

# *Fusarium circinatum* and *Ceratocystis eucalypticola* diagnostics in South Africa

Darryl Herron, Scion, New Zealand



# FABI

# ADSW 2023

9 - 11 MAY | CANBERRA





**Australian Government**

**Department of Agriculture,  
Fisheries and Forestry**

The National Plant Biosecurity Diagnostic and Surveillance Professional Development and Protocols Projects are coordinated and delivered by Plant Health Australia and are funded by the Department of Agriculture, Fisheries and Forestry.

The objectives of the Projects are to enhance and strengthen Australia's diagnostic and surveillance capacity and capability to identify priority plant pests that impact on plant industries, environment and the community.

**ADSW 2023**

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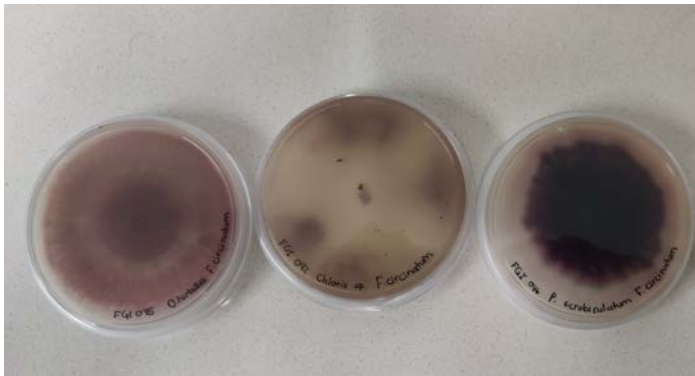
# *Fusarium circinatum* (Nirenberg & O'Donnell 1998)

*Fusarium moniliforme* var. *subglutinans* (Wollenw. & Reinking)

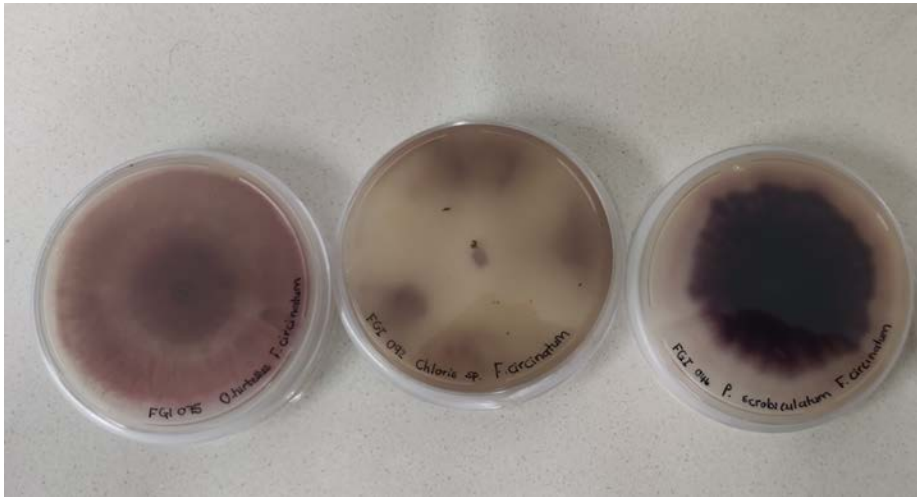
*Fusarium lateritium* f. sp. *pini* (Snyder et al.)

