



HAL
open science

Tache noire de l'ananas : déterminisme du processus infectieux par approches moléculaire et biochimique

Manon Vignassa

► **To cite this version:**

Manon Vignassa. Tache noire de l'ananas : déterminisme du processus infectieux par approches moléculaire et biochimique. Sciences agricoles. Université de la Réunion, 2021. Français. NNT : 2021LARE0013 . tel-03765217

HAL Id: tel-03765217

<https://theses.hal.science/tel-03765217v1>

Submitted on 31 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

UNIVERSITÉ DE LA RÉUNION

ÉCOLE DOCTORALE SCIENCES, TECHNOLOGIES ET SANTÉ

CIRAD – UNITÉ MIXTE DE RECHERCHE QUALISUD

THÈSE

Présentée à l'Université de La Réunion en vue de l'obtention du
grade de Docteur d'Université

Discipline : Biochimie et Biologie moléculaire

Spécialité : Biologie végétale

Manon VIGNASSA

Tache noire de l'ananas : Déterminisme du processus infectieux par approches moléculaire et biochimique

Sous la direction de Marc CHILLET et Sabine SCHORR-GALINDO

Soutenue le 08 juin 2021, devant le jury composé de:

| | | |
|-----------------------------------|---|--|
| Luc DE LAPEYRE DE BELLAIRE | Directeur de recherche, CIRAD | Rapporteur |
| Christian STEINBERG | Directeur de recherche, INRAE | Rapporteur et Président du jury |
| Elsa BALLINI | Maître de conférence, Montpellier SupAgro | Examinatrice |
| Isabelle FOCK-BASTIDE | Maître de conférence, Université de La Réunion | Examinatrice |
| Marc CHILLET | Directeur de recherche, CIRAD | Directeur de thèse |
| Sabine SCHORR-GALINDO | Professeur, Université de Montpellier | Co-directrice de thèse |
| Jean-Christophe MEILE | Chercheur, CIRAD | Co-encadrant de thèse |

LETTRÉ D'ENGAGEMENT DE NON-PLAGIAT

Je soussignée Manon Vignassa en ma qualité de doctorante de l'Université de La Réunion, déclare être consciente que le plagiat est un acte délictueux passible de sanctions disciplinaires. Aussi, dans le respect de la propriété intellectuelle et du droit d'auteur, je m'engage à systématiquement citer mes sources, quelle qu'en soit la forme (textes, images, audiovisuel, internet), dans le cadre de la rédaction de ma thèse et de toute autre production scientifique, sachant que l'établissement est susceptible de soumettre le texte de ma thèse à un logiciel anti-plagiat.

Fait à SAINT-PIERRE, le 08/05/2021

Signature :



Extrait du Règlement intérieur de l'Université de La Réunion
(validé par le Conseil d'Administration en date du 11 décembre 2014)

Article 9. Protection de la propriété intellectuelle – Faux et usage de faux, contrefaçon, plagiat

L'utilisation des ressources informatiques de l'Université implique le respect de ses droits de propriété intellectuelle ainsi que ceux de ses partenaires et plus généralement, de tous tiers titulaires de tels droits.

En conséquence, chaque utilisateur doit :

- utiliser les logiciels dans les conditions de licences souscrites ;
- ne pas reproduire, copier, diffuser, modifier ou utiliser des logiciels, bases de données, pages Web, textes, images, photographies ou autres créations protégées par le droit d'auteur ou un droit privatif, sans avoir obtenu préalablement l'autorisation des titulaires de ces droits.

La contrefaçon et le faux

Conformément aux dispositions du code de la propriété intellectuelle, toute représentation ou reproduction intégrale ou partielle d'une œuvre de l'esprit faite sans le consentement de son auteur est illicite et constitue un délit pénal.

L'article 444-1 du code pénal dispose : « Constitue un faux toute altération frauduleuse de la vérité, de nature à causer un préjudice et accomplie par quelque moyen que ce soit, dans un écrit ou tout autre support d'expression de la pensée qui a pour objet ou qui peut avoir pour effet d'établir la preuve d'un droit ou d'un fait ayant des conséquences juridiques ».

L'article L335_3 du code de la propriété intellectuelle précise que : « Est également un délit de contrefaçon toute reproduction, représentation ou diffusion, par quelque moyen que ce soit, d'une œuvre de l'esprit en violation des droits de l'auteur, tels qu'ils sont définis et réglementés par la loi. Est également un délit de contrefaçon la violation de l'un des droits de l'auteur d'un logiciel (...) ».

Le plagiat est constitué par la copie, totale ou partielle d'un travail réalisé par autrui, lorsque la source empruntée n'est pas citée, quel que soit le moyen utilisé. Le plagiat constitue une violation du droit d'auteur (au sens des articles L 335-2 et L 335-3 du code de la propriété intellectuelle). Il peut être assimilé à un délit de contrefaçon. C'est aussi une faute disciplinaire, susceptible d'entraîner une sanction.

Les sources et les références utilisées dans le cadre des travaux (préparations, devoirs, mémoires, thèses, rapports de stage...) doivent être clairement citées. Des citations intégrales peuvent figurer dans les documents rendus, si elles sont assorties de leur référence (nom d'auteur, publication, date, éditeur...) et identifiées comme telles par des guillemets ou des italiques.

Les délits de contrefaçon, de plagiat et d'usage de faux peuvent donner lieu à une sanction disciplinaire indépendante de la mise en œuvre de poursuites pénales.

TABLE DES MATIÈRES

| | |
|--|----|
| LISTE DES ABRÉVIATIONS | 4 |
| LISTE DES FIGURES..... | 7 |
| LISTE DES TABLEAUX | 9 |
| INTRODUCTION BIBLIOGRAPHIQUE | 10 |
| 1. Résistance et sensibilité, les concepts fondateurs de l'immunité des plantes | 11 |
| 1.1. Les défenses constitutives des plantes | 11 |
| 1.1.1. Défenses constitutives directes..... | 11 |
| 1.1.2. Défenses constitutives indirectes..... | 12 |
| 1.2. Perception de l'agent infectieux et initiation d'un dialogue moléculaire complexe | 13 |
| 1.3. Transduction des signaux de défense selon les voies de signalisation hormonale..... | 16 |
| 1.3.1. Synthèse des ROS | 16 |
| 1.3.2. Voie de l'acide salicylique..... | 17 |
| 1.3.3. Voie de l'acide jasmonique..... | 19 |
| 1.3.4. Voie de l'éthylène..... | 19 |
| 1.3.5. Autres phytohormones | 20 |
| 1.4. Les protéines PR | 21 |
| 1.4.1. Structures des protéines PR | 22 |
| 1.4.2. Diversité fonctionnelle des protéines PR | 22 |
| 1.5. Adaptation des agents infectieux et modulation des ajustements cellulaires de l'hôte .. | 24 |
| 1.5.1. Les effecteurs, un arsenal complexe au service de la pathogénicité | 24 |
| 1.5.2. Stratégies de modulation de l'immunité des plantes | 25 |
| 2. Les agents pathogènes fongiques | 27 |
| 2.1. Organisation des communautés fongiques et enjeux infectieux..... | 27 |
| 2.1.1. Co-occurrence d'espèces fongiques en fonction des habitats..... | 28 |
| 2.1.2. Caractérisation des flux d'inoculum | 30 |
| 2.1.2.1. Phase sexuée du genre <i>Fusarium</i> | 30 |
| 2.1.2.2. Phase sexuée du genre <i>Talaromyces</i> | 32 |
| 2.1.2.3. Phase asexuée des genres <i>Fusarium</i> et <i>Talaromyces</i> | 33 |
| A) Microconidies | 33 |
| B) Macroconidies | 34 |
| C) Chlamydoconidies | 34 |
| 2.1.2.4. Modes de dispersion dans l'environnement..... | 35 |
| 2.2. Régulation du métabolisme secondaire chez les champignons filamenteux | 36 |
| 2.2.1. Clusters de gènes de biosynthèse des mycotoxines (BGC) | 37 |

| | | |
|---|---|-----|
| 2.2.2. | Initiation des mécanismes de signalisation | 40 |
| 2.3. | Toxinogénicité, des potentiels en interaction | 41 |
| 2.3.1. | Mycotoxines impliquées dans les processus infectieux | 41 |
| 2.3.2. | Influence des interactions bactérie-champignon sur la biosynthèse de mycotoxines 42 | |
| 2.3.3. | Influence des interactions champignon-champignon sur la biosynthèse de mycotoxines | 43 |
| 3. | Maladie de la tache noire de l'ananas : un pathosystème pluripartite ?..... | 44 |
| 3.1. | Définition du pathosystème | 45 |
| 3.1.1. | L'ananas, un hôte singulier..... | 45 |
| 3.1.1.1. | Caractéristiques morphologiques | 45 |
| 3.1.1.2. | Cycle cultural | 47 |
| 3.1.2. | La mécanique de l'ombre des genres <i>Fusarium</i> et <i>Talaromyces</i> | 49 |
| 3.1.2.1. | Processus infectieux | 50 |
| 3.1.2.2. | Diversité des contaminants fongiques et distribution géographique..... | 51 |
| 3.2. | Déterminants de la sensibilité de l'ananas 'Queen Victoria' | 53 |
| 3.2.1. | Caractéristiques morpho-anatomiques..... | 53 |
| 3.2.2. | Accumulation de composés phénoliques..... | 55 |
| 3.2.3. | Influence des facteurs abiotiques | 56 |
| 4. | Problématique scientifique et objectifs de travail | 57 |
| CHAPITRE I Approche contexte-spécifique de l'épidémiologie de la tache noire à la Réunion..... | | 60 |
| Article 1 - Pineapple mycobiome related to Fruitlet Core Rot occurrence and the influence of fungal species dispersion patterns..... | | 61 |
| Supplementary data – Article 1..... | | 91 |
| CHAPITRE II Influence des interactions inter-pathogènes sur la croissance et la toxinogénèse | | 103 |
| Article 2 - Interactions between fungal pathogens of pineapple Fruitlet Core Rot modulate their mycotoxigenic potential | | 104 |
| Supplementary data – Article 2..... | | 124 |
| CHAPITRE III Réponses moléculaires précoces conditionnant la sensibilité de l'ananas 'Queen Victoria' à la tache noire | | 126 |
| Article 3 - Early genetic markers of 'Queen Victoria' pineapple susceptibility to Fruitlet Core Rot ... | | 127 |
| 1. | Introduction..... | 130 |
| 2. | Materials and Methods | 133 |
| 2.1. | Plant material and inoculation | 133 |
| 2.2. | Total RNA extraction | 134 |
| 2.3. | Primers design | 134 |
| 2.4. | Primers validation..... | 134 |

| | | |
|-----------------------------|---|-----|
| 2.4.1. | DNA Extraction | 134 |
| 2.4.2. | Polymerase Chain Reaction | 135 |
| 2.5. | Reference gene determination | 135 |
| 2.6. | cDNA synthesis and RT-qPCR assays | 137 |
| 2.7. | Computational analysis | 137 |
| 3. | Results | 138 |
| 3.1. | Correlations between gene expression and experimental conditions..... | 138 |
| 3.2. | Effects of cultivar and infection by <i>F. ananatum</i> on the genetic responses of host defenses | 140 |
| 3.3. | Shift of pineapple gene expression between 72- and 96-hours post-inoculation | 143 |
| 4. | Discussion | 148 |
| 4.1. | Contrasting constitutive expression levels between pineapple cultivars | 148 |
| 4.2. | <i>Fusarium ananatum</i> repressed host defenses until 72 hours post-inoculation | 150 |
| 4.3. | Shift of pineapple responses to <i>F. ananatum</i> and wounding between 72 and 96 hpi ... | 152 |
| 5. | Conclusion | 153 |
| 6. | References..... | 154 |
| | Supplementary data – Article 3..... | 162 |
| CHAPITRE IV | Discussion générale et perspectives | 166 |
| 1. | Distribution spatiale et détermination de nouveaux agents pathogènes | 167 |
| 2. | Influence des interactions inter-pathogènes sur la croissance et la toxinogénicité..... | 170 |
| 3. | Transduction et modulation des signaux de défense | 173 |
| 4. | Perspectives de recherche et d’application pour la filière de production..... | 175 |
| RÉFÉRENCES BIBLIOGRAPHIQUES | | 179 |
| CONTRIBUTION SCIENTIFIQUE | | 202 |
| ABSTRACT | | 203 |
| RÉSUMÉ | | 204 |

