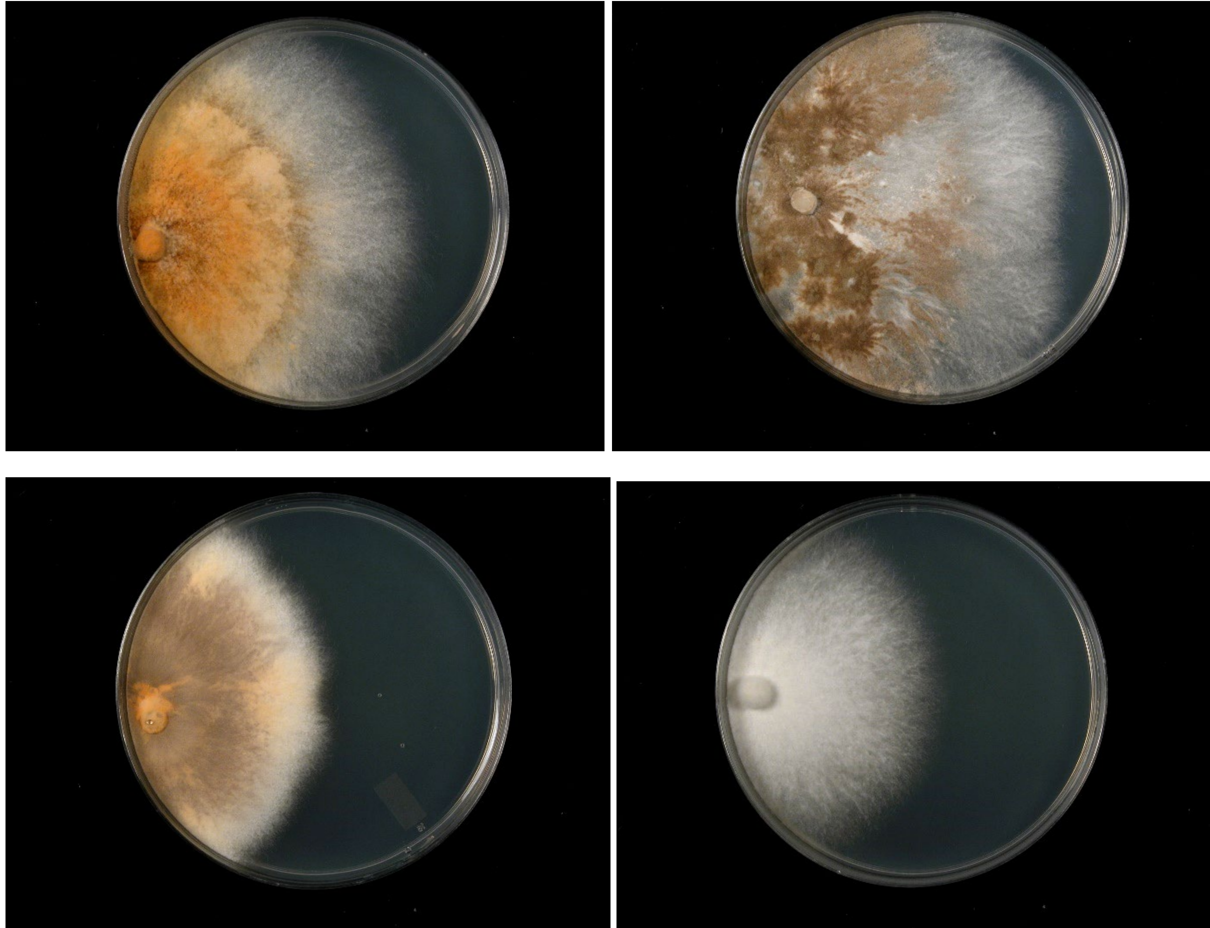
Moniliophthora roreri is shaped by agriculture. New Phytologist:
https://bsppjournals.onlinelibrary.wiley.com/doi/10.1111/ppa.13603?af=R

Moniliophthora roreri: Culture morphology



Moniliophthora roreri in culture. Young cultures are frosty white (bottom right). As cultures age they can develop orange pigments (left cultures). As conidia develop, cultures will turn superficially brown from the conidia (top right & left and bottom left).