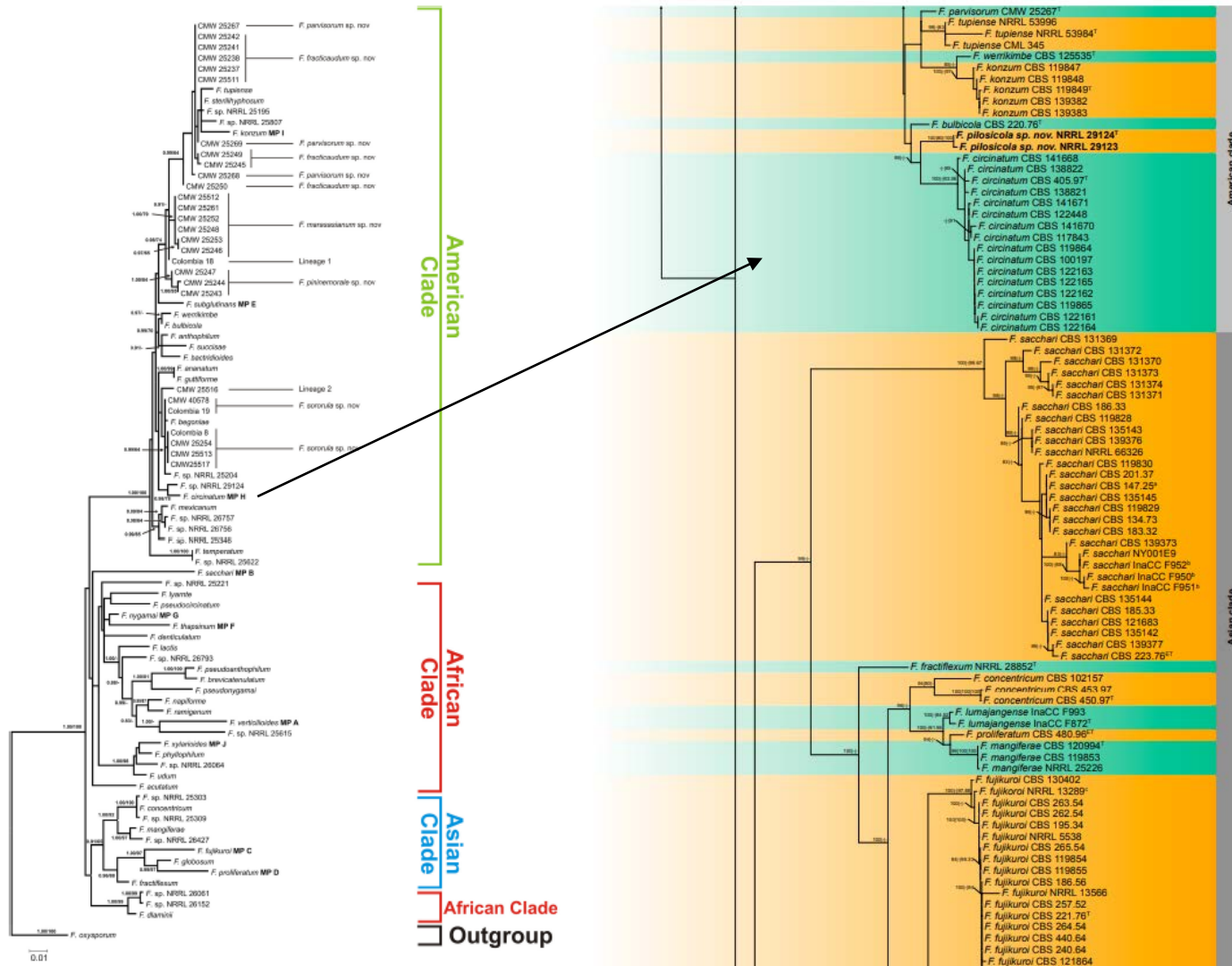
*Fusarium subglutinans* f. sp. *pini* (Correll et al.)

*Gibberella circinata* (Nirenberg & O'Donnell 1998)



# *Fusarium circinatum*





# *Fusarium fujikuroi* species complex +70 phylogenetic species

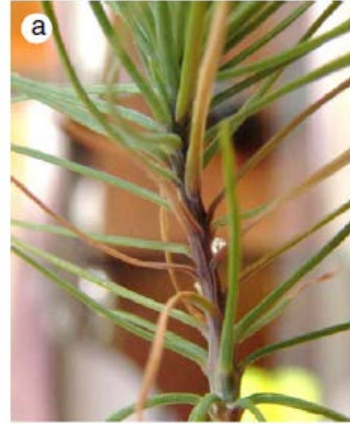
Article

*Fusarium mindanaoense* sp. nov., a New *Fusarium* Wilt Pathogen of Cavendish Banana from the Philippines Belonging to the *F. fujikuroi* Species Complex

Shunsuke Nozawa <sup>1</sup>, Yosuke Seto <sup>2</sup>, Yoshiki Takata <sup>1</sup>, Lalaine Albano Narreto <sup>3</sup>, Reynaldo R. Valle <sup>4</sup>, Keiju Okui <sup>5</sup>, Shigeya Taida <sup>5</sup>, Dionisio G. Alvindia <sup>6</sup>, Renato G. Reyes <sup>7</sup> and Kyoko Watanabe <sup>1,4,\*</sup>