LISTE DES ABRÉVIATIONS

ABA: Abscisic Acid
Abm: Antibiotic biosynthesis monooxygenase
ADN: Acide Désoxyribonucléique
ALT: Altenuène
AME: Alternariol Monométhyl Ether
AOH: Alternariol
ARN: Acide Ribonucléique
ARR2: *Arabidopsis* Response Regulator 2
ATP: Adénosine Triphosphate
Avr: Avirulence
 a_w : Activité de l'eau
BA2H: Benzoic-Acid-2-Hydroxylase
BAK1: Brassinosteroid insensitive 1-Associated receptor Kinase 1
BALDH: Benzaldehyde Dehydrogenase
Bea: Beauvericine
BGC: Biosynthesis Gene Cluster
BIK1: *Botrytis*-Induced Kinase 1
BRF: Bois Raméal Fragmenté
C4H: Cinnamate 4-Hydroxylase
CAT: Catalase
CC: Coiled-Coil
Cmu1: Chorismate mutase 1
COI1: Coronatine Insensitive 1
CreA: Catabolite responsive elements A
CRN8: Crinkler 8
Cys: Cystéine
DGGE: Elctrophorèse sur gel en gradient dénaturant
DON: Déoxynivalénol
ECD: Extracellular domain
EGF: Epidermal Growth Factor
EIN: Ethylene-Insensitive
ERF: Ethylene-Responsive element binding Factor
ESYN1: Enniatin Synthase 1
ETI: Effector-Trigierred-Immunity
ETR1: Ethylene-Resistant 1
ETS: Effector-Trigierred-Susceptibility
FB: Fumonisines B
FCR: Fruitlet Core Rot
FFSC: *Fusarium fujikuroi* Species Complex
FHB: *Fusarium* Head Blight
FUG1: Fungal Unknown Gene 1
FUM: Fumonisines
G: Guanine nucleotide-binding protein
gpaB: G protein alpha subunit B

GPX: Glutathion Peroxydase
GTP: Guanosine Triphosphate
His: Histidine
HPLC: High Performance Liquid Chromatography
HR: Hypersensitive Response
IAA: Indole-3-Acetic Acid
ICS1: Isochorismate Synthase 1
IPL: Isochorismate Pyruvate Lyase
ITS: Internal Transcribed Spacer
JA: Jasmonic Acid
JA-Ile: Jasmonoyl-L-Isoleucine
JAZ: Jasmonate ZIM-domain protein
K: Kinase
LC/MS-MS: Liquid Chromatography tandem Mass-Spectrometry
LOD: Limit of Detection
LOQ: Limit of Quantification
LRR: Leucine-Rich Repeat
LSU: Large-Subunit
LysM: Lysin Motif
LZ: Leucine-Zipper
MAMP: Microbe-Associated Molecular Patterns
MLO: Mildew resistance Locus O
MON: Moniliformine
MTI: MAMP-Triggered Immunity
MYC: Myelocytomatosis oncogene
NAC: NAM, ATAF and CUC
nag1: N-acetyl- β -D-glucosaminidase 1
NBS: Nucleotide-Binding Site
NIS1: Necrosis-Inducing Secreted protein 1
NPR1: Nonexpressor of Pathogenesis-Related gene 1
OTA: Ochratoxine A
PacC: loss or reduction in Phosphatase Activity at ACid but not at alkaline pH
PAL: Phenylalanine Ammonia-Lyase
PAMP: Pathogen Associated Molecular Patterns
PCD: Programmed Cell Death
PCR: Polymerase Chain Reaction
PDA: Potato Dextrose Agar
PGIP: Polygalacturonase Inhibiting Proteins
PGPR: Plant Growth-Promoting Rhizobacteria
pH: potentiel Hydrogène
PR: Pathogenesis-Related
PRR: Pattern Recognition Receptors
PTFE: polytetrafluoroethylene
PTI: PAMPs-Trigerrred Immunity
R: Résistance
Redox: Réaction d'oxydoréduction
RLK: Receptor-Like Kinases

RLP: Receptor-Like Proteins
ROS: Reactive Oxygen Species
RT-qPCR: Real Time-quantitative Polymerase Chain Reaction
S: Sensibilité
SA: Salicylic Acid
SAR: Systemic Acquired Resistance
Se: Sépale
SET1: Su(var)3-9, Enhancer-of-zeste, Trithorax 1
Shy1: Salicylate hydroxylase 1
sid2: SA induction deficient-2
SOD: Superoxyde Dismutase
SSU: Small-Subunit
TEF-1 α : Translation Elongation Factor-1 α
TIF: Traitement d'Induction Florale
TIR: Toll-Interleukin-Receptor
TMV: Tobacco Mosaic Virus
Tri: Trichothécènes
UV: Ultraviolet
ZEA: Zéaralénone

LISTE DES FIGURES

- Figure 1.** Structure des parois cellulaires des cellules fongiques composées en partie interne de chitines et de glucanes interconnectés ainsi que d'une couche externe constituée d'un réseau de mannoprotéines riche en glycoprotéines (Geoghegan *et al.*, 2017). 14
- Figure 2.** Représentation schématique de la structure des RLKs et RLPs. Les LRR, LysM, EGF et lectines constituent les différents ectodomains (ECD) identifiés chez les PRRs. Les classes de ligands reconnues sont spécifiées pour chaque ectodomaine (d'après Betham *et al.*, 2020). RLK, Receptor-Like Kinase; RLP, Receptor-Like Protein; ECD, Extracellular domain; K, Kinase; LRR, Leucine-Rich Repeat; LysM, Lysin Motif; EGF, Epidermal Growth Factor..... 15
- Figure 3.** Biosynthèse de l'acide salicylique selon la voie des shikimate-phénylpropanoïdes. IPL, Isochorismate Pyruvate Lyase; PAL, Phenylalanine Ammonia-Lyase; BALDH, Benzaldehyde Dehydrogenase; C4H, Cinnamate 4-Hydroxylase; BA2H, Benzoic-Acid-2-Hydroxylase (Per *et al.*, 2017)..... 18
- Figure 4.** Organisation des principaux domaines protéiques constituant les protéines PR de type NBS-LRR. NBS, Nucleotide-Binding Site; LRR, Leucine Rich Repeat; TIR, Toll-Interleukin-Receptor; CC, Coiled-Coil; LZ, Leucine-Zipper. 22
- Figure 5.** Cycle de reproduction sexuée du genre *Fusarium* (Peraza-Reyes and Berteaux-Lecellier, 2013). 31
- Figure 6.** Cycle de reproduction sexuée du genre *Talaromyces* (www.biologydiscussion.com/fungi/penicillium-description-structure-and-reproduction). ... 32
- Figure 7.** Cycle de reproduction asexuée du genre *Talaromyces* analogue à celui des *Fusarium* (www.biologydiscussion.com/fungi/penicillium-description-structure-and-reproduction). ... 34
- Figure 8.** Symptôme caractéristique de la maladie de la tache noire observé sur le cultivar 'Queen Victoria' (photos : M. Vignassa)..... 45
- Figure 9.** Coupe longitudinale de fleur d'ananas (www.biologievegetale.be/morphologie-vegetale/angiospermes/angiospermes-organographie/fruits/)..... 46
- Figure 10.** Principales étapes de l'itinéraire technique établi pour la culture de l'ananas à la Réunion. BRF, Bois Raméal Fragmenté; (photos : P. Fournier, M. Darnaudery et M. Vignassa). 48
- Figure 11.** Coupe transversale de fruit simple présentant un brunissement de la zone intercarpellaire (photo : M. Vignassa). 51

Figure 12. Coupe longitudinale de cavité florale d'ananas 'Queen Victoria' (Barral *et al.*, 2019b). Les flèches noires indiquent les zones de fusion imparfaite entre les sépales; Se, sépale; Echelle = 1 cm. 53

Figure 13. Images d'autofluorescence de coupes transversales de fruits simples d'ananas visualisées par microscope à épifluorescence (Barral *et al.*, 2019b). (A) Cultivar résistant 'MD-2', (B) Cultivar sensible 'Queen Victoria' ; les flèches blanches indiquent la zone de fusion entre les loges carpellaires ; les cercles blancs désignent les nectaires..... 54

Figure 14. Principal component analysis (PCA) of pineapple fruit experimental conditions according to their mean ΔCt value. (A) Respective contribution of the genes of interest (variables). (B), (C) and (D) highlight the dispersion of data following cultivar, kinetics and inoculation modality, respectively. *NPR1*, Non-Expressor of Pathogenesis Related gene 1; *NPR3*, Non-Expressor of Pathogenesis Related gene 3; *PR-1*, Pathogenesis-Related gene 1; *PR-2*, Pathogenesis-Related gene 2; *COI1*, Coronatine-insensitive 1; *PR-3*, Pathogenesis-Related gene 3; *PR-12*, Pathogenesis-Related gene 12; *EIN3*, Ethylene Insensitive 3; hpi, hours post-inoculation..... 139

Figure 15. Fuzzy clustering based on principal component analysis (PCA). Conditions are show as the combination of pineapple fruits of cultivar 'MD-2' and 'Queen Victoria' (QV) considering no inoculation (Mock), sterile water inoculation (H₂O) or *F. ananatum* inoculation (fusa) and sampled at four kinetic times (24, 48, 72 and 96 hours post-inoculation). Black trajectories illustrate the time line for each condition of inoculation. 141

Figure 16. Relative expression of eight defense genes in two pineapple fruit cultivars 'MD-2' and 'Queen Victoria' (QV) characterized by a contrasted susceptibility to FCR in response to *Fusarium ananatum* and sterile water inoculations. Black dots with bars represent average relative expression levels ($\Delta\Delta Ct$) with asymptotic confidence intervals at 95% ($n = 24$). Differences in expression levels between 'MD-2' and 'Queen Victoria' cultivars were either significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) for each gene of interest. *NPR1*, Non-Expressor of Pathogenesis Related gene 1; *NPR3*, Non-Expressor of Pathogenesis Related gene 3; *PR-1*, Pathogenesis-Related gene 1; *PR-2*, Pathogenesis-Related gene 2; *COI1*, Coronatine-insensitive 1; *PR-3*, Pathogenesis-Related gene 3; *PR-12*, Pathogenesis-Related gene 12; *EIN3*, Ethylene Insensitive 3..... 142

Figure 17. Relative expression dynamics of four defense genes involved in salicylic acid signaling pathway during the early stages of pineapple fruit responses to *Fusarium ananatum* (*Fa*) or sterile water (H₂O) inoculation. Black dots with bars show mean relative expression level ($\Delta\Delta Ct$) with asymptotic confidence intervals at 95% ($n = 3$). Differences in expression following inoculation with *F. ananatum* or sterile water were either significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) for each target gene and each kinetic time. Letters show significant differences in average expression levels between the kinetic points for each condition of inoculation and each cultivar according to Tukey's multiple comparison test at $p < 0.05$. (A) and (B) *NPR1*, Non-Expressor of Pathogenesis Related gene 1; (C) and (D)

NPR3, Non-Expressor of Pathogenesis Related gene 3; (E) and (F) PR-1, Pathogenesis-Related gene 1; (G) and (H) PR-2, Pathogenesis-Related gene 2; QV, 'Queen Victoria' 145

Figure 18. Relative expression dynamics of four defense genes involved in jasmonic acid and ethylene signaling pathways during the early stages of pineapple fruit responses to *Fusarium ananatum* (*Fa*) or sterile water (H₂O) inoculation. Black dots with bars show mean relative expression level ($\Delta\Delta Ct$) with asymptotic confidence intervals at 95% ($n = 3$). Differences in expression following inoculation with *F. ananatum* or sterile water were either significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) for each target gene and each kinetic time. Letters show significant differences in average expression levels between the kinetic points for each condition of inoculation and each cultivar according to Tukey's multiple comparison test at $p < 0.05$. (A) and (B) *COI1, Coronatine-insensitive 1; (C) and (D) PR-3, Pathogenesis-Related gene 3; (E) and (F) PR-12, Pathogenesis-Related gene 12; (G) and (H) EIN3, Ethylene Insensitive 3; QV, 'Queen Victoria' 146*

LISTE DES TABLEAUX

| | |
|--|-----|
| Tableau 1. Familles de protéines PR identifiées (van Loon <i>et al.</i> , 2006)..... | 23 |
| Tableau 2. Fonctions des composantes du cluster de biosynthèse des trichothécènes (Proctor <i>et al.</i> , 2018)..... | 37 |
| Tableau 3. Fonctions des composantes du cluster de biosynthèse des fumonisines (Alexander <i>et al.</i> , 2009)..... | 38 |
| Tableau 4. Fonctions des composantes du cluster de biosynthèse de la beauvericine (Niehaus <i>et al.</i> , 2016)..... | 39 |
| Table 5. Primers used in this study. | 136 |

INTRODUCTION BIBLIOGRAPHIQUE

1. Résistance et sensibilité, les concepts fondateurs de l'immunité des plantes

Dans leur environnement, les plantes sont soumises à de nombreuses contraintes biotiques pouvant mener au développement de pathologies qui mettent en péril leur survie et/ou leur reproduction. Ces attaques sont causées par une grande diversité de ravageurs et d'agents pathogènes capables de coloniser les différents organes de leur hôte. Avec plus de 8 000 espèces caractérisées comme pathogènes, les champignons sont responsables d'une majeure partie des épidémies recensées et constituent dès lors, le groupe le plus impliqués dans les maladies des plantes devant les bactéries et les virus (Gladioux *et al.*, 2011). L'établissement d'une interaction hôte - pathogène est fondé sur des intérêts contraires qui induisent la mise en place de stratégies de défense pour la plante hôte et de contournement ou de manipulation pour le pathogène. Dans ce contexte de course à l'armement, la plante hôte dispose de défenses constitutives et induites.