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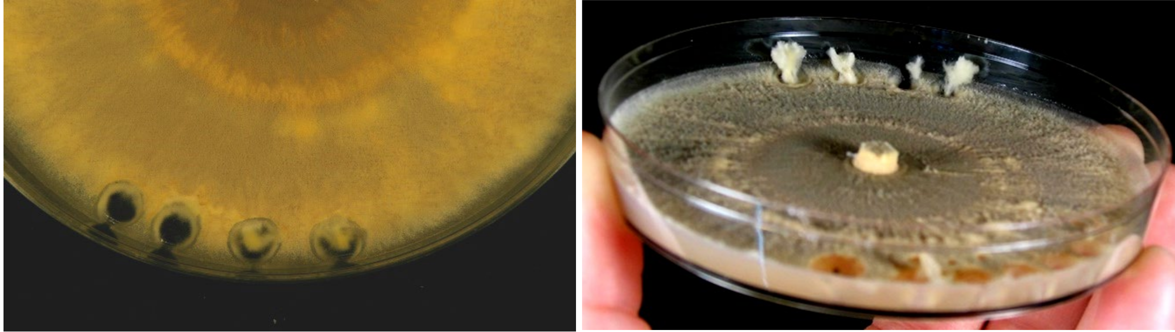
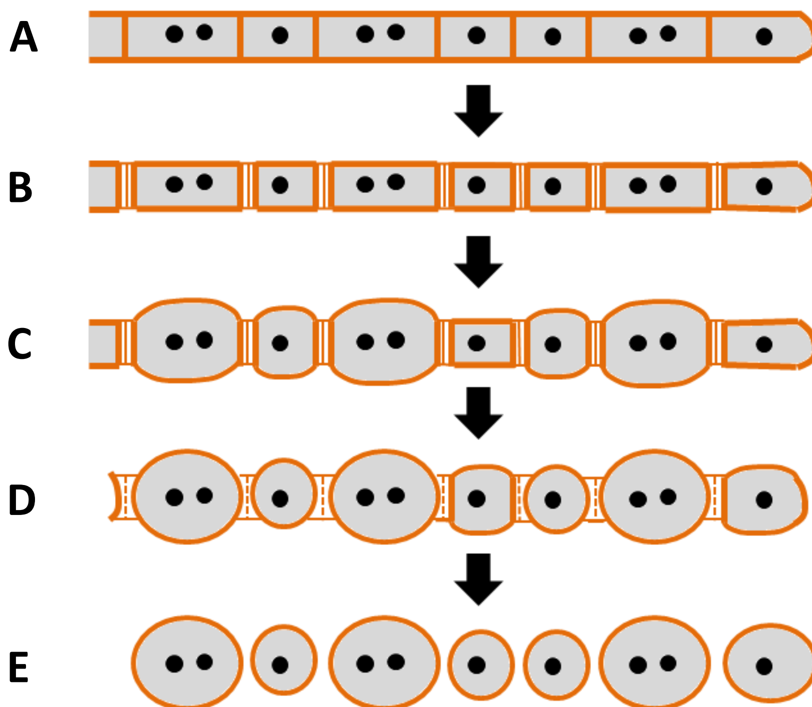


Very old cultures develop hyphal tufts at the growing margin.



Model of the thallic rhexolytic conidiogenesis in *M. roreri*. This type of conidiogenesis is diagnostic (different than other cacao pathogens). (A) Monokaryotic hyphae with varying number of nuclei; (B) plasma contraction of hyphal cells and formation of internal periclinal cell walls delimiting plasma-free compartments; (C) swelling of conidiogenous cells during maturation of conidia; (D) conidia secession; and (E) mature conidia. *From: Diaz-Valderrama JR, Aime MC. 2016. The cacao pathogen Moniliophthora roreri (Marasmiaceae) produces*

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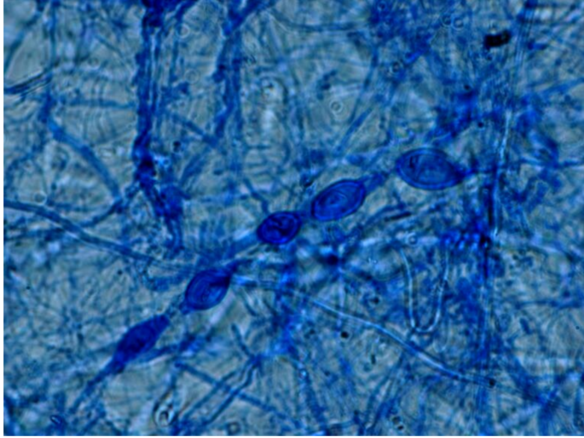
rhexolytic thallic conidia and their size is influenced by nuclear condition. Mycoscience 57:208-216. doi: 10.1016/j.myc.2016.01.004



Conidia stained in cotton blue.

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