Herron et al. 2015 *Studies in Mycology*; Yilmaz et al. 2021 *Persoonia*

# Fusarium wilt and pitch canker



Steenkamp et al. 2012 *Australasian Plant Pathology*



# Fusarium wilt and pitch canker

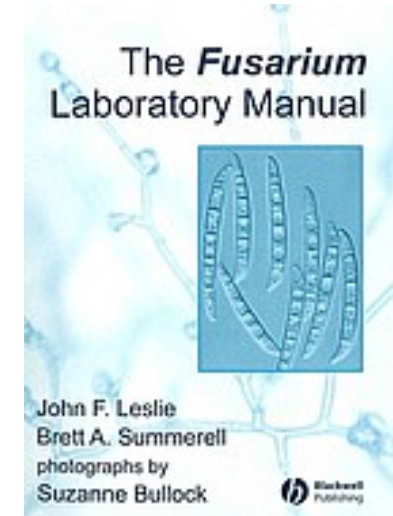




# Sample collection



# Sample processing



# Sample processing



FSM – Fusarium Selective Media

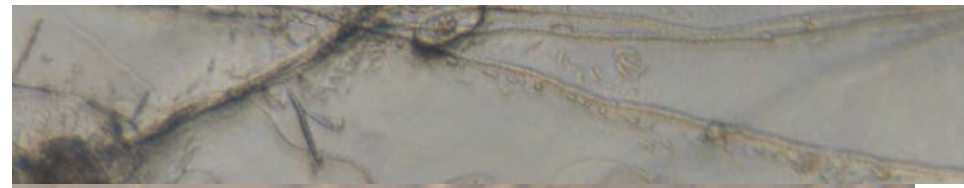
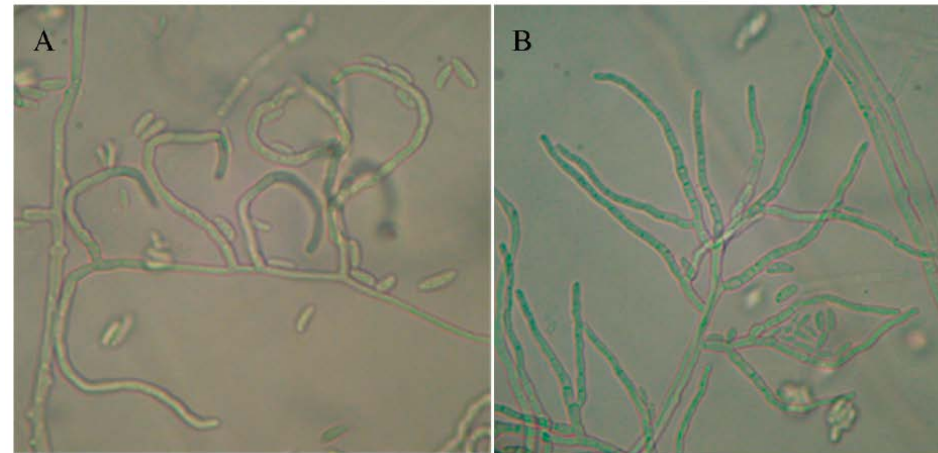
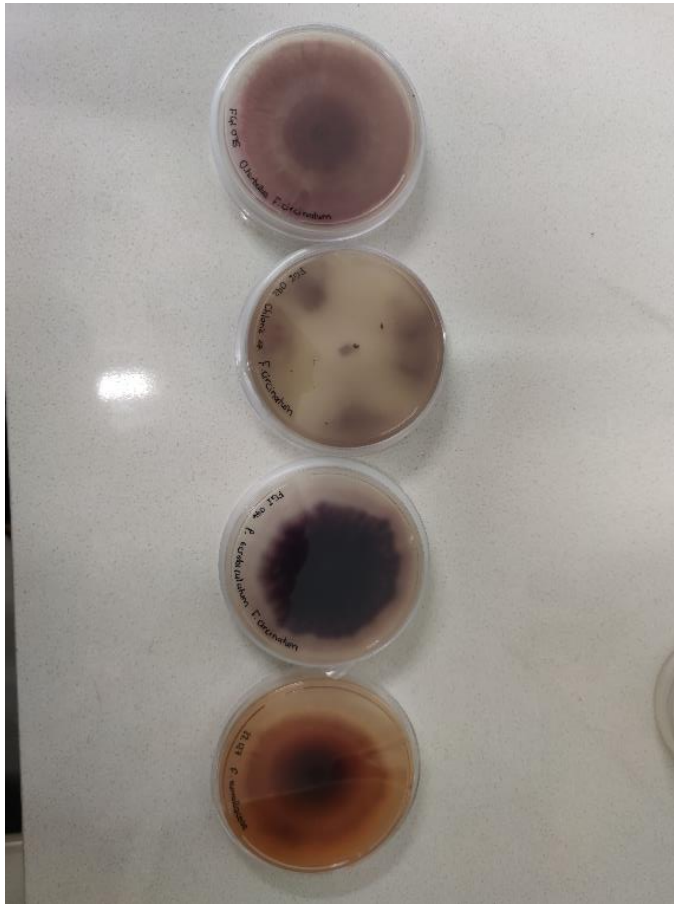
KCL – WA with KCL