1.1. Les défenses constitutives des plantes

Pour se protéger des phytopathogènes, les plantes possèdent des barrières morpho-anatomiques et biochimiques préformées. Ces défenses résultent du processus de coévolution et peuvent être considérées selon deux catégories.

1.1.1. Défenses constitutives directes

Les défenses constitutives directes font partie intégrante de la plante hôte et ne nécessitent donc pas d'induction par l'attaque d'un pathogène. Une première ligne de défense est caractérisée à l'échelle morpho-anatomique selon différentes stratégies. La présence d'une cuticule lisse et épaisse à la surface de l'épiderme supérieur permet notamment d'accroître la robustesse et la résistance des tissus (Ziv *et al.*, 2018). Des dépôts de cires, de phénols cristallisés et de silice peuvent également venir renforcer la structure épidermique du système foliaire (Karabourniotis *et al.*, 2020). A l'échelle cellulaire, le réseau constitué de microfibrilles de cellulose, d'hémicellulose et de pectines est densifié constituant une paroi cellulaire épaisse plus difficile à pénétrer par un pathogène (Pogorelko *et al.*, 2013). L'apposition d'épines ou de trichomes glandulaires contraint également l'accès aux tissus pour les ravageurs (insectes et herbivores) (Huchelmann *et al.*, 2020).

Ces stratégies de défense sont, selon les espèces considérées, observées seules ou en combinaison limitant ainsi les possibilités pour le pathogène de parvenir à l'initiation d'un processus infectieux.

Dans un second temps, les défenses constitutives sont considérées à l'échelle du métabolisme de la plante. Les métabolites secondaires impliqués sont communément distingués selon trois catégories : les composés phénoliques, les composés azotés et les terpènes (Freeman and Beattie, 2008). Ces composés influencent des paramètres tels que l'amertume, l'astringence ou encore la dureté de tissus et peuvent être fortement toxiques pour les bioagresseurs qui les consomment. A titre d'exemple, les alcaloïdes et les glycosides sont des composés présentant une forte amertume combinée à une toxicité élevée pour une majeure partie des bioagresseurs des plantes (Matsuura and Fett-Neto, 2015). Parallèlement, d'autres tactiques disruptives sont mises en place *via* la biosynthèse constitutive d'inhibiteurs de protéases. En se liant aux sites actifs de certaines protéases, les inhibiteurs parviennent à interférer dans les processus digestifs des phytophages allant jusqu'à causer des retards de croissance et de développement (Kim *et al.*, 2009). Les parois végétales peuvent également être constituées d'inhibiteurs d'endo-polygalacturonases (PGIP - Polygalacturonase Inhibiting Proteins) qui provoquent chez les champignons pathogènes, la synthèse d'oligomères pectiques de grande taille aisément reconnus par la plante lui permettant ainsi d'initier ses mécanismes de défenses (Sarrocco *et al.*, 2017).

1.1.2. Défenses constitutives indirectes

Chez les plantes, des stratégies de défenses basées sur des relations de mutualisme ou de symbiose sont définies comme constitutives indirectes. Les espèces végétales représentent une niche écologique favorisant l'accès aux nutriments pour les prédateurs d'agents pathogènes. Ce type d'interaction est notamment observé chez les fourmis vivant en mutualisme avec les acacias (*Vachellia drepanolobium*) et bâtissant leurs fourmilières sur les tiges et dans les tubercules (Palmer *et al.*, 2017). Elles représentent un allié de taille pour la plante grâce à leurs attaques envers des herbivores tels que les chenilles. Réciproquement, cette symbiose est également favorable aux fourmis qui disposent du nectar produit au niveau des nectaires.

Les interactions de symbiose telles que celles observées avec les rhizobactéries favorisant la croissance des plantes (PGPR – Plant Growth-Promoting Rhizobacteria) font également l’objet de recherches accrues pour leur potentiel de biocontrôle (Liu *et al.*, 2017; Shameer and Prasad, 2018). En effet, la sécrétion de sidérophores par des bactéries appartenant aux genres *Rhizobium* et *Pseudomonas* induit la réduction de la disponibilité en fer présent dans la rhizosphère. Il s’instaure donc une compétition pour l’accessibilité aux nutriments parmi le microbiote racinaire (Weller, 2007). Les sidérophores des PGPR possèdent cependant une affinité plus forte que les chélateurs libérés par les autres microorganismes (Ahmed and Holmström, 2014). De cette manière, le développement de pathogènes fongiques est limité tandis que la croissance de la plante est favorisée en partie *via* la restriction des pathologies pouvant impacter son système racinaire (Verma *et al.*, 2011).

1.2. Perception de l’agent infectieux et initiation d’un dialogue moléculaire complexe

La reconnaissance immunitaire est une condition essentielle pour la structuration des défenses de l’hôte. Les agents pathogènes sont le plus souvent détectés grâce à des motifs moléculaires très conservés nommés PAMPs (Pathogen Associated Molecular Patterns). Chez les pathogènes fongiques, les PAMPs les plus largement décrits sont les glucanes, la chitine et les xylanases (Nürnberg and Kemmerling, 2009). Les glucanes représentent 50 à 60% des parois cellulaires et sont essentiellement constitués de liaisons de types β -1,3- puis β -1,6- ainsi que β -1,4- en de plus faibles proportions (Kang *et al.*, 2018). La chitine est quant à elle constituée de chaînes de résidus de type N-acétylglucosamine s’apparentant à des microfibrilles. Ces dernières constituent un réseau et sont liées de façon covalente aux β - 1,3- glucanes conférant aux parois fongiques la souplesse et la robustesse nécessaires pour pallier les stress d’ordres biotiques ou abiotiques (Figure 1 ; Gow *et al.*, 2017). Malgré son rôle prépondérant dans la structuration des parois, la chitine reste un composant minoritaire (Bowman and Free, 2006). Conjointement, les xylanases représentent à la fois une arme et une faiblesse pour le pathogène. Son activité hydrolytique permet la dégradation de l’hémicellulose constituant les parois des cellules végétales et facilite ainsi la pénétration du pathogène dans les tissus hôtes (Paccanaro *et al.*, 2017).

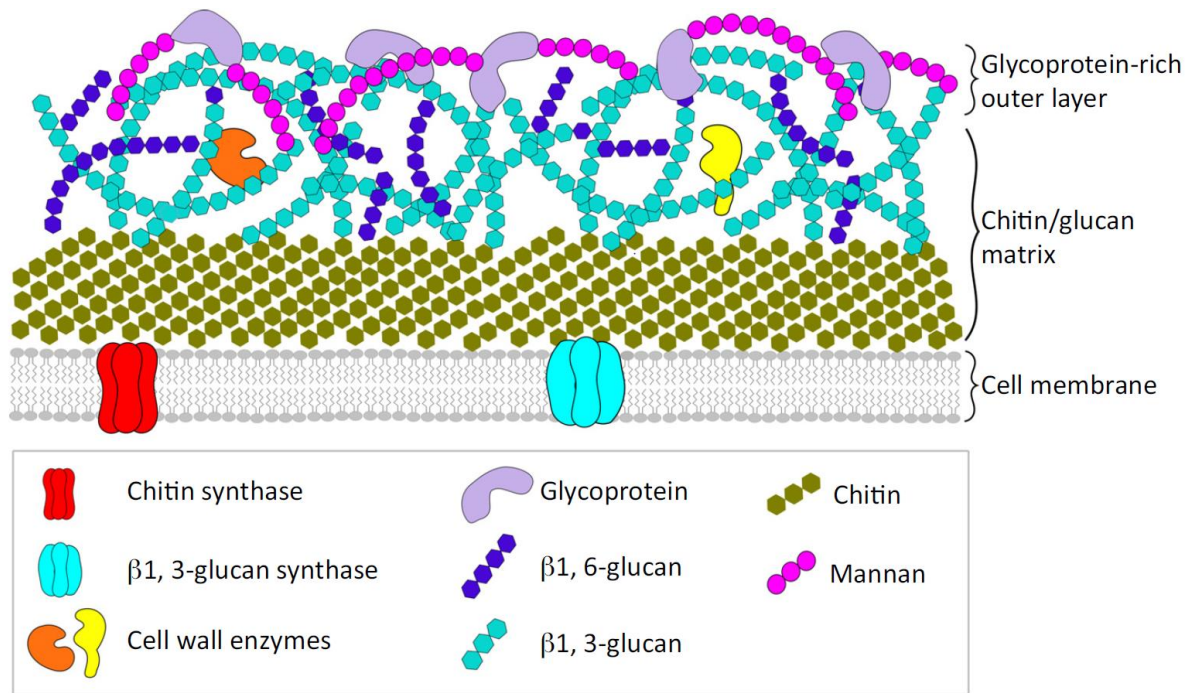


Figure 1. Structure des parois cellulaires des cellules fongiques composées en partie interne de chitines et de glucanes interconnectés ainsi que d’une couche externe constituée d’un réseau de mannoprotéines riche en glycoprotéines (Geoghegan *et al.*, 2017).

La reconnaissance extracellulaire de ces molécules ‘signal’ d’origine fongique est effectuée par des PRRs (Pattern Recognition Receptors) classifiés selon deux groupes distincts, les RLKs (Receptor-Like Kinases) possédant un domaine kinase intracellulaire et les RLPs (Receptor-Like Proteins) ne comportant pas de domaine kinase (Wang and Chai, 2020). Les ectodomains (ECD) des récepteurs PRRs présentent des motifs différenciés qui déterminent le type de ligand reconnu et activent ainsi la transduction des signaux d’alerte (Figure 2). La structure des PRRs peut ainsi être établie selon des domaines extracellulaires de types LRR (Leucine-Rich Repeat), des motifs analogues aux EGF (Epidermal Growth Factor), des domaines LysM (Lysin Motif) ou lectines (Saijo *et al.*, 2018 ; Lu and Tsuda, 2021).

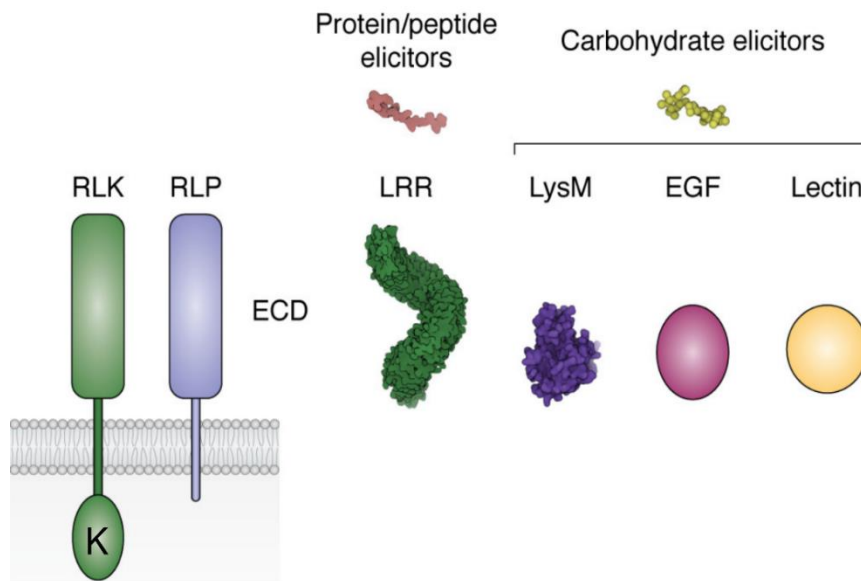


Figure 2. Représentation schématique de la structure des RLKs et RLPs. Les LRR, LysM, EGF et lectines constituent les différents ectodomaines (ECD) identifiés chez les PRRs. Les classes de ligands reconnues sont spécifiées pour chaque ectodomaine (d'après Betham *et al.*, 2020). RLK, Receptor-Like Kinase; RLP, Receptor-Like Protein; ECD, Extracellular domain; K, Kinase; LRR, Leucine-Rich Repeat; LysM, Lysin Motif; EGF, Epidermal Growth Factor.

