

Diseases of cacao in Colombia: What we know and what we need to know.

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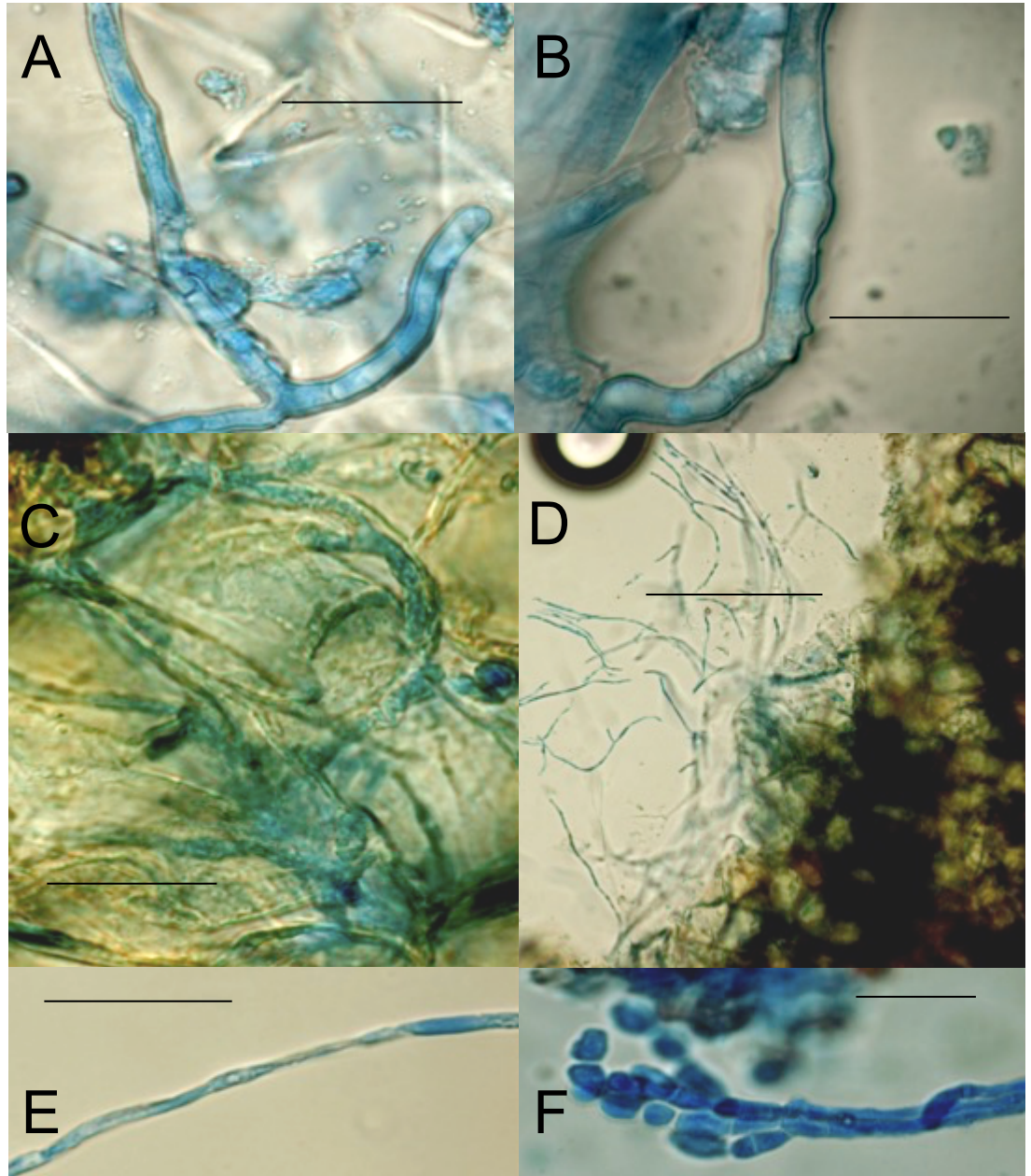
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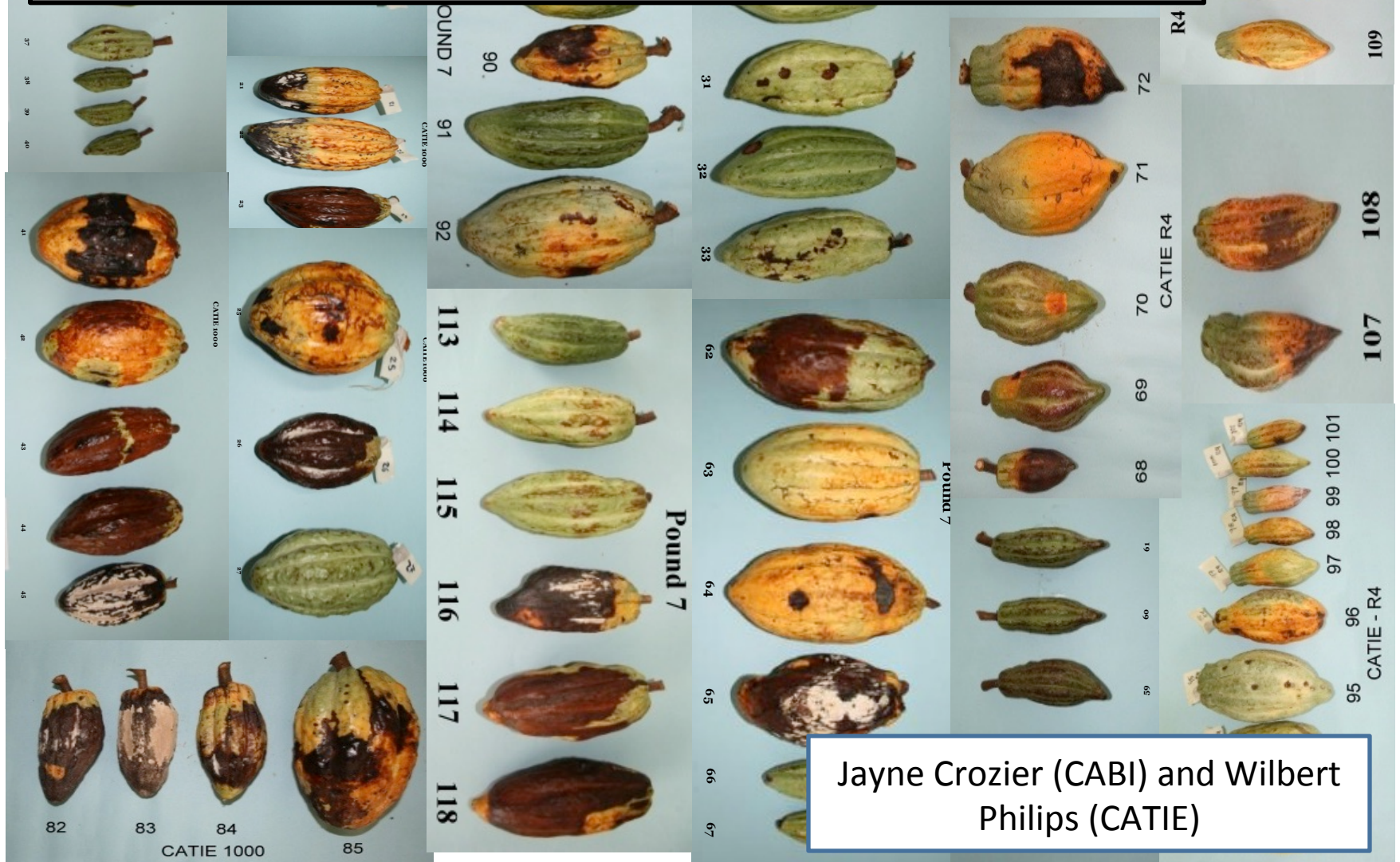
(A, B, C) Biotrophic *Mr* Mycelia from malformed green pods (3)