PDA – Full and ½ strength

SNA – Spezieller Nährstoffarmer Agar

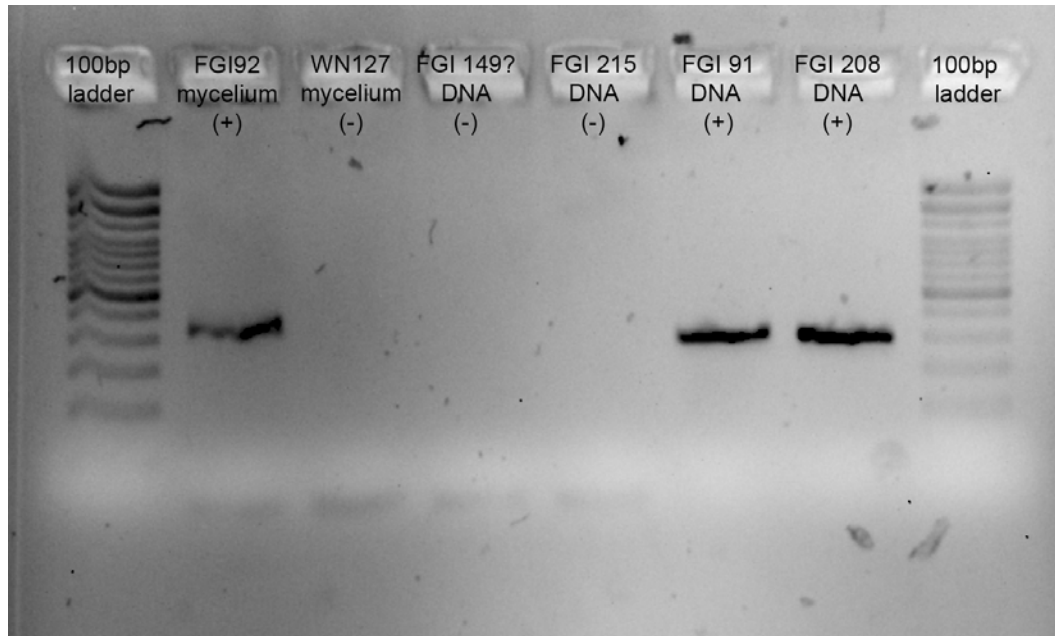
CLA – Carnation Leaf Agar

# Diagnosics – morphology



Mullet et al. 2017 *Forests*

# Diagnosics – molecular



Diagnostic end point PCR  
-CIRC1A / 4A (IGS)

Sanger sequencing

-EF1 (5' ATGGGTAAGGARGACAAGAC-3')

-EF2 (5'-GGARGTACCAGTSATCATGTT-3')

O'Donnell et al. 1998; Schweigkofler et al. 2004 *Applied and Environmental Microbiology*; loos et al. 2019 *Scientific reports*

# Diagnosics – molecular

Protocol	End point PCR		SYBR Green real-time PCR			Hydrolysis probe real-time PCR				Significance of the difference between protocols
	p1	p9	p4	p5	p6	p2	p3	p7	p8	
Number of laboratories involved	6	6	6	5	4	6	6	4	6	
Number of samples analyzed and retained	474	472	473	393	393	474	474	316	473	
Number of indeterminate results <sup>ab</sup>	2 A (2 AB)	4 A (2 AB)	7 A (4 AB)	6 A (0 A)	18 B (16 C)	5 A (1 AB)	5 A (4 AB)	3 A (0 AB)	9 AB (8 BC)	P < 0.001 (P < 0.001)
Negative Accord (NA) <sup>bc</sup>	181 (180)	183 (179)	187 (183)	154 (154)	146 (130)	171 (171)	192 (191)	128 (128)	163 (161)	—
Positive Accord (PA) <sup>bc</sup>	223 (221)	229 (231)	230 (227)	194 (189)	192 (191)	230 (226)	236 (232)	156 (153)	221 (220)	—
Negative Deviation (ND) <sup>bc</sup>	53 (55)	43 (44)	45 (48)	34 (40)	35 (37)	46 (50)	40 (44)	28 (31)	55 (56)	—
Positive Deviation (PD) <sup>bc</sup>	17 (18)	17 (19)	11 (15)	11 (11)	19 (35)	27 (27)	6 (7)	4 (4)	34 (37)	—
Diagnostic Sensitivity % (SE) <sup>bc</sup>	80.8 (80.1)	84.3 (83.6)	83.6 (82.5)	85.0 (82.8)	84.0 (83.6)	83.3 (81.9)	85.5 (84.1)	84.8 (83.2)	80.1 (79.7)	P = 0.71 (P = 0.88)
Diagnostic Specificity % (SP) <sup>bc</sup>	91.4 ABCD (90.9 AB)	92.4 ABCD (90.4 AB)	94.4 AC (92.4 AB)	93.3 ABC (93.3 AB)	88.5 ABD (78.8 C)	86.4 BD (86.4 AC)	97.0 C (96.6 B)	97.0 C (97.0 B)	82.6 D (81.6 C)	P < 0.001 (P < 0.001)
Diagnostic accuracy % (AC) <sup>bc</sup>	85.2 AB (84.6 ABC)	87.7 AB (86.4 AB)	88.2 A (86.7 AB)	88.5 A (87.3 ACD)	85.9 AB (81.6 ABC)	84.6 AB (83.8 ABC)	90.3 A (89.2 B)	89.9 A (88.9 D)	81.2 B (80.5 CD)	P = 0.02 (P < 0.001)