La première phase de défense active nommée PTI (PAMPs-Triggered Immunity) est alors initiée (Zipfel, 2009). Cette étape va permettre la restriction spatiale du pathogène pour tenter d'inhiber la colonisation des tissus hôtes. Selon ces conditions, la plante peut alors être considérée comme résistante. Cet état est communément associé à une réponse hypersensible (HR) qui se définit par l'apparition de nécroses localisées au niveau de la zone de contact primaire avec le pathogène (Coll *et al.*, 2011). Cette stratégie va alors permettre de circonscrire l'infection. La résistance peut être définie selon deux classes. Elle est dite non-hôte lorsqu'une espèce végétale est en mesure de résister à l'ensemble des souches d'une même espèce d'agent pathogène, tandis que la résistance spécifique caractérise une espèce végétale qui n'est capable de résister qu'à une souche particulière d'agent pathogène (Lee *et al.*, 2017 ; Periyannan *et al.*, 2017). Selon ces possibilités d'interactions s'articulant autour d'un même hôte, le basculement vers un « état » résistant ou sensible va alors dépendre des souches d'un pathogène. Dans ces conditions, la résistance est alors décrite comme génotype dépendante (Ashraf *et al.*, 2009 ; Mina *et al.*, 2020). Ces éléments constituent les fondements du modèle théorique de la relation gène pour gène établi par Flor : à un gène de résistance spécifique (*R*) de la plante hôte correspond un gène d'avirulence (*Avr*) présent chez l'agent infectieux (Flor, 1971). Ainsi, la reconnaissance *Avr/R* mène à une relation d'incompatibilité et

de facto, à la résistance de la plante. A l'inverse, pour toute autre combinaison, la relation est dite compatible et conduit au développement de la maladie.

1.3. Transduction des signaux de défense selon les voies de signalisation hormonale

1.3.1. Synthèse des ROS

Les pathogènes fongiques parviennent dans de rares cas à contourner les structures de défenses constitutivement exprimées. La plante va alors percevoir l'agent infectieux et mettre en place une nouvelle stratégie de défense visant à contrer la colonisation de ses tissus par le pathogène. Cette reconnaissance va alors amorcer chez la plante hôte de nombreuses réponses cellulaires régulées dans le temps et dans l'espace.

Lors des étapes les plus précoces, l'activation des RLKs conduit à la production abondante de molécules toxiques et se traduit par l'établissement d'un burst-oxydatif (Kimura *et al.*, 2017). Ce phénomène provoque d'importantes modifications de la physiologie cellulaire *via* l'accumulation d'espèces réactives de l'oxygène (ROS). Une accumulation d'ions superoxydes (O_2^-), de peroxyde d'hydrogène (H_2O_2) et de radicaux hydroxyles (OH) est notamment observée au niveau de la zone de contact primaire. Un rôle supposé de senseur des processus redox est attribué à ces récepteurs. Toutefois, le mécanisme de ces interactions n'est encore que partiellement connu. L'accumulation des ROS va alors permettre l'initiation d'une signalisation moléculaire complexe menant à l'établissement de la HR ainsi qu'à la mort cellulaire programmée (PCD – Programmed Cell Death) (del Rio, 2015). Les potentiels de biosynthèse du H_2O_2 ainsi que ses capacités de diffusion au travers des membranes suggèrent qu'il pourrait constituer un messager secondaire (Torres *et al.*, 2006). Ainsi, le burst-oxydatif provoque un épaissement des parois cellulaires et l'induction par le H_2O_2 (impliqué dans les voies de signalisation longues distances) de l'expression de facteurs géniques caractérisés comme sensibles aux variations du potentiel redox (Kovacs *et al.*, 2015 ; Niu and Liao, 2016). L'homéostasie intracellulaire est assurée par le biais d'enzymes et de molécules antioxydantes. Les mécanismes enzymatiques permettant le maintien de la balance redox sont fondés sur l'activité de la superoxyde dismutase (SOD), de la glutathion peroxydase (GPX), de catalases (CAT) et de la peroxirédoxine (Apel and Hirt, 2004 ; Nantapong *et al.*, 2019). Conjointement, les composés antioxydants les plus abondants dans les cellules hôtes sont l'acide ascorbique et le glutathion sous sa forme oxydée (Foyer and Noctor, 2011).

L'association des voies de signalisation des ROS et de l'acide salicylique (SA) est indispensable à l'établissement de la SAR (Systemic Acquired Resistance) (Durrant and Dong, 2004).

1.3.2. Voie de l'acide salicylique

Les mécanismes de signalisation sont régis par la capacité d'une plante à percevoir puis interpréter les signaux exogènes ou endogènes détectés à l'échelle cellulaire. Ces signaux doivent ensuite être décodés pour permettre l'activation des programmes génétiques menant à l'établissement de réponses physiologiques en adéquation avec les contraintes environnementales. Chez les végétaux, de nombreuses molécules assurent ces fonctions de messagers intercellulaires et peuvent être décrites en tant que molécules 'signal'. Dans le contexte d'une réponse à une attaque pathogène, les phytohormones jouent un rôle essentiel dans la conversion du signal externe en réponse cellulaire.

L'acide salicylique est un composé phénolique présent sous une forme libre ou conjuguée. Sa biosynthèse est induite lors d'une infection par des champignons pathogènes de types biotrophes ou hémibiotrophes. Chez les monocotylédones, l'accumulation basale de SA est plus élevée que chez les dicotylédones, les rendant insensibles à un apport exogène de SA (Umemura *et al.*, 2009). Des études menées sur le riz (*Oryza sativa* spp. *japonica*) suggèrent que le SA endogène protège la plante du burst-oxydatif en modulant la balance redox (Yang *et al.*, 2004). Les mécanismes de biosynthèse dérivent de la voie des shikimate-phénylpropanoïdes selon deux processus distincts (Figure 3). Par la β -oxydation et l'hydroxylation de l'acide trans-cinnamique issu de la conversion de la phénylalanine ou *via* la conversion de l'isochorismate (Boatwright and Pajerowska-Mukhtar, 2013). Cette seconde voie de synthèse est préférentiellement activée pour l'accumulation du SA et l'établissement de la SAR en cas d'attaque pathogène. En effet, chez *Arabidopsis thaliana*, des mutants *sid2* (*SA induction deficient-2*) porteurs de mutations sur le gène *ICS1* (*Isochorismate Synthase 1*) possèdent des potentiels d'accumulation représentant 5 à 10% des capacités totales des plants sauvages ainsi qu'une déficience dans la mise en place de la SAR (Nawrath and Métraux, 1999 ; Wildermuth *et al.*, 2001). De telles incapacités dans la synthèse et l'accumulation du SA entraînent une augmentation des niveaux de sensibilité face à certains pathogènes.

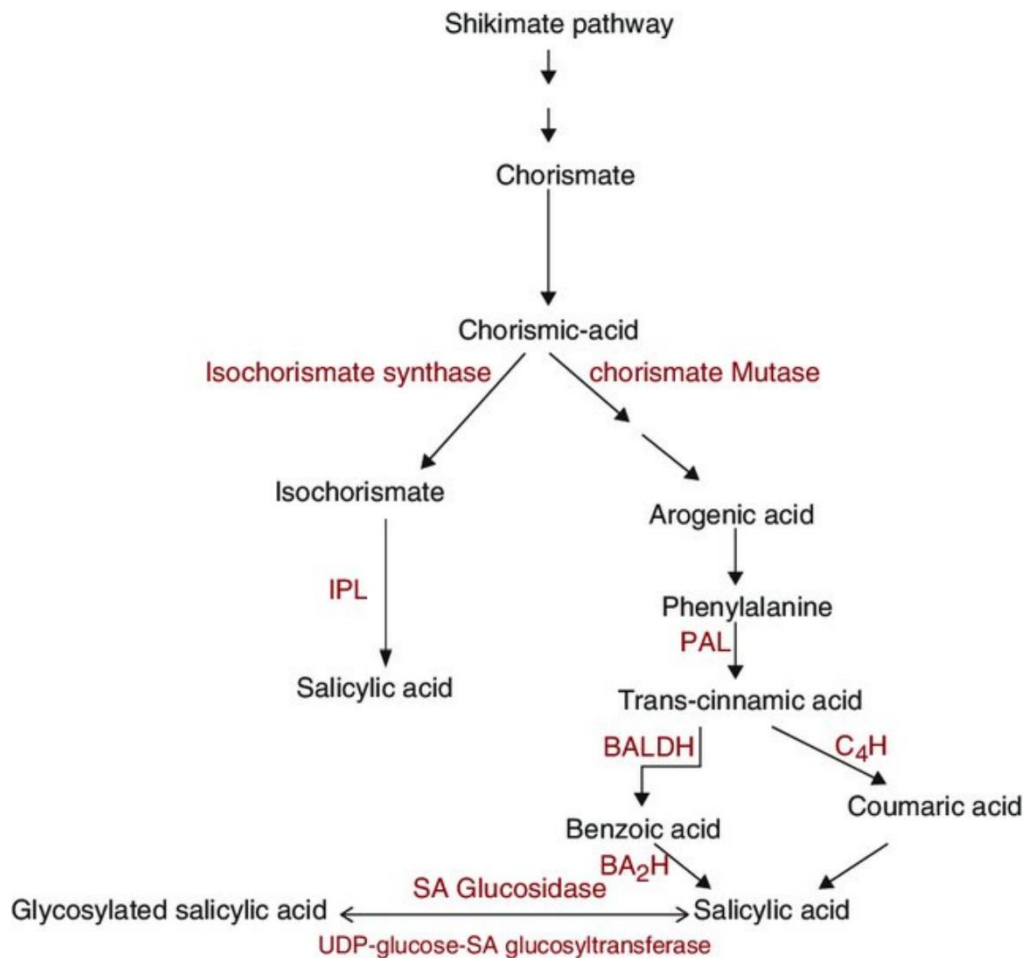


Figure 3. Biosynthèse de l'acide salicylique selon la voie des shikimate-phénylpropanoïdes. IPL, Isochorismate Pyruvate Lyase; PAL, Phenylalanine Ammonia-Lyase; BALDH, Benzaldehyde Dehydrogenase; C4H, Cinnamate 4-Hydroxylase; BA2H, Benzoic-Acid-2-Hydroxylase (Per *et al.*, 2017).

La transduction des signaux du SA engendre des modifications post-traductionnelles de facteurs géniques clés menant à l'établissement des réponses de défenses systémiques. *NPR1* (*Non-expressor of Pathogenesis-Related gene 1*) possède un rôle important dans ces réactions en cascade. Sa translocation vers le noyau active de manière indirecte plusieurs gènes *R* (Backer *et al.*, 2019). Bien qu'étant activé par la voie de l'acide salicylique, celui-ci intervient également dans la régulation des voies de l'acide jasmonique et de l'éthylène. En effet, l'inter-régulation des voies de signalisation par antagonisme ou synergie permet une modulation fine des réponses de défense (Salzman *et al.*, 2005 ; Li *et al.*, 2019).

1.3.3. Voie de l'acide jasmonique

Sous sa forme naturelle, l'acide jasmonique (JA) possède un cycle cyclopentanone associé à deux chaînes latérales α (acide carboxylique) et ω (chaîne pentényle). Des modifications peuvent intervenir sur ces structures constituant une famille de composés naturels regroupés sous le nom de jasmonates. Largement décrit chez les plantes pour son rôle de phytohormone impliquée dans la croissance et le développement, le JA fait également partie des substances engagées dans l'établissement de la résistance. A l'inverse du SA, le JA est synthétisé et accumulé lors d'une attaque par un champignon pathogène nécrotrophe ou hémibiotrophe. Sa synthèse est initiée au sein des chloroplastes à partir d'acides gras insaturés puis est suivie par la conversion de composés intermédiaires en JA dans les peroxyosomes. Le JA est ensuite métabolisé dans le cytoplasme en sa forme active le jasmonoyl-L-Isoleucine (JA-Ile) ainsi qu'en méthyl-jasmonate. La translocation du JA-Ile vers le noyau permet l'activation du gène *COI1* (*Coronatine Insensitive 1*) et entraîne la dégradation des protéines JAZ (Jasmonate ZIM-domain proteins) (Sheard *et al.*, 2010). La levée du pouvoir répresseur des JAZ permet la libération de facteurs de transcription de types *WRKYs*, *MYCs* (*Myelocytomatosis oncogenes*), *ERFs* (*Ethylene-Responsive element binding Factor*) et *NACs* (*NAM*, *ATAF* and *CUC*), indispensables à la régulation des gènes *R* (Fu and Dong, 2013). La signalisation du JA peut s'effectuer selon deux échelles. Tout d'abord une transduction opérant sur de courtes distances et restreinte aux zones adjacentes au site d'infection primaire (Ruan *et al.*, 2019). Puis, une transduction sur de plus longues distances grâce au système vasculaire permettant ainsi la mise en place de la SAR (Ryan and Moura, 2002 ; Heil and Ton, 2008).