(D,E) Necrotrophic *Mr* mycelia from necrotic sporulating pods (4)

(F) Meiophore? from sporulating pod (4)



The pathogen occasionally infects and causes disease on even highly tolerant clones. How?



Jayne Crozier (CABI) and Wilbert Philips (CATIE)



So, the pathogen is altering its gene expression pattern during infection of tolerant clones. What is happening to the plant's gene expression?

What responses does the pathogen elicit from the host when causing disease in a tolerant clone?

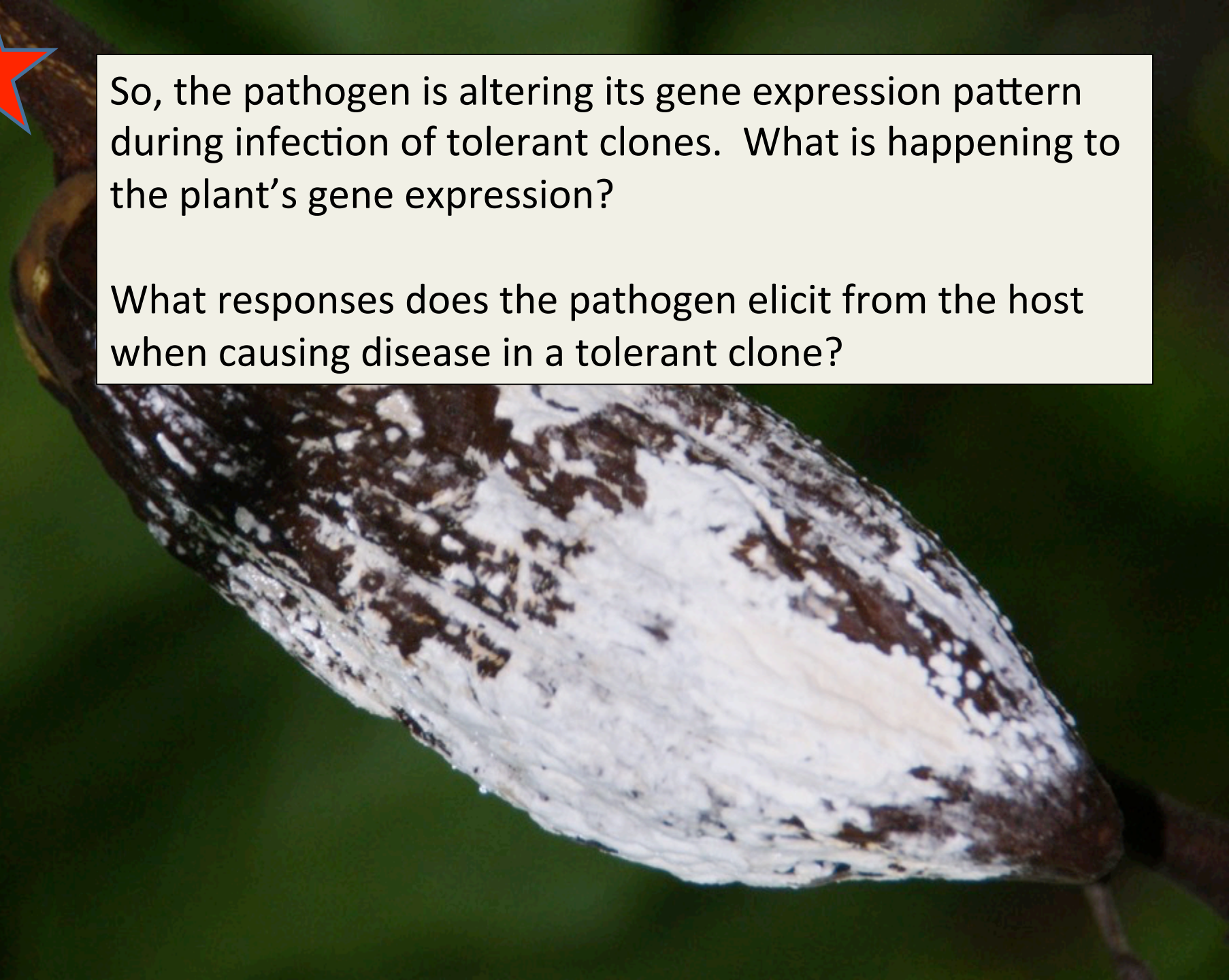
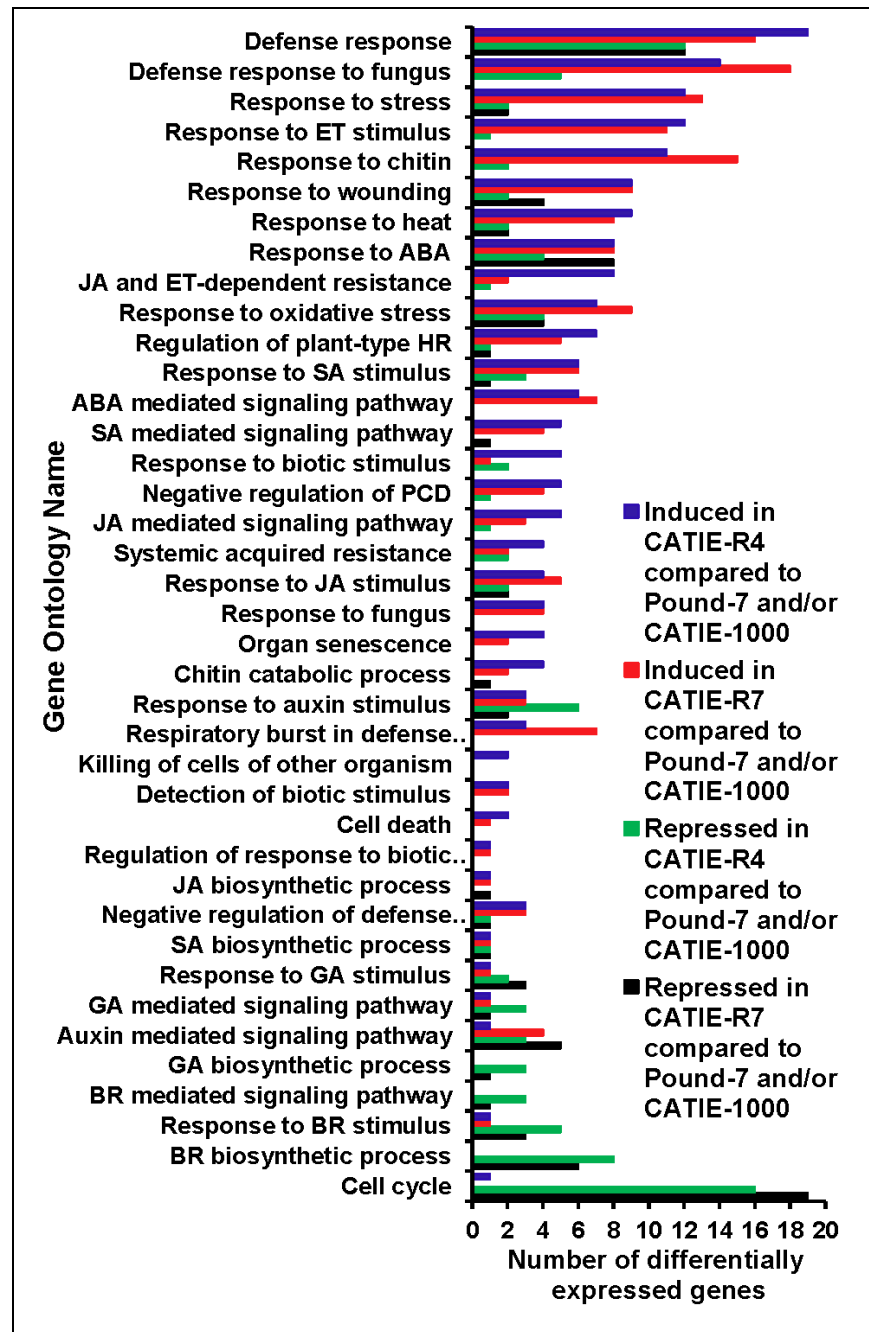


Figure 2. Shift in the number of differentially expressed genes among key biological processes involved in disease response between tolerant and susceptible *Theobroma cacao* clones in response to *Moniliophthora roreri* infection in pods. Differentially expressed genes were identified using RNA-Seq analysis between two tolerant clone CATIE-R4 and CATIE-R7 and two susceptible clones CATIE-1000 and Pound-7.





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The *M. roreri* genetic diversity in Costa Rica is very narrow. How does this compare to other countries? Why is this an important question?