Ioos et al. 2019 *Scientific reports*

**Good enough** depending on your situation

Issues with cross-reactivity, sensitivity, inclusivity

-New and unknown closely related species

-Inconsistencies with detection of *Fusarium circinatum* from inoculated material (seed)

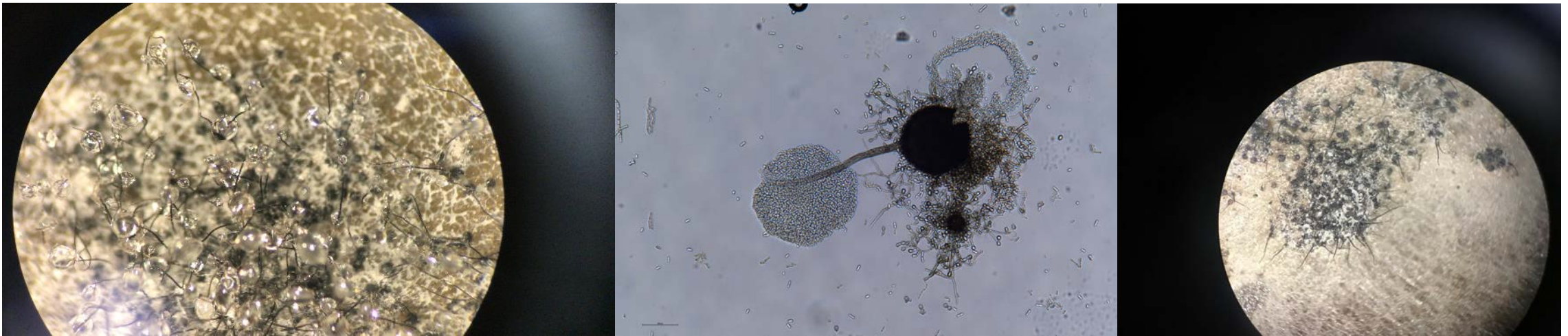
-Inconsistencies with different strains of *Fusarium circinatum*