1.3.4. Voie de l'éthylène

Conjointement à la transduction de signaux par le JA, la voie de signalisation de l'éthylène est également impliquée dans le cadre de maladies cryptogamiques causées par des pathogènes nécrotrophes ou hémibiotrophes.

Les mécanismes de biosynthèse de l'éthylène, établis d'après le cycle de Yang, sont initiés à partir de la méthionine et suivent un enchaînement de conversions enzymatiques (Rzewuski and Sauter, 2008). En l'absence d'éthylène, la transduction de signaux est inhibée par l'activité kinase du récepteur ETR1 (Ethylene-Resistant 1) localisé sur la membrane du réticulum

endoplasmique. Suivant son accumulation, l'éthylène est alors perçu par ETR1 levant son potentiel inhibiteur et permettant la déphosphorylation de la protéine transmembranaire EIN2 (Ethylene-Insensitive 2) (Bisson *et al.*, 2009). La partie C-terminale portée par EIN2 va alors être clivée puis transloquée vers le noyau (Qiao *et al.*, 2012). La perception de ce signal par le facteur de transcription *EIN3* (*Ethylene-Insensitive 3*) permet l'activation de l'expression de facteurs géniques sous-jacents menant *in fine* à l'induction des gènes de défense de la voie de signalisation de l'éthylène.

La mise en œuvre de mécanismes de défense nécessite une régulation fine dans le temps et dans l'espace. Des modulations croisées s'opèrent entre les voies du SA, JA et de l'éthylène suivant des patterns d'antagonisme ou de synergie. Il a été montré que l'activation de la voie du SA consécutive à une primo-infection d'*Arabidopsis thaliana* par le pathogène hémibiotrophe *Pseudomonas syringae* entraîne par la suite des niveaux de sensibilité plus élevés aux infections causées par *Alternaria brassicae* qui nécessitent, de par son profil nécrotrophe, la mise en place des voies du JA et de l'éthylène alors réprimées (Spoel *et al.*, 2007). A l'inverse, les voies du JA et de l'éthylène peuvent également restreindre les défenses induites par le SA. Des mutations du gène *COI1* et de certains facteurs *MYCs* ont permis d'observer chez *A. thaliana* une accumulation du SA plus importante menant à des niveaux de résistance plus élevés suite à une infection par *Pseudomonas syringae* (Spoel and Dong, 2008). Toutefois, ces potentiels de co-régulation restent en grande partie méconnus malgré leur rôle prépondérant dans la plupart des interactions plante – pathogène.

1.3.5. Autres phytohormones

Parallèlement aux voies préalablement exposées, des phytohormones telles que l'acide abscissique (ABA), l'auxine ou acide indole-3-acétique (IAA), les cytokinines, les acides gibbérelliques ainsi que les brassinostéroïdes peuvent également être impliquées dans les processus de défenses. L'ABA induit par exemple l'apposition de callose au site d'infection. Ce processus permet de limiter la colonisation des tissus hôtes par le pathogène ainsi que la ponction des nutriments présents dans le milieu intracellulaire (Oide *et al.*, 2013). Cependant, l'ABA présente des interactions antagonistes avec les processus de biosynthèse du JA et de l'éthylène entraînant une inhibition des défenses induites et par conséquent, une augmentation de la sensibilité de l'hôte (LeNoble *et al.*, 2004 ; Checker *et al.*, 2018). Autre exemple, les cytokinines sont décrites pour leur capacité d'induction de la synthèse et de

l'accumulation du SA. Notamment, le facteur *ARR2* (*Arabidopsis Response Regulator 2*) impliqué dans la voie des cytokinines, est nécessaire à la régulation de l'activité du facteur *NPR1* (O'Brien and Benková, 2013). Les cytokinines ont également été caractérisées pour leur pouvoir inducteur sur la synthèse *de novo* des phytoalexines, composés directement dirigés vers l'agent pathogène (Großkinsky *et al.*, 2011). Lors d'une attaque par un agent pathogène, certains diterpènes tels que les acides gibbérelliques interviennent dans la modulation des signaux moléculaires menant à l'établissement des réponses de défense. Il est établi que la liaison entre les acides gibbérelliques et les protéines DELLA (aspartate – glutamate – leucine – leucine – alanine motif) participe à la régulation des signaux transduits parmi les voies du SA et du JA (Yang *et al.*, 2012 ; Vleeschauwer *et al.*, 2016). Par ailleurs, la présence de co-régulations entre les phytohormones a été mise en évidence. En effet, l'auxine/IAA induit l'accumulation d'acides gibbérelliques nécessaires à son transport de cellule en cellule (Willige *et al.*, 2011). Le rôle de l'IAA dans les mécanismes de résistance contre les pathogènes nécrotrophes est notamment supposé *via* l'induction de gènes impliqués dans la biosynthèse du JA (Fu and Wang, 2011).

1.4. Les protéines PR

Suite à la détection du pathogène, un remaniement profond de la physiologie cellulaire est amorcé et permet l'induction de gènes *R* codant pour les protéines PR (Pathogenesis-Related Protein). Ces dernières ne sont synthétisées que dans des situations de transitions physiologiques importantes comme lors de la floraison ou de la sénescence, mais aussi lors de stress biotiques tels qu'une attaque par un agent pathogène (Tamás *et al.*, 1998 ; Akiyama *et al.*, 2004 ; Lim *et al.*, 2007 ; Ali *et al.*, 2018). Les protéines PR sont indispensables à l'établissement de deux mécanismes de défense précédemment décrits, la HR et la résistance systémique acquise (SAR). Ces protéines PR résistent et restent solubles à des niveaux de pH très faibles. De plus, elles présentent une forte résistance à l'action des protéases produites par le pathogène et par la plante elle-même. Ces propriétés leur confèrent donc une grande stabilité et facilitent leur accumulation dans les vacuoles et les espaces intercellulaires. Leur synthèse constitue une source de défense active pour la plante mais leur potentiel peut toutefois être contrecarré *via* des mécanismes de manipulation élaborés mis en place par les pathogènes.

1.4.1. Structures des protéines PR

La structure primaire des protéines PR est caractérisée par la présence de domaines protéiques très conservés au sein du règne végétal. Une classification des gènes *R* a donc pu être établie sur la base de cinq domaines structuraux majeurs.

Ainsi, la région C-terminale de protéines PR est constituée d'un domaine LRR (Leucine-Rich-Repeat) contribuant à la spécificité de liaison de l'interaction ligand – récepteur et aux réactions de phosphorylation associées à la transduction de signaux (Figure 4). Ce domaine est suivi par un site NBS (Nucleotide-Binding Site) très conservé et facilitant la fixation et l'hydrolyse des nucléosides triphosphates ATP et GTP. La région N-terminale est, quant à elle, majoritairement structurée selon trois domaines possibles: LZ (Leucine-Zipper) impliqué dans l'homo- ou l'hétérodimérisation des protéines, TIR (Toll-Interleukin-Receptor) engagé dans la signalisation cellulaire ou CC (Coiled-Coil) facilitant les interactions protéines – protéines (Tao *et al.*, 2000 ; DeYoung and Innes, 2006 ; McHale *et al.*, 2006).

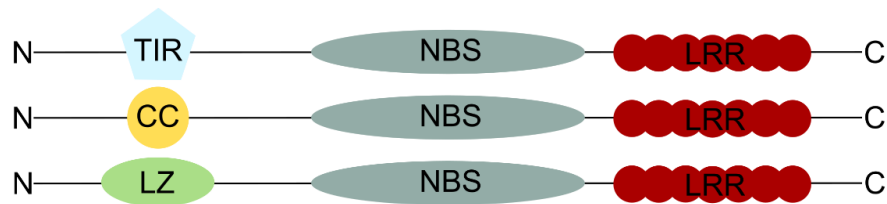


Figure 4. Organisation des principaux domaines protéiques constituant les protéines PR de type NBS-LRR. NBS, Nucleotide-Binding Site; LRR, Leucine Rich Repeat; TIR, Toll-Interleukin-Receptor; CC, Coiled-Coil; LZ, Leucine-Zipper.

De nombreux gènes *R* codent pour des protéines PR de type TIR-NBS-LRR. Cependant, seules des séquences non-TIR ont été mises en évidence chez les monocotylédones appartenant à l'Ordre des Poales duquel dépend l'ananas (Tarr and Alexander, 2009).

1.4.2. Diversité fonctionnelle des protéines PR

Les protéines PR ont été caractérisées pour la première fois chez le tabac dans le contexte d'une infection par le virus de la mosaïque du tabac (TMV) (van Loon and van Kammen, 1970). Actuellement, 17 familles ont été recensées et se distinguent par leurs activités peroxydases,

inhibiteurs de protéases ou oxalates oxydases mais aussi par leurs fonctions thionines ou analogues à la thaumatine (Tableau 1, van Loon *et al.*, 2006). Parmi les plus étudiées, les PR-1 constituent une famille protéique caractérisée par une forte activité antifongique et sont notamment décrites pour leur capacité à lier et séquestrer les stérols des membranes fongiques inhibant dès lors la croissance et le développement du pathogène (Gamir *et al.*, 2017). Les parois cellulaires fongiques sont également soumises à l'action de glucanases (PR-2) et de chitinases de classes I, II et III (PR-3, PR-4, PR-8 et PR-11) (van Loon and van Strien, 1999 ; Dimitriev *et al.*, 2017). Conjointement, les défensines (PR-12) possèdent un spectre d'activité diversifié. Leur activité antifongique mène à l'altération de la morphologie des hyphes perturbant ainsi la croissance et la capacité de pénétration du pathogène (Lacerda *et al.*, 2014). La littérature leur attribue également des potentiels d'inhibition de la synthèse protéique et de certaines enzymes digestives (α -amylases) (Stotz *et al.*, 2009).

Tableau 1. Familles de protéines PR identifiées (van Loon *et al.*, 2006).

| Family | Type member | Properties |
|---------------|-------------------------------------|--------------------------------------|
| PR-1 | Tobacco PR-1a | Unknown |
| PR-2 | Tobacco PR-2 | β -1,3-glucanase |
| PR-3 | Tobacco P, Q | Chitinase type I, II, IV, V, VI, VII |
| PR-4 | Tobacco 'R' | Chitinase type I, II |
| PR-5 | Tobacco S | Thaumatins-like |
| PR-6 | Tomato Inhibitor I | Proteinase-inhibitor |
| PR-7 | Tomato P69 | Endoproteinase |
| PR-8 | Cucumber chitinase | Chitinase type III |
| PR-9 | Tobacco "lignin-forming peroxidase" | Peroxidase |
| PR-10 | Parsley "PR1" | Ribonuclease-like |
| PR-11 | Tobacco "class V" chitinase | Chitinase, type I |
| PR-12 | Radish Rs-AFP3 | Defensin |
| PR-13 | Arabidopsis THI2.1 | Thionin |
| PR-14 | Barley LTP4 | Lipid-transfer protein |
| PR-15 | Barley OxOa (germin) | Oxalate oxidase |
| PR-16 | Barley OxOLP | Oxalate-oxidase-like |
| PR-17 | Tobacco PRp27 | Unknown |

1.5. Adaptation des agents infectieux et modulation des ajustements cellulaires de l'hôte

1.5.1. Les effecteurs, un arsenal complexe au service de la pathogénicité

Pendant les étapes précoces du dialogue moléculaire, la reconnaissance *Avr/R* induit la HR qui peut alors être définie comme SAR. Ce type d'interaction est essentiel à l'établissement de mécanismes de défense tels que la PTI. Toutefois, cette première barrière défensive peut être contournée par le champignon pathogène en adaptant ses facteurs de virulence. En effet, des molécules effectrices (*Avr*) sont émises dans le but de contrôler plusieurs mécanismes indispensables aux défenses de l'hôte (Han and Kahmann, 2019). Les effecteurs se définissent comme des molécules de faible poids moléculaire sécrétées par le pathogène dans le but de moduler les défenses de l'hôte. Les protéines effectrices constituent des composantes clés dans les mécanismes de manipulation des défenses de la plante grâce à la présence de séquences d'adressage définissant le compartiment cellulaire cible (Kamoun, 2006). Une partie de ces effecteurs va interférer dans la PTI faisant basculer l'hôte dans un « état » sensible nommé ETS (Effector-Triggered-Susceptibility). La sensibilité se définit comme l'incapacité de l'hôte à contrôler efficacement le développement et la croissance de l'agent pathogène. Cette seconde phase d'attaque initie la mise en place chez la plante hôte de nouveaux dispositifs de défense. Les récepteurs NBS-LRR sont alors disposés à la surface des cellules et vont, de façon analogue aux PRRs, reconnaître spécifiquement les molécules effectrices. Cette liaison de type ligand-récepteur établit une seconde phase de défense appelée ETI (Effector-Triggered-Immunity) qui se traduit par la sécrétion d'un nouvel ensemble de protéines PR (Jones and Dangl, 2006).