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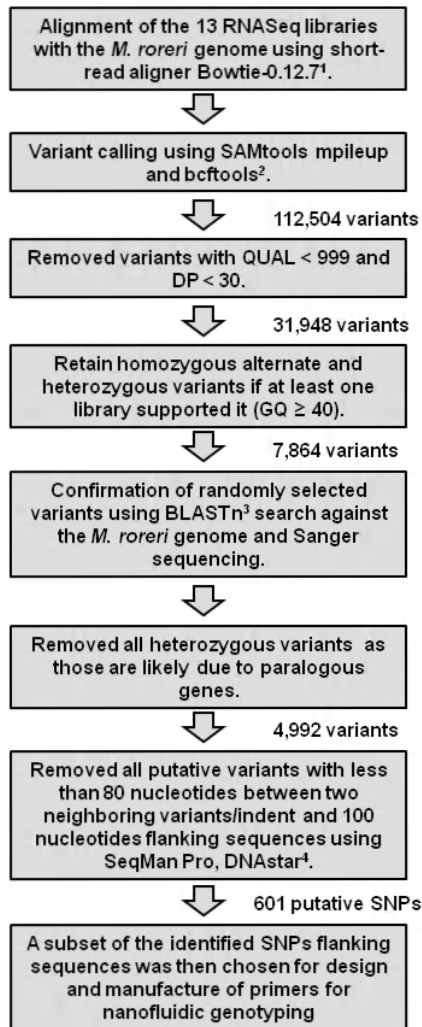
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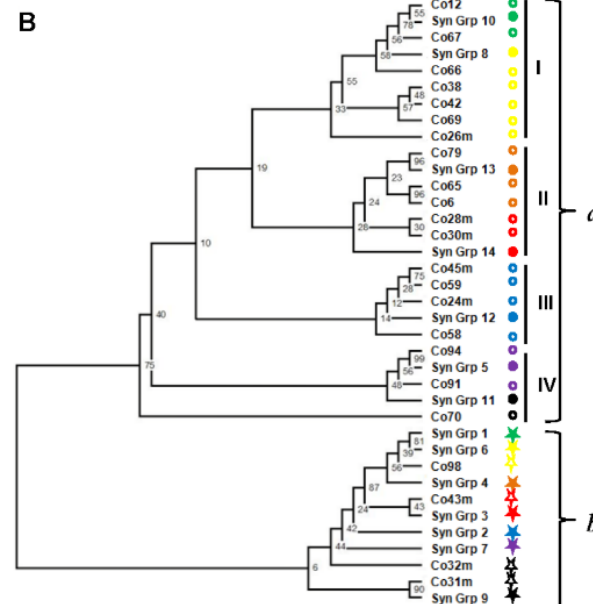
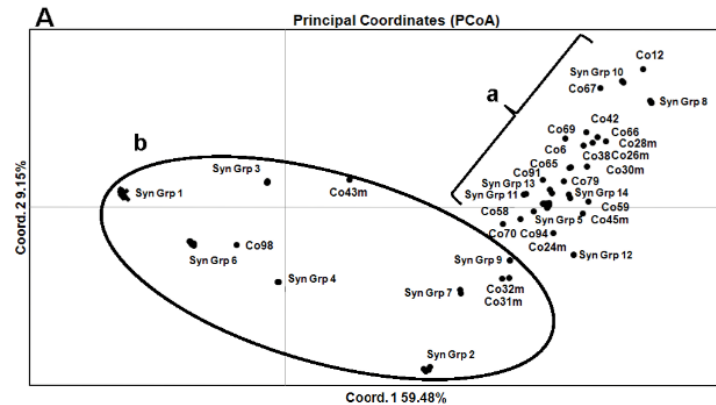
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Combination of RNAseq and SNP nanofluidic array reveals the center of genetic diversity of cacao pathogen *Moniliophthora roreri* in the upper Magdalena Valley of Colombia and its clonality.



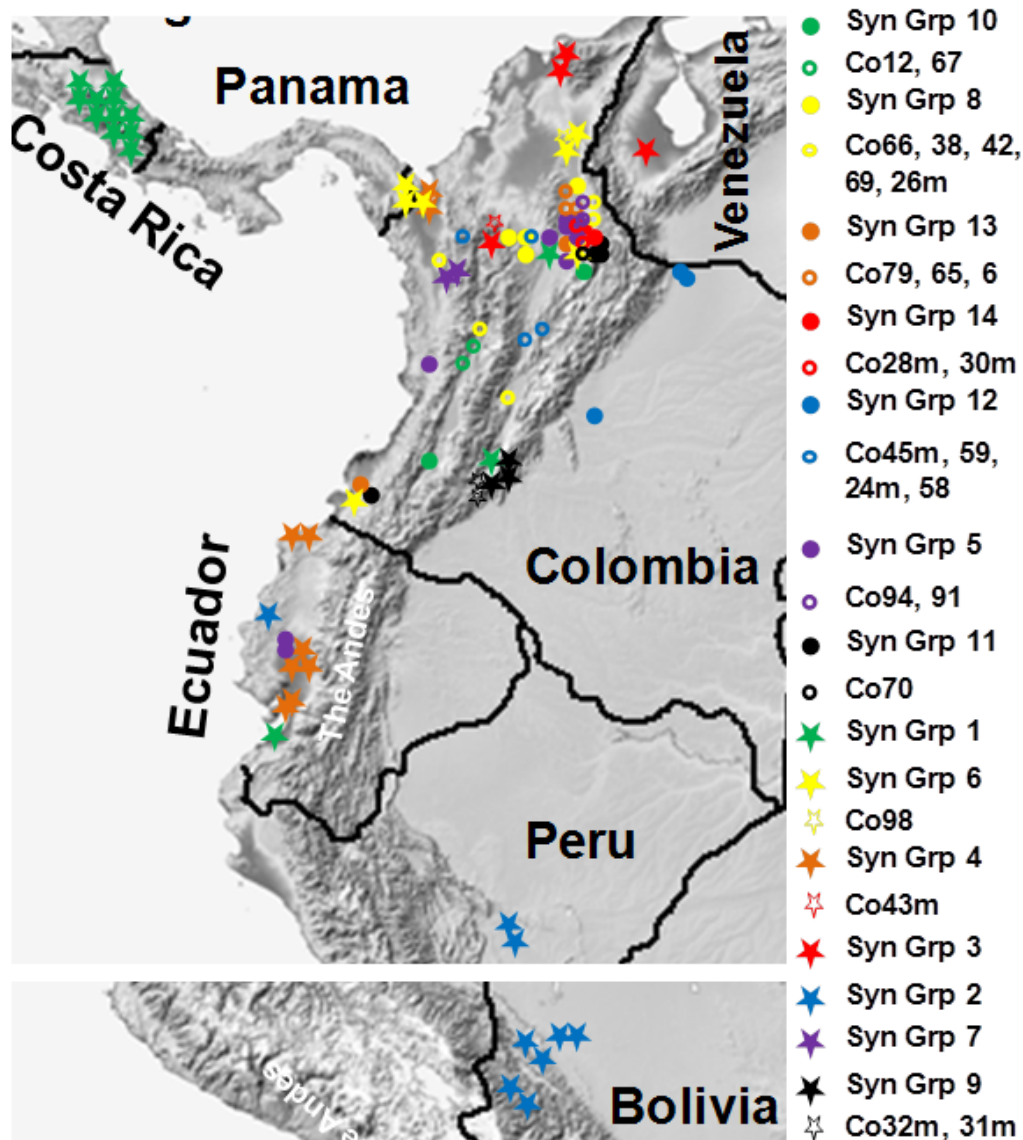
The overview of the SNP mining strategy from *M. roreri* RNASeq libraries



Genetic relationships based on 88 SNP markers among 172 *M. roreri* isolates collected from frosty pod rot affected areas of South and Central America

Wilbert Philips (CATIE)

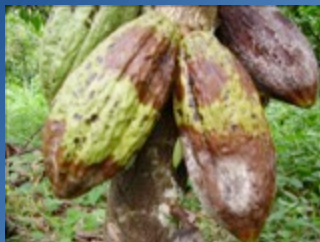
Moniliophthora roreri SNP



Geographical distributions of *M. roreri* isolates collected from frosty pod rot affected areas of South and Central America and their relation.