-Panel of isolates to provide certainty and confidence

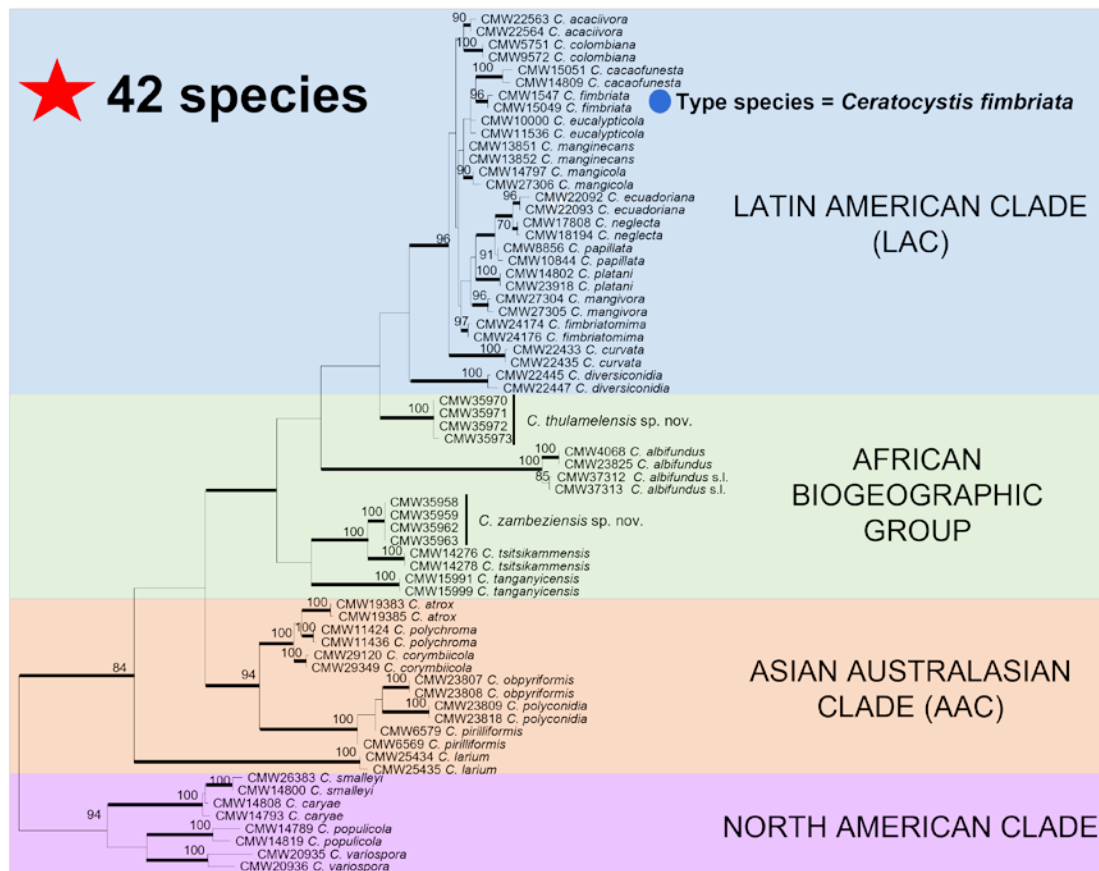
# *Ceratocystis eucalypticola* (van Wyk & Wingfield, 2012)

First described in South Africa from wounded *Eucalyptus* trees in 2012

First report of *Eucalyptus* GU tree mortality from *C. eucalypticola* in South Africa 2019/2020