Pour le pathogène, la sécrétion d'effecteurs est indispensable à l'établissement et la continuité du processus infectieux. Suite à leur émission, les molécules effectrices de nature protéique vont être spécifiquement adressées vers un compartiment cellulaire de l'hôte tel que le noyau, les chloroplastes, le réticulum endoplasmique ou encore les mitochondries (Fabre *et al.*, 2019). Les effecteurs ciblent des molécules de l'hôte et manipulent ainsi de nombreux processus biochimiques et physiologiques. Les molécules ciblées par les effecteurs sont donc définies comme des facteurs de sensibilité car elles conditionnent malgré elles le basculement de l'hôte vers un « état » sensible. A titre d'exemple, l'effecteur CRN8 (Crickler 8) sécrété par le pathogène *Phytophthora infestans* responsable du mildiou de la pomme de terre, présente une séquence d'adressage dirigée vers le noyau des cellules végétales.

Dans le noyau de la cellule hôte, son activité kinase provoque la phosphorylation des protéines végétales et induit, dans certains cas, la mort cellulaire chez la plante ayant été infectée (van Damme *et al.*, 2012). De façon analogue, les effecteurs peuvent modifier l'expression de facteurs génétiques clés chez l'hôte entraînant une sous ou sur-proportion des produits de ces gènes alors définis comme gènes de sensibilité ou gènes *S* et causant des interférences dans la signalisation des voies de défense (van Schie and Takken, 2014). Le gène *MLO* (*Mildew resistance Locus O*) chez l'orge (*Hordeum vulgare*) en est un exemple. Ce gène code pour une protéine transmembranaire possédant une fonction putative de régulateur négatif de la mort cellulaire. Certains génotypes d'orge qui possèdent des allèles mutés du gène *Mlo* se caractérisent par une résistance élevée à l'agent causal de l'oïdium de l'orge, *Blumeria graminis*. Cette résistance est conditionnée par l'apposition d'une papille à la zone de pénétration du pathogène limitant la colonisation des tissus hôtes (Zheng *et al.*, 2016). Chez le cultivar possédant la mutation *mlo*, l'implication des produits du gène est postulée dans la régulation négative de la transduction du signal calcique qui possède un rôle inhibiteur des mécanismes de défenses immunitaires (Acevedo-Garcia *et al.*, 2014). Ces stratégies de contournement et de manipulation permettent aux champignons pathogènes de détourner les ressources nutritives de la plante hôte.

1.5.2. Stratégies de modulation de l'immunité des plantes

Le processus de coévolution instauré entre les pathogènes fongiques et les végétaux a permis l'établissement de mécanismes complexes de défense chez les plantes hôtes. En réponse, les champignons pathogènes présentent des stratégies de manipulation tout aussi sophistiquées. Des tactiques de contournement et de modulation des défenses ont pu être caractérisées dès les étapes les plus précoces d'une interaction. En effet, il a été démontré que l'effecteur NIS1 (Necrosis-Inducing Secreted protein 1) provenant de souches de *Colletotrichum* spp. et de *Magnaporthe oryzae* supprime la réponse hypersensible et le burst-oxydatif *via* l'abolition de l'activité kinase des RLKs BAK1 (Brassinosteroid insensitive 1-Associated receptor Kinase 1) et BIK1 (Botrytis-Induced Kinase 1) (Irieda *et al.*, 2018). Le large spectre de molécules effectrices confère aux pathogènes la possibilité d'intervenir dans la signalisation des principales phytohormones et ainsi d'établir des conditions favorables à la colonisation des tissus de la plante. Ainsi, en présence de SA, *Ustilago maydis* sécrète l'effecteur Shy1 (Salicylate hydroxylase 1) possédant une activité salicylate hydrolase.

Cet effecteur permet la dégradation du SA et de ses dérivés produits par l'hôte et engendre une inhibition de sa voie de biosynthèse (Patkar and Naqvi, 2017). Chez la tomate, le pathogène nécrotrophe *Botrytis cinerea* sécrète un exopolysaccharide qui élicite la voie du SA et réprime la voie du JA, pourtant impliquée dans la réponse à ce type de pathogène. Le pathogène tire ainsi partie de l'antagonisme opérant entre les voies de biosynthèse des hormones végétales (El Oirdi *et al.*, 2011). La complexité des mécanismes de manipulation repose également sur un mimétisme très élaboré visant à duper l'hôte. Le pathogène *Ustilago maydis* sécrète dans les cellules hôtes la chorismate mutase (Cmu1) dont la proximité avec le chorismate de la plante est telle que cette dernière est intégrée dans la voie de biosynthèse du SA. Cmu1 ne sera cependant pas convertie en isochorismate mais en préphénate inhibant ainsi l'accumulation du SA (Lanver *et al.*, 2017). De plus, le mimétisme métabolique peut intervenir lors de plusieurs étapes du processus infectieux établi par le pathogène. Au cours de la phase de pénétration des cellules hôtes, les cellules appressoriales de *Magnaporthe oryzae* activent la synthèse de l'Abm (Antibiotic biosynthesis monooxygenase). Ce composé intervient dans la conversion de l'acide jasmonique endogène aux cellules fongiques en dérivés hydroxylés (12OH-JA) qui seront sécrétés dans le milieu cellulaire de l'hôte. Ces dérivés vont alors interférer dans la perception de l'accumulation de JA par la plante entraînant une inhibition de la transduction des signaux de défenses de la voie du JA. Ce court-circuit dans la signalisation du JA permet ainsi au pathogène de s'assurer la pénétration dans le milieu cellulaire de l'hôte (Patkar *et al.*, 2015). Dans un second temps, cette stratégie est également mise en place en convertissant cette fois, le JA endogène aux cellules végétales en 12OH-JA grâce à l'action de l'Abm. Cette nouvelle phase de manipulation des défenses facilite la colonisation des tissus hôtes et le basculement du riz (*Oryza sativa*) vers un état sensible (Chanclud and Morel, 2016). Dans le cadre de ce pathosystème impliquant *Magnaporthe oryzae* et le riz, le 12OH-JA et l'Abm sont alors respectivement considérés comme des effecteurs métabolique (lors de la phase de pénétration) et peptidique (lors du processus de colonisation de l'hôte) menant à l'établissement de la pyriculariose (Shen *et al.*, 2018).

2. Les agents pathogènes fongiques

Parmi les microorganismes pathogènes des plantes, les champignons révèlent une importante diversité. Leur représentation au sein des écosystèmes naturels démontre un large spectre d'hôtes traduisant une importante pression infectieuse. Les cultures d'intérêt agronomique sont fortement impactées par les maladies cryptogamiques qui provoquent chaque année d'importantes pertes de rendements et/ou l'altération des produits d'origine végétale destinés à l'alimentation humaine et animale. Les environnements biotiques et abiotiques ont une influence directe sur le développement de pathologies et constituent des paramètres clés pour la compréhension des interactions plantes - pathogènes. L'impact du contexte environnemental est d'autant plus complexe à déterminer en zones tropicales et subtropicales pour lesquelles une importante biodiversité fongique est recensée sous de nombreux microclimats.

2.1. Organisation des communautés fongiques et enjeux infectieux

En association avec les bactéries et les levures, les champignons contribuent à la structuration de la flore microbienne des plantes. Regroupées sous le terme de microbiome, les communautés microbiennes sont établies sous forme de réseau d'interactions finement modulées par de nombreux échanges biochimiques (Fitzpatrick *et al.*, 2020). Cette communication est façonnée par la perception des signaux environnementaux mais également par la nature des interactions entre les microorganismes (Barriuso *et al.*, 2018). En effet, la composition d'une communauté peut être directement affectée par la présence d'une ou plusieurs espèces et ce, par le biais de relations antagoniste ou bénéfique (Durán *et al.*, 2018, Uroz *et al.*, 2019). Les différentes structures anatomiques des plantes présentent des microbiomes spécifiques qui possèdent un rôle essentiel dans les processus de croissance et de développement (Bai *et al.*, 2015). Les interactions entre les plantes et les microorganismes sont le plus souvent décrites au travers des interactions plante – pathogène. Toutefois, les défenses des plantes modulent également les communautés microbiennes non pathogènes. En effet, des mutants d'*Arabidopsis thaliana* caractérisés par une surexpression des défenses associées aux MAMPs (Microbe-Associated Molecular Patterns) réduisent les capacités de colonisation du système racinaire du champignon mutualiste *Piriformospora indica* (Jacobs *et al.*, 2011). Par opposition, des perturbations de la taille et de la composition des communautés

endophytes des feuilles sont mises en évidence chez des mutants d'*Arabidopsis* présentant une incapacité de mise en place de la MTI (MAMP-Triggered Immunity) (Chen *et al.*, 2020). Par ailleurs, le microbiome peut constituer une importante source de stimulation de l'immunité des plantes. En effet, la perception par la plante de ses endophytes permet le maintien de l'expression des réponses de défense menant dès lors à une augmentation de la résistance face aux attaques pathogènes (Pieterse *et al.*, 2014).

2.1.1. Co-occurrence d'espèces fongiques en fonction des habitats

Au sein d'un organe végétal, la structuration des communautés fongiques peut être définie par le terme mycobiome. L'essor des outils « -omiques » a permis la caractérisation des flores fongiques et des associations d'espèces régissant les interactions au sein des communautés (Anal *et al.*, 2020). Ces interactions sont conditionnées selon des mécanismes décrits comme intraspécifique, interspécifique ou de symbiose. Parmi certains écosystèmes, ces relations sont observées lors d'une concurrence pour l'accessibilité en nutriments. Les ressources nutritives sont en effet indispensables pour l'amélioration de la valeur sélective d'une espèce au sein d'une communauté (Gonçalves *et al.*, 2020). Ainsi, une interaction intraspécifique définit des échanges entre souches fongiques appartenant à la même espèce tandis qu'une relation interspécifique décrit des phénomènes d'interaction entre des souches d'espèces différentes mais partageant une même niche écologique (Shearer, 1995 ; Connolly *et al.*, 2001). Parmi ces modalités d'interaction, deux stratégies de compétition peuvent être élaborées : la compétition d'exploitation, fondée sur une exploitation intensive des ressources en nutriments du milieu dans le but d'en limiter la disponibilité pour les espèces concurrentes et la compétition d'interférence, qui se définit par une inhibition de la croissance et la restriction du processus de propagation du concurrent (Wicklow, 1992 ; Ghoul and Mitri, 2016). Au sein des pathosystèmes, la définition des espèces antagonistes et de leur profil de compétition constitue une source de biocontrôle pour la gestion agroécologique des risques épidémiologiques (Pellan *et al.*, 2020). Chez les champignons, les échanges conditionnant ces modalités d'interaction reposent en partie sur la sécrétion de métabolites secondaires parmi lesquels les pigments et les mycotoxines sont les plus communément rapportés dans la littérature (Yu and Keller, 2005 ; Fox and Howlett, 2008 ; Keller, 2018). Ces molécules vont permettre à l'espèce possédant le pouvoir antagoniste dominant de s'assurer une niche écologique *via* la conquête d'un habitat (Fox and Howlett, 2008).