Black pod



Phytophthora palmivora,
P. megakarya

Phytophthora Species Causing Black Pod Rot on Cacao

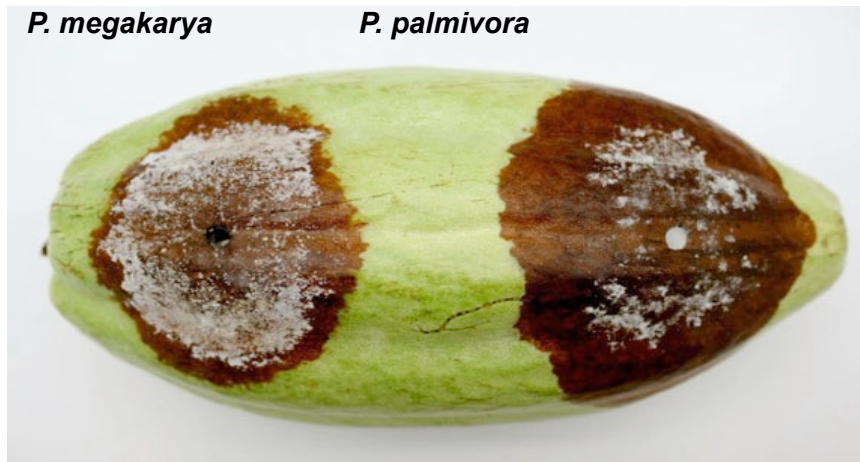
P. megakarya and black pod in Africa

- *P. megakarya* was first identified in Nigeria in 1979 and by 1981 became the predominant.
- In recent years, *P. megakarya* seems to have displaced *P. palmivora* from Cameroon and Nigeria.



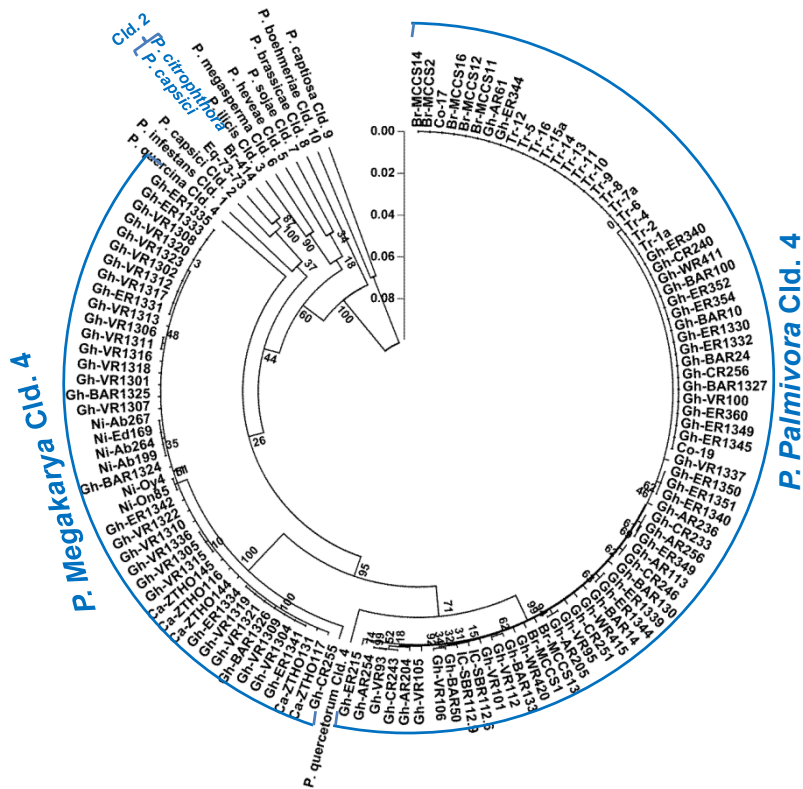
The Ghana situation

- Until the mid 1980s, black pod disease was known to be caused by *P. palmivora* only causing 4.9-19% pod loss.
- From 1985, pod loss due to black pod increased to 60-100% in the infected areas.
- In the year 2012 it lost more than 200,000 MT of cacao beans (25% of annual output) due to black pod.

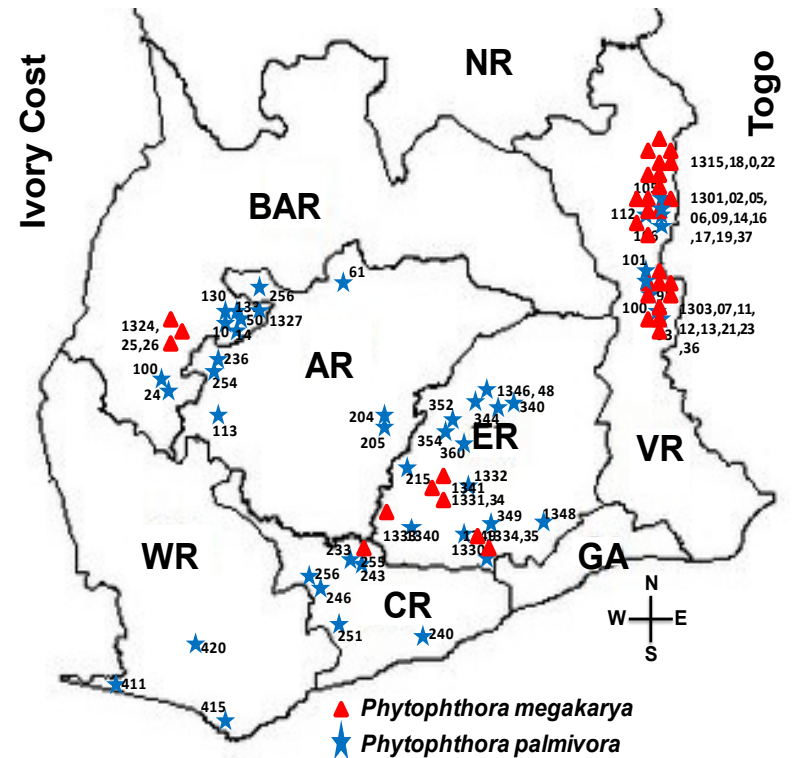


Difference in black pod lesions caused by *P. megakarya* and *P. palmivora* under similar conditions