# Ceratocystis eucalypticola

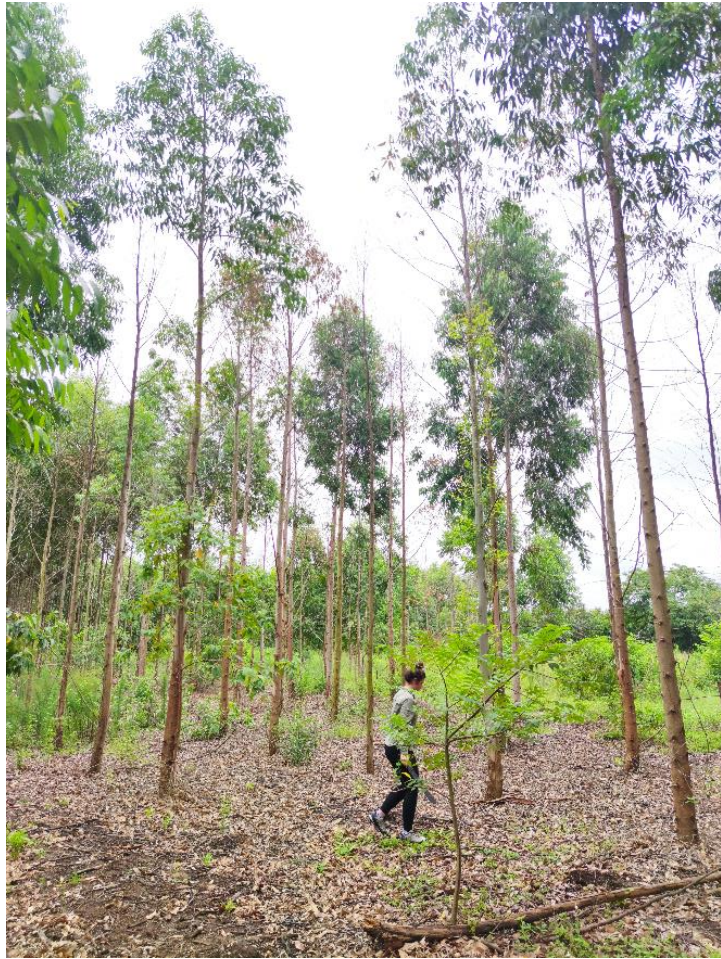


*Ceratocystis fimbriata*  
s. lato species complex  
Latin American Clade

Irene Barnes, FABI



# Ceratocystis wilt



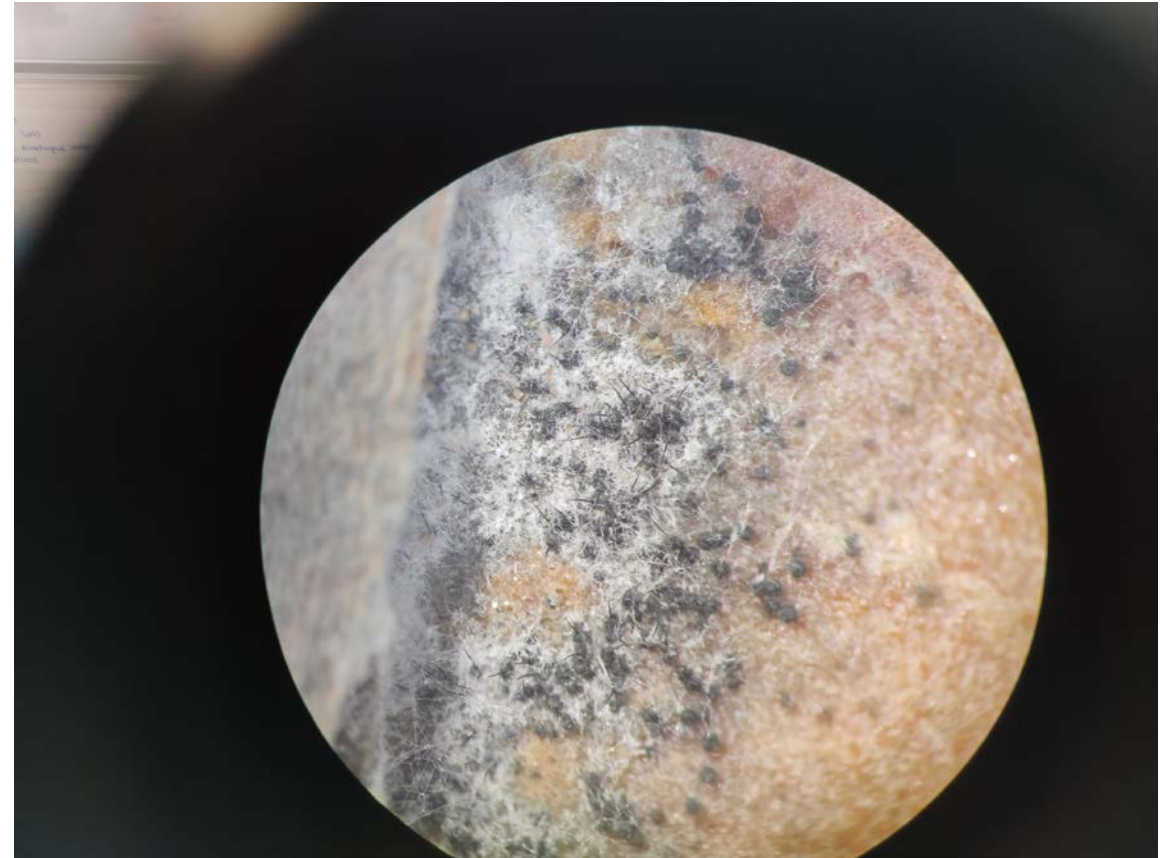
# Sample collection



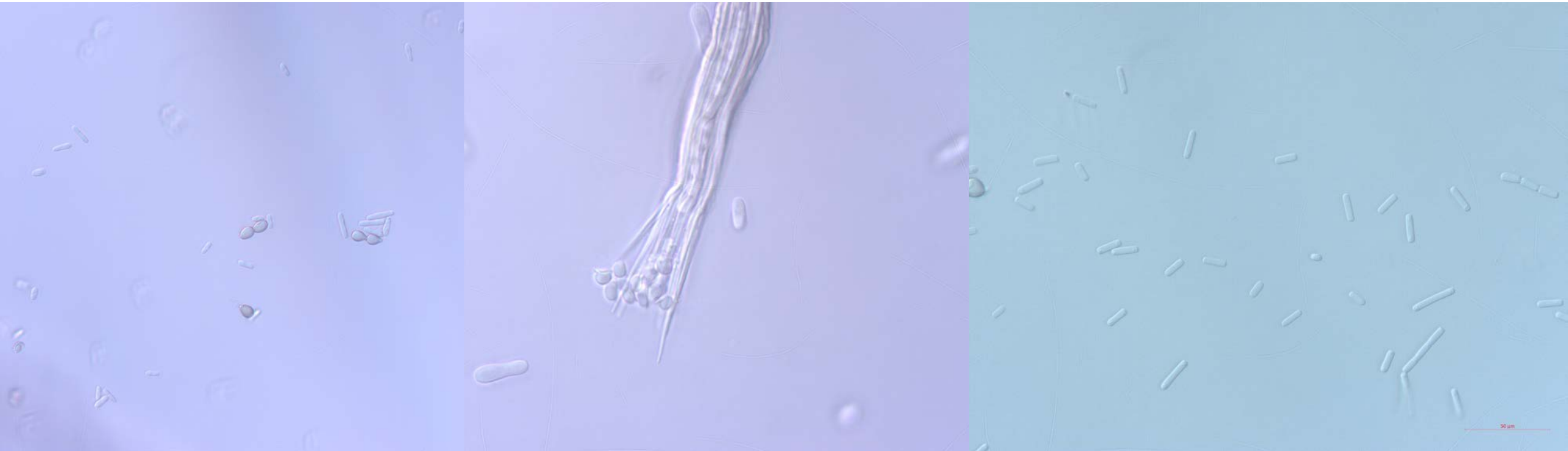
# Sample processing



# Diagnostics – morphology

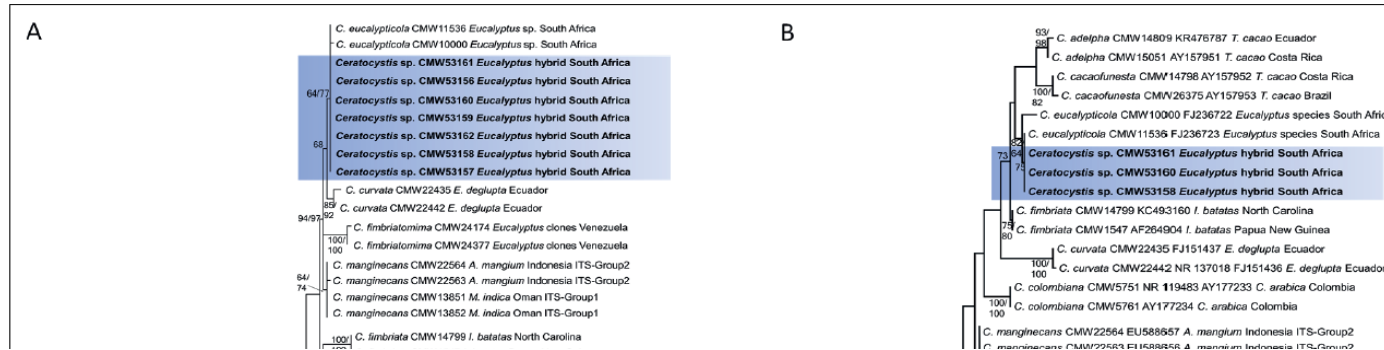