A l'échelle du règne végétal, de nombreux phytopathogènes coexistent. Cette co-occurrence repose sur une colonisation différentielle des niches écologiques différée à la fois dans le temps et dans l'espace. Toutefois, certains pathogènes peuvent initier une compétition au niveau du site d'infection primaire qui constitue une zone d'intérêt pour la ponction des nutriments de l'hôte. Parmi les espèces de champignons pathogènes des plantes, le genre *Fusarium* présente une importante diversité phylogénétique (Summerell and Leslie, 2011). L'étude des composantes fongiques à l'échelle des écosystèmes démontre une répartition variée à travers des habitats tels que les végétaux cultivés, les sols et les résidus de cultures. L'implication dans les pathosystèmes d'espèces de *Fusarium* de types biotrophe et hémibiotrophe traduit une forte dépendance aux facteurs biotiques. A titre d'exemple, l'infection du blé tendre (*Triticum aestivum*) par le pathogène *Fusarium graminearum* intervient majoritairement lors de la floraison, étape du développement considérée comme fortement sensible à la fusariose de l'épi (*Fusarium* Head Blight, FHB). Ce stade ontogénique s'accompagne d'une accumulation dans les anthères de glycine bêtaïne et de choline, composés impliqués dans la stimulation de la germination et de la croissance de *F. graminearum* (Strange *et al.*, 1974 ; Pearce *et al.*, 1976). Par ailleurs, la capacité des pathogènes fongiques à initier un processus infectieux dépend intrinsèquement des contraintes abiotiques de leur environnement. Les espèces fongiques privilégient des niches écologiques humides. En effet, des paramètres tels que la température et une hygrométrie élevée favorisent la germination des spores et l'infection de tout ou partie d'un hôte (Doohan *et al.*, 2003 ; Leyronas and Nicot, 2013 ; Crandall and Gilbert, 2017).

Les conditions environnementales façonnent la distribution et la prédominance des espèces pathogènes à l'échelle des aires de production. Une étude *in vitro* menée sur des souches pathogènes du FHB provenant de différentes régions productrices d'Europe, a montré que les températures optimales de croissance des souches sont positivement corrélées au climat de leur zone géographique d'origine (Brennan *et al.*, 2003). En effet, parmi les aires de productions des céréales à grains, le FHB peut être induit par différentes espèces fongiques. Leur prédominance varie selon les zones géographiques en corrélation avec le climat. Ainsi, *F. graminearum* est décrit comme le pathogène majoritaire des zones tempérées d'Europe centrale et du sud-est tandis que *Fusarium culmorum* domine le nord-est de l'Europe présentant un climat maritime plus froid (Osborne and Stein, 2007). En Asie, les rotations de

cultures basées sur l'alternance riz / blé favorisent la prévalence de *Fusarium asiaticum* dans le complexe du FHB (Zhang *et al.*, 2012 ; Yang *et al.*, 2018). Toutefois, les représentants d'un cortège pathogène peuvent montrer des sensibilités aux traitements fongicides et des potentiels pathogéniques très contrastés (Müllenborn *et al.*, 2008 ; Wollenberg *et al.*, 2018). Une meilleure compréhension de la structuration des communautés fongiques apparaît alors essentielle pour la gestion des risques associés aux maladies des plantes. En effet, une connaissance exhaustive de la flore fongique en présence peut notamment permettre de caractériser avec précision les réponses des pathogènes aux stratégies contrôles. La détermination des conditions environnementales associées aux périodes d'infection peut également permettre de prédire et d'anticiper l'impact des changements climatiques sur les rendements et les risques sanitaires des cultures de demain (Moretti *et al.*, 2019).

2.1.2. Caractérisation des flux d'inoculum

Les cultures tropicales n'échappent pas à la pression infectieuse imposée par les champignons ascomycètes. La compréhension des risques épidémiologiques repose en partie sur la détermination des flux d'espèces et de leurs modalités de dispersion à travers les paysages agricoles. La propagation d'une espèce fongique peut s'effectuer sur de courtes ou de longues distances selon le contexte environnemental des cultures (Sapoukhina *et al.*, 2010 ; Golan and Pringle, 2017). En effet, des paramètres tels que les espèces végétales environnantes, le précédent cultural, le relief et les vents dominants peuvent directement influencer le développement et la progression spatiale d'une épidémie (Parnell *et al.*, 2010). Les champignons produisent différents types de spores spécifiques à leurs phases de reproduction. Ces spores sont dispersées dans l'environnement lorsque les conditions climatiques optimales (température et humidité relative) sont rencontrées.

2.1.2.1. Phase sexuée du genre *Fusarium*

Chez certains ascomycètes, une phase de reproduction sexuée peut être décrite. La reproduction sexuée des *Fusarium* se définit selon plusieurs téléomorphes appartenant aux genres *Gibberella*, *Albonectria* ou encore *Haematonectria* (Leslie and Summerell, 2006). Toutefois, certaines espèces telles que *F. oxysporum* ou *F. ananatum* ne possèdent pas de forme parfaite. Chez des espèces telles que *F. verticillioides*, *F. proliferatum* ou *F. fujikuroi*, cette phase est initiée à partir d'hyphes septés du mycélium végétatif. Pour cela, une cellule

de l'hyphe se différencie en ascogone et constitue le gamète femelle (Figure 5). L'agrégation de plusieurs hyphes autour de l'ascogone entraîne la formation d'une enveloppe de protection qui constitue le périthèce (Lord and Read, 2011). La fécondation résulte de la rencontre du matériel génétique mâle et femelle dans l'ascogone. Cette dernière est assurée par des hyphes ou des conidies. Toutefois, certains champignons présentent des cellules différenciées constituant les gamètes mâles. Plusieurs méioses ainsi qu'une mitose se succèdent et conduisent à la formation d'ascospores regroupés au sein d'un asque (Peraza-Reyes and Berteaux-Lecellier, 2013).

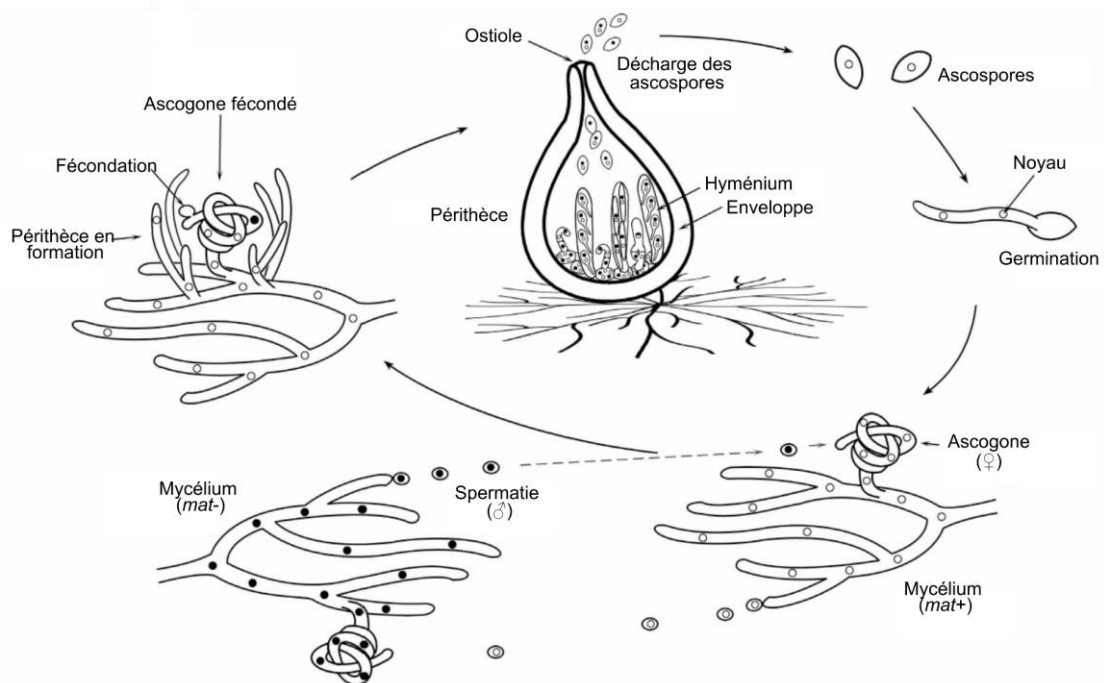


Figure 5. Cycle de reproduction sexuée du genre *Fusarium* (Peraza-Reyes and Berteaux-Lecellier, 2013).

Les asques ainsi formés sont maturés dans les périthèces, structures conférant une protection et favorisant leur survie sur les résidus de cultures. La combinaison de températures et de niveaux d'humidité relative favorables (le plus souvent après des épisodes pluvieux), entraîne l'éjection par l'ostiole des ascospores matures sur de courtes distances. Leur dispersion dans l'environnement permet le développement d'un nouveau mycélium végétatif.

2.1.2.2. Phase sexuée du genre *Talaromyces*

En 2011, la nomenclature des *Penicillium* a été révisée sur la base du concept « un champignon : un nom » menant au basculement du sous-genre *Biverticillium* vers un nouveau groupe monophylétique correspondant au genre *Talaromyces* connu jusqu'alors comme la forme téléomorphe de certains *Penicillium* (Samson *et al.*, 2011 ; Yilmaz *et al.*, 2014). Ceci explique que la définition du cycle de reproduction sexuée des *Talaromyces* spp. se rapporte encore actuellement aux *Penicillium*. De façon analogue aux ascomycètes appartenant au genre *Fusarium*, la reproduction sexuée des espèces appartenant au genre *Talaromyces* est initiée à partir du mycélium végétatif. La phase sexuée débute par la formation d'un ascogone (gamète femelle) à partir d'une cellule mycélienne uninucléée formant une structure tubulaire à la surface d'un hyphe (Figure 6).

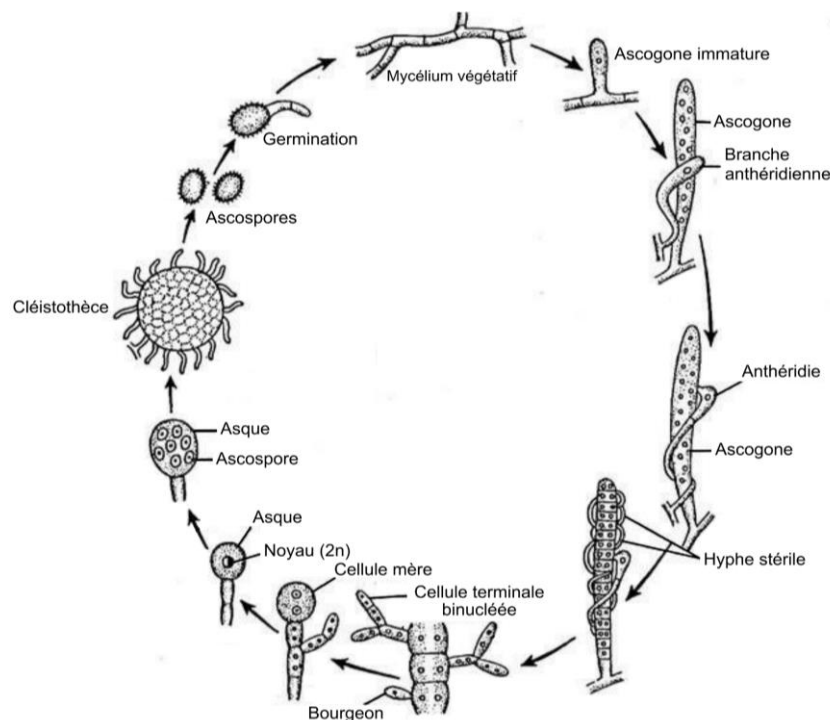


Figure 6. Cycle de reproduction sexuée du genre *Talaromyces* (www.biologydiscussion.com/fungi/penicillium-description-structure-and-reproduction).

Suivant l'élongation de l'ascogone, plusieurs divisions nucléaires s'opèrent. Une seconde structure nommée anthéridie (gamète mâle) est alors constituée à partir du même hyphe ou d'un hyphe adjacent issu du même mycélium. La structure anthérienne s'enroule autour de l'ascogone et entre en contact par son extrémité avec la paroi ascogonale (Benjamin, 1955). Un pore est ensuite formé par dégradation de la paroi au niveau de la zone d'interaction des

gamètes. Les noyaux de chaque gamète se rassemblent et subissent un arrangement par paire déclenchant la division en septa de l'ascogone. Ceci entraîne un bourgeonnement au niveau de chaque septum. La cellule en position terminale du bourgeon se différencie et constitue la cellule mère du futur asque. Au cours du développement des asques, plusieurs méioses se succèdent donnant lieu à la production d'ascospores (Yilmaz *et al.*, 2016). Parallèlement, un hyphes stérile se développe à la base de l'ascogone dont l'élongation va suivre un enroulement autour de la structure reproductrice. Cette étape génère un entrelacement d'hyphes appelé péridium permettant la protection des asques (Pöggeler *et al.*, 2018). La structure ainsi formée constitue le cléistothèce au sein duquel la maturation des ascospores est effectuée (Yilmaz *et al.*, 2016). A maturité, les asques sont dissous libérant les ascospores dans le cléistothèce. La décharge des ascospores se produit par rupture du cléistothèce lorsque que la température et l'humidité relative sont optimales. Un nouveau mycélium végétatif peut alors être établi grâce à la germination des ascospores sur le substrat.