Ishmael Amoako-Attah, Andrews Y. Akrofi (CRIG)



Molecular phylogenetic analysis of *Phytophthora* isolates collected from infected cacao pods from West Africa, Central and South America



Geographical distributions of *Phytophthora megakarya* and *P. palmivora* isolates causing black pod of cacao in Ghana.

Comparative genomics of *Phytophthora megakarya* and *P. palmivora*

Thanks to all our collaborators

Brett Tyler, Brent
Kronmiller and



Mark Gultinan



David Lary



Danyu Shen

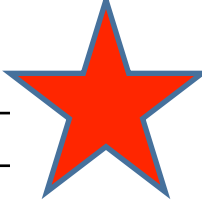


Funding source



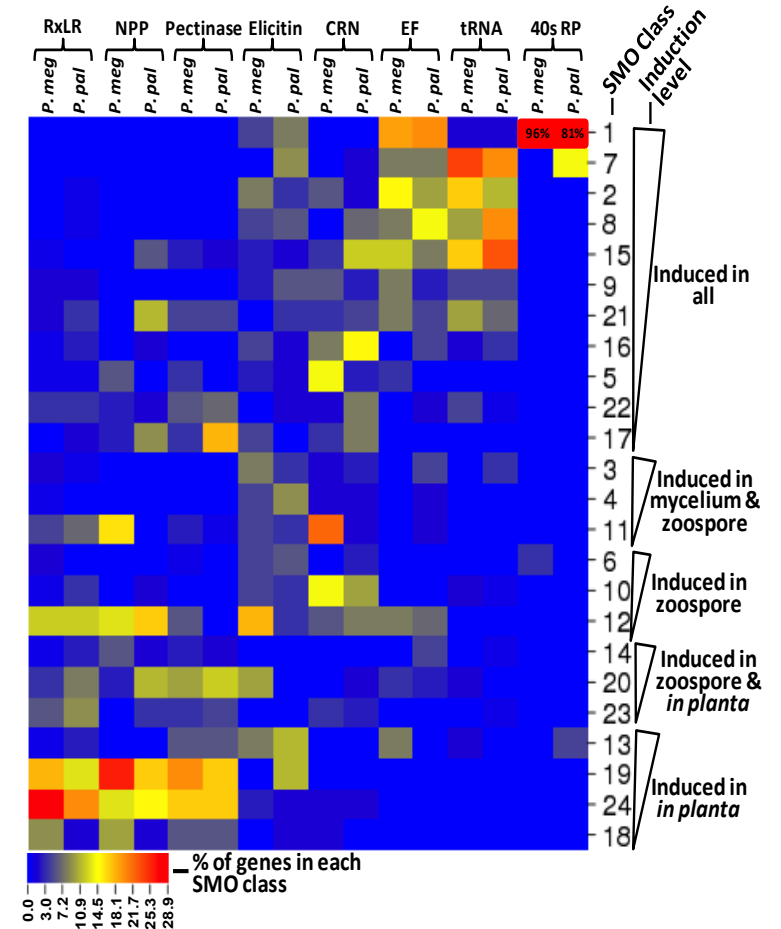
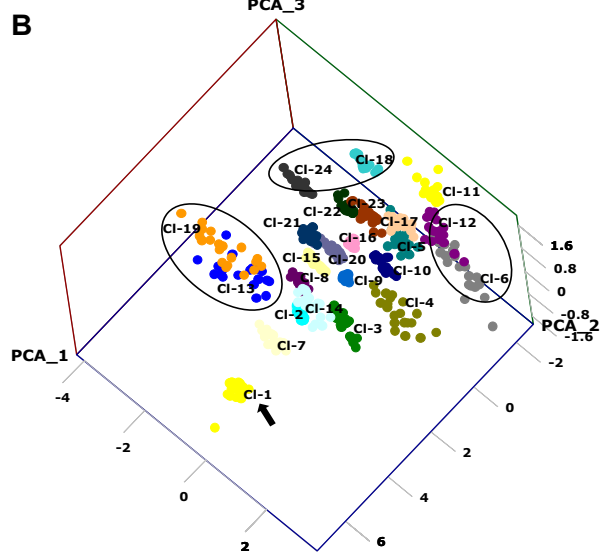
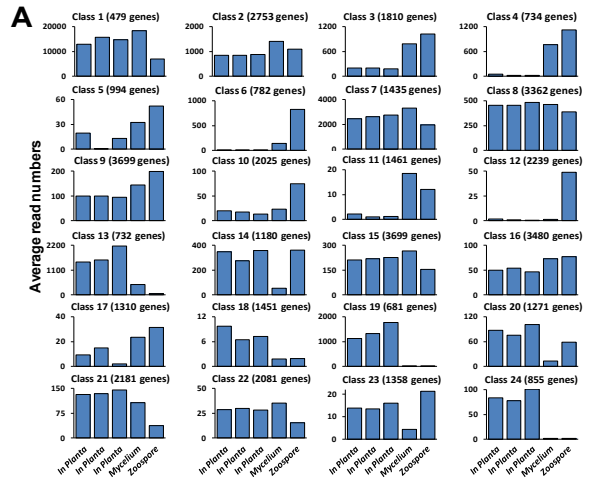
Other collaborators