# Diagnosics – morphology



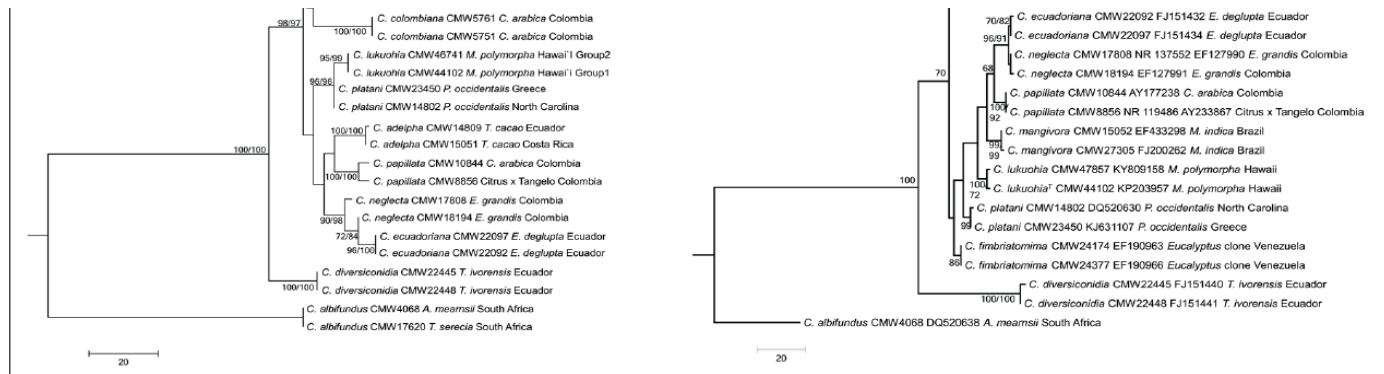
# Diagnosics – molecular

ITS, *bt1*, *ef1*,  
*ms204*, *rpb2*



ITS

RT-qPCR being developed at FABI to distinguish between species in LAC



Roux et al. 2020 *Southern Forests*

# Acknowledgements



Kira Lynn



Wilma Nel



Irene Barnes



Cassie Carstens (MTO)



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