1. Cocoa Research Institute of Ghana, Akim New-Tafo, Ghana
2. Cocoa Research Centre, the University of the West Indies, St Augustine, Trinidad and Tobago
3. Cocoa Research Institute of Nigeria, Ibadan, Nigeria
4. Plant Pathology Department, University of Florida, Gainesville, FL, USA
5. Regional Laboratory for Biological and Applied Microbiology, IRAD, Yaoundé, Cameroon
6. CIRAD, UPR 106 Bioagresseurs, Montpellier, France
7. Department of Agriculture and Agroforestry, CATIE, Turrialba, Costa Rica

**Table 1** Genome assembly and annotation statistics

	<i>P. megakarya</i>	<i>P. palmivora</i>
Genome		
Estimated genome sizes	126.88 Mb	151.23 Mb
Total Contig length	101,182,312	107,423,419
Contig numbers	27,143	28,632
CEGMA Completeness (%)	94.35	96.37
GC content	48.92%	48.91%
N50 Contig length	6,902	6,456
Scaffold number	24,070	24,815
K-mer analysis		
Read data	5,233 Mb	8,053 Mb
Average read length	90	90
K-mer length	15	15
Coverage depth	33.41	44.63
K-mer number	4,388,028,112	5,057,947,212
K-mer depth	28.01	28.03
Contig representation	64.58%	59.53%
Gene model		
Gene number	41,992	44,305
Total gene length	42,723,254	45,995,141
Average gene length	969.78	1038.15
Average gene density [#]	0.272	0.254
Number of expressed genes [*]	14,611	25,319
Genes with GO annotation [‡]	15,431	21,276
Genes within KEGG pathway	14,789	15,717
Core orthologous genes [±]	15,088	15,088
Genes with unknown functions/hypothetical protein	27,133	22,208

[#]CDS bases/total genome bases^{*} Only gene models with ≥ 10 reads, either in mycelia, zoospore or *in planta* are reported.[‡]Gene models with $E < 10^{-4}$ for BLASTn against Uniport Gene Ontology database.[±] Core orthologous were estimated based on bidirectional best BLASTp hits. To be considered as ortholog, BLASTp with least 50% of the sequence should align with E-value less than $1e-10$.



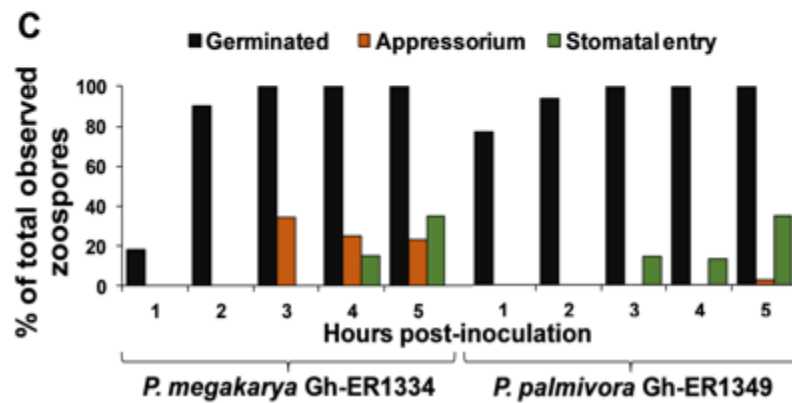
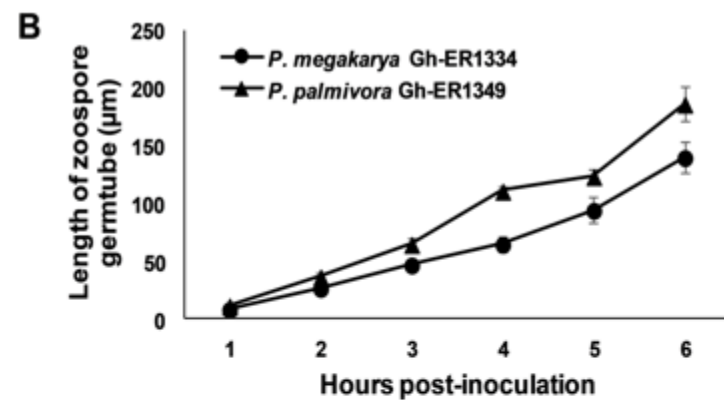
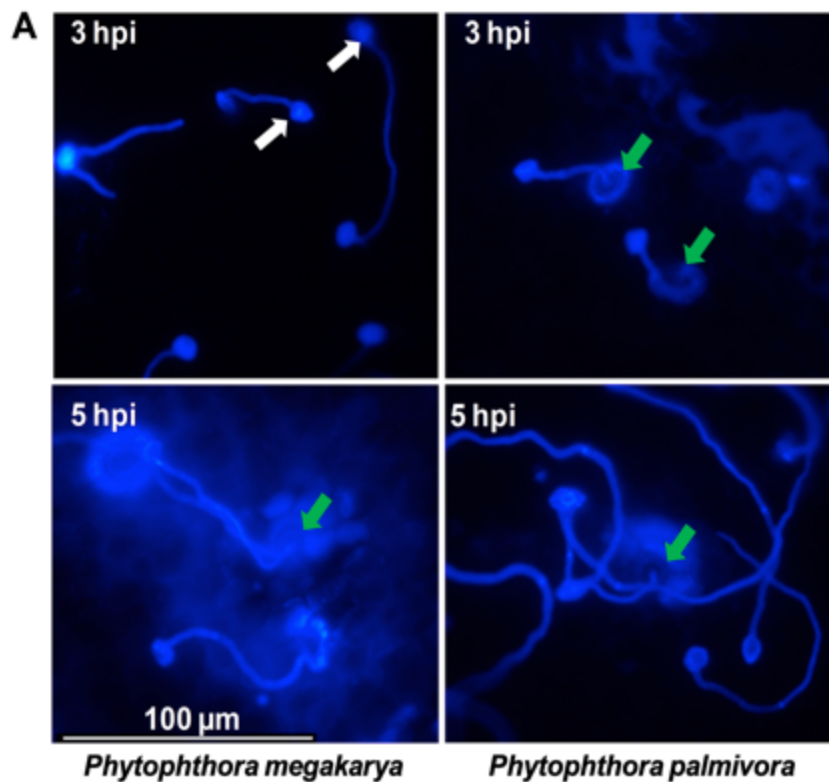
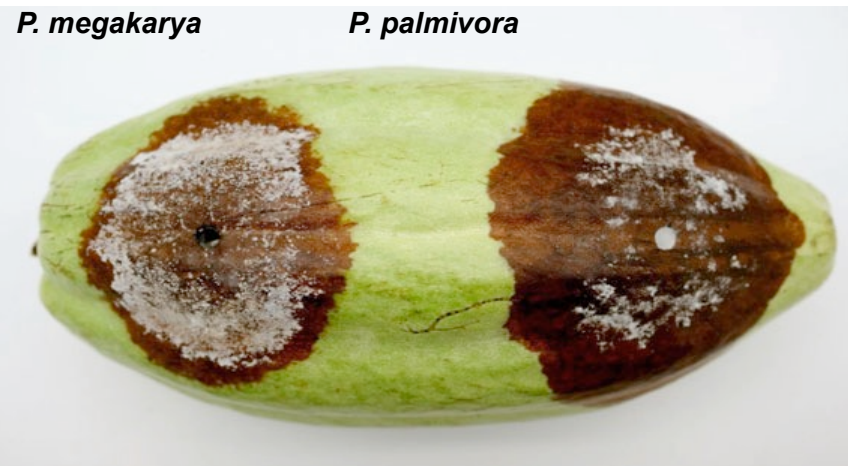
Differential distributions of gene models among the 24 self-organizing map (SOM) classes for different gene families.

Self-organizing map (SOM) classification of *P. megakarya* and *P. palmivora* gene models based on the RNA-Seq count data and their relationship.

Box 2

Basic differences in the biology of *P. megakarya* and *P. palmivora*.

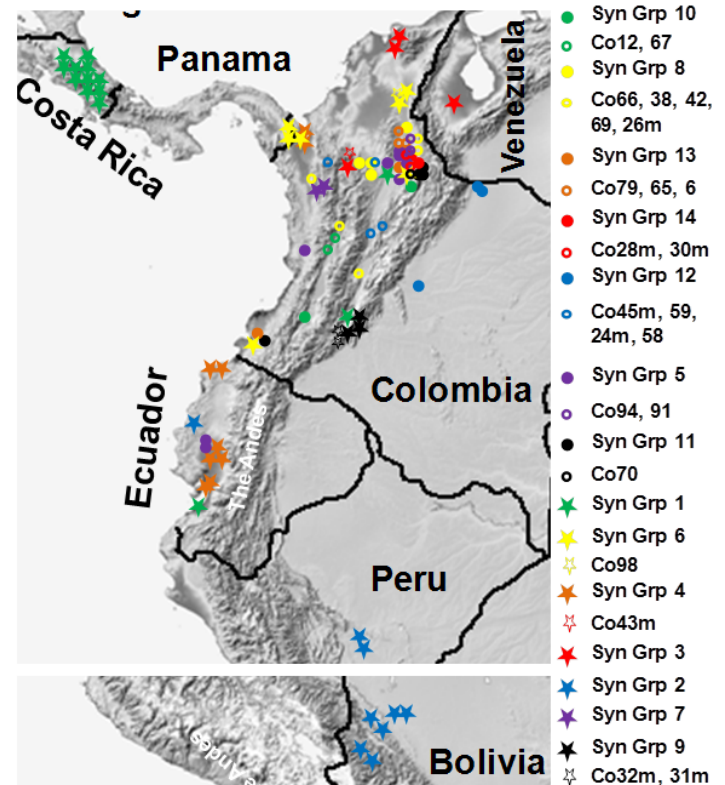
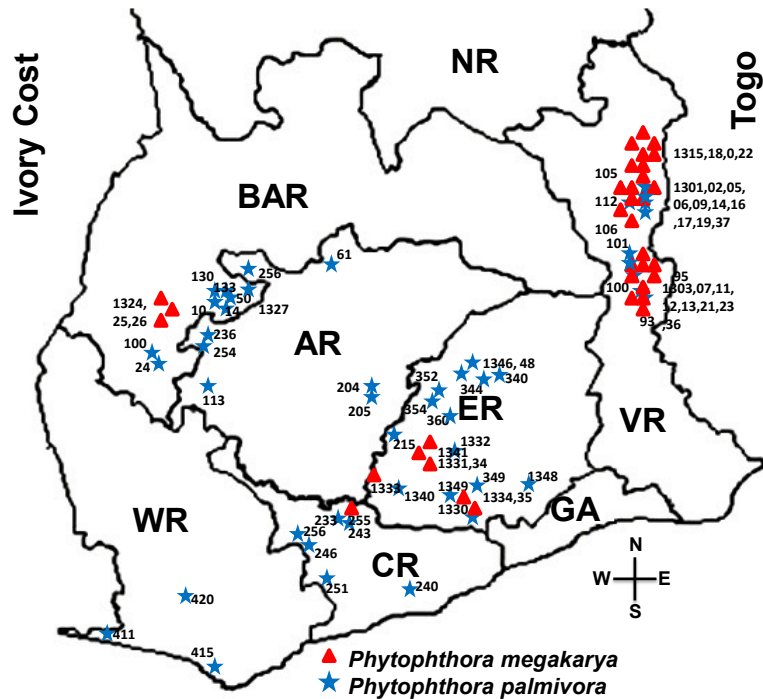






Breeders are working on a moving playing field where the pathogens they are trying to manage and the genetic diversity within those pathogens are always changing.

100um



We need to know the genetic diversity of all major pathogens of cacao in Colombia and the region relative to the areas where germplasm selection and breeding is being carried out. Imagine figures like those above detailing the diversity of *Moniliophthora* spp., *Phytophthora* spp., *Lasiodiplodia* spp., *Ceratocystis* spp., etc. overlaid onto the cacao genetic diversity being screened for resistance.



Thank You