2.1.2.3. Phase asexuée des genres *Fusarium* et *Talaromyces*

A) *Microconidies*

La propagation des ascomycètes dans l'environnement est assurée selon des phases de multiplication végétative. Les microconidies se définissent comme des cellules de petite taille et unicellulaires. Leur production est initiée par la différenciation d'une cellule du mycélium végétatif et déclenche la mise en place de conidiophores (Figure 7). Ces structures sont formées à partir de filaments non cloisonnés et dont l'extrémité supporte une vésicule qui constitue le siège de la production de conidies. En partie basale, la vésicule soutient des cellules conidiogènes nommées phialides (Leslie and Summerell, 2006). La formation des microconidies est assurée par le bourgeonnement en chaîne des phialides également appelée structure en balai ou polyphialide. Ainsi, la plus jeune microconidie est en contact direct avec la phialide tandis que la plus ancienne est retrouvée à l'extrémité de la chaîne de bourgeonnement. Le détachement des microconidies en position terminale entraîne la germination et la propagation de l'espèce fongique sur un nouveau substrat.

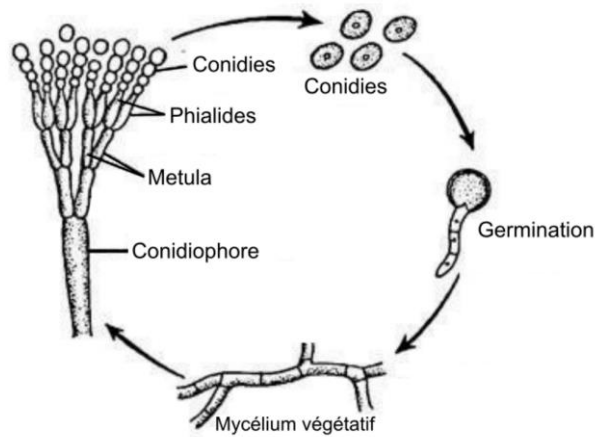


Figure 7. Cycle de reproduction asexuée du genre *Talaromyces* analogue à celui des *Fusarium* (www.biologydiscussion.com/fungi/penicillium-description-structure-and-reproduction).

B) Macroconidies

Les macroconidies sont des cellules pluricellulaires de grande taille produites par le genre *Fusarium*. Leur production s'effectue par le développement de conidiophores à la surface d'un entrelacement d'hyphes défini sous le terme de sporodochium. De façon analogue aux microconidies, le bourgeonnement des phialides mène à la mise en place de structures en balai dont se détachent les macroconidies en position terminale (Leslie and Summerell, 2006). Cette étape du cycle de reproduction asexuée des ascomycètes peut être réalisée directement sur l'hôte grâce à l'insertion de sporodochium dans l'épiderme de la plante.

C) Chlamydospores

Très fréquente chez les Mucorales, la formation de chlamydospores n'est pas étendue à l'ensemble des ascomycètes. A titre d'exemple, elle peut être observée parmi le genre *Fusarium* chez des espèces telles que *Fusarium solani* et *Fusarium oxysporum* (Mondal and Hyakumachi, 1998 ; Hou *et al.*, 2020). Leur production est effectuée à partir d'une cellule du mycélium végétatif. Ces spores restent connectées à l'hyphe et sont disposées de manière intercalée, sessile (latérale) ou terminale. Les chlamydospores possèdent une paroi épaisse et ne sont pas dispersées dans l'environnement. Elles constituent une forme de résistance pour les champignons telluriques.

2.1.2.4. Modes de dispersion dans l'environnement

Dans l'environnement, la propagation des maladies cryptogamiques résulte de modèles de dissémination établis selon des échelles locales ou globales. La caractérisation des pathogènes et des modes de dispersion qui leur sont associés sont des paramètres clés de la compréhension des épidémies. Les profils de dispersion sont tout d'abord considérés selon les habitats privilégiés par les espèces fongiques. En effet, les systèmes foliaires et les sols constituent deux interfaces majeures pour les cultures et comportent des communautés fongiques spécifiques.

Ainsi, les profils de dispersion sont tout d'abord corrélés aux types de spores produites. Les macro- et les microconidies sont principalement véhiculées par les eaux de pluie par effet de « splashing » ou de ruissellement (Hörberg, 2002 ; Perryman *et al.*, 2014). A la Réunion, ces profils de propagation sont notamment observés sur les vergers de manguiers soumis à une forte pression de la part du pathogène *Colletotrichum gloeosporioides*, agent causal de l'antracnose (Kamle and Kumar, 2016). A maturité, les fruits infectés présentent un développement de nécroses sous forme de « coulures » provenant du transport de l'inoculum du pédoncule vers la peau du fruit (Nelson, 2008). Plus généralement, les phénomènes de ruissellement se caractérisent par une dispersion localisée à l'échelle d'un même organe ou d'organes adjacents. Par ailleurs, les conidies des champignons dits telluriques peuvent être transportées par le biais des systèmes d'irrigation (Dita *et al.*, 2018). Les épisodes pluvieux et l'irrigation par aspersion induisent également une augmentation de l'humidité relative et favorisent la décharge des ascospores dans l'environnement (Mondal *et al.*, 2003 ; Cordo *et al.*, 2017). Chez les champignons filamenteux, les ascospores sont éjectées par le biais de l'ostiole situé à l'apex des périthèces. Consécutivement à leur décharge, ces ascospores vont être transportées par le vent sur des distances variables. Par ailleurs, le détachement des macro- et microconidies est également favorisé par l'action mécanique des flux aériens ou hydriques. La concentration en spores d'un environnement donné diminue à mesure que la distance depuis la source de l'inoculum augmente (Holb *et al.*, 2004 ; Rieux *et al.*, 2014). Ainsi, la dispersion aérienne constitue un paramètre prépondérant de la propagation d'un pathogène sur les cultures.

Les phénomènes de dispersion s'établissent dans le temps et dans l'espace grâce aux formes de résistance que constituent les spores (Wyatt *et al.*, 2013). Leur capacité de survie dépend

des conditions climatiques de leur substrat. En effet, des paramètres tels que des températures extrêmes, une faible humidité relative et des radiations de types UV-A et UV-B (fort ensoleillement) retardent la germination et réduisent la virulence ainsi que la survie des conidies (Braga *et al.*, 2015). Sur des systèmes culturaux fortement ensoleillés, la survie des spores de classe hyaline est estimée à quelques heures (longévité variable selon l'espèce considérée) tandis que des spores pigmentées peuvent persister plusieurs jours sur un substrat (Nascimento *et al.*, 2010 ; Lagomarsino Oneto *et al.*, 2020). La détermination des capacités de survie en fonction de ces variables est donc indispensable à la modélisation ainsi qu'à la prédiction des flux épidémiologiques. Ces données constituent par ailleurs d'importants leviers pour la prévention et la maîtrise des maladies cryptogamiques.

2.2. Régulation du métabolisme secondaire chez les champignons filamenteux

Le métabolisme secondaire des champignons est finement régulé au cours des différents stades de leur développement. Les fonctions associées aux métabolites secondaires d'origine fongique ne sont pour l'heure que partiellement connues mais ils constituent déjà des composés d'intérêt pour les industries alimentaires et pharmaceutiques.

Lors d'une interaction plante – pathogène, la sécrétion active de métabolites secondaires par le champignon révèle l'importance de ces composés dans l'induction de la sensibilité chez l'hôte. Des enzymes hydrolytiques ou PCWDEs (Plant Cell Wall Degrading Enzymes) telles que les cutinases, les cellulases, les xylanases ou les endo-polygalacturonases permettent le contournement des barrières morpho-anatomiques des plantes et la dégradation des parois cellulaires favorisant la colonisation des tissus hôtes (Annis and Goodwin, 1997). De plus, les pigments et les mycotoxines sont décrits pour leurs activités antibiotique, antivirale et antifongique mais également pour leur rôle de facteurs de virulence (Nirlane da Costa Souza *et al.*, 2016 ; Venkatesh and Keller, 2019 ; Reverberi *et al.*, 2010). Les mycotoxines peuvent constituer un risque important pour la santé humaine et animale de par leur toxicité et leur potentiel de contamination des productions végétales. La consommation récurrente de produits contaminés par ces toxines peut engendrer d'importantes répercussions sur la santé humaine. Des concentrations seuil ont donc été définies par la communauté européenne pour les produits à grains destinés à l'alimentation humaine et animale (AFSSA, 2009). Par ailleurs, les teneurs maximales à considérer pour les fruits et les légumes sont exclusivement définies

pour la patuline et l'ochratoxine A (Règlement (CE) N°1881/2006, 2006). La diversité du genre *Fusarium* se traduit également dans la multiplicité des potentiels toxigènes résultant de différentes voies de biosynthèse.

2.2.1. Clusters de gènes de biosynthèse des mycotoxines (BGC)

La biosynthèse des mycotoxines est conditionnée par une succession de facteurs génétiques exprimés chez le champignon avant la récolte mais également lors du stockage en silo ou en chambre froide des productions végétales d'intérêt alimentaire. Ces contaminations pré- et/ou post-récolte sont notamment causées par des espèces fongiques appartenant aux genres *Fusarium*, *Penicillium* et *Aspergillus*. Au sein des génomes fongiques, les gènes responsables de la biosynthèse de toxines sont organisés selon plusieurs clusters spécifiques aux mycotoxines produites. Notamment, la production des trichothécènes résulte de l'expression du cluster de gènes *Tri* (Proctor *et al.*, 2018). Son implication a été mise en évidence dans les potentiels toxigènes d'espèces appartenant aux genres *Fusarium*, *Stachybotrys* et *Trichoderma* (Cardoza *et al.*, 2011 ; Semeiks *et al.*, 2014). Ce cluster est constitué d'un nombre de gènes variable selon les genres et les espèces considérés (Tableau 2). Ce cluster est impliqué dans la biosynthèse du déoxynivalénol (DON), du nivalénol et des trichothécènes B (Alexander *et al.*, 2009 ; Boutigny *et al.*, 2009).

Tableau 2. Fonctions des composantes du cluster de biosynthèse des trichothécènes (Proctor *et al.*, 2018).

| Gene | Functional category | Function in trichothecene biosynthesis |
|---------------|---|---|
| <i>Tri3</i> | acetyl transferase | acetylation at C15 (<i>Fusarium</i>) |
| <i>Tri4</i> | cytochrome P450 monooxygenase | oxygenation of Trichodiene at C2, C11, and C13 (<i>Myrothecium</i> , <i>Trichoderma</i>), or C2, C3, C11, and C13 (<i>Fusarium</i>) |
| <i>Tri5</i> | terpene synthase | cyclization of farnesyl pyrophosphate to Trichodiene (<i>Fusarium</i> , <i>Trichoderma</i>) |
| <i>Tri6</i> | Zn2His2 transcription factor | transcriptional regulation of <i>Tri</i> gene expression (<i>Fusarium</i>) |
| <i>Tri7</i> | acetyl transferase | acetylation at C4 (<i>Fusarium</i>) |
| <i>Tri8</i> | esterase | deacetylation at C3 or C15 (<i>Fusarium</i>) |
| <i>Tri9</i> | unknown | unknown |
| <i>Tri10</i> | transcriptional regulator | transcriptional regulation of <i>Tri</i> gene expression (<i>Fusarium</i>) |
| <i>Tri11</i> | cytochrome P450 monooxygenase | hydroxylation at C15 (<i>Fusarium</i>) |
| <i>Tri12</i> | major facilitator superfamily transporter | Trichothecene efflux pump (<i>Fusarium</i>) |
| <i>Tri13</i> | cytochrome P450 monooxygenase | hydroxylation at C4 (<i>Fusarium</i>) |
| <i>Tri14</i> | unknown | unknown; not required for synthesis in culture (<i>Fusarium</i>) |
| <i>Tri16</i> | acyl transferase | acylation at C8 (<i>Fusarium</i>) |
| <i>Tri17</i> | polyketide synthase | synthesis of polyketide esterified at C4 (predicted in <i>Stachybotrys</i>) |
| <i>Tri18</i> | acyl/acetyl transferase | unknown |
| <i>Tri19</i> | terpene synthase | <i>Tri5</i> paralog |
| <i>Tri22d</i> | cytochrome P450 monooxygenase | hydroxylation at C4 (<i>Trichoderma</i>) |
| <i>Tri101</i> | acetyl transferase | acetylation at C3 (<i>Fusarium</i>) |

Ces mycotoxines sont considérées comme des facteurs de virulence essentiels au processus infectieux. En effet, la mutation du gène *Δtri5* (trichodiène synthase) invalidant la production de trichothécènes, conduit à une réduction de la virulence des souches de *F. graminearum* mutées (Jansen *et al.*, 2005). Dans le contexte de l'établissement de la fusariose de l'épi (FHB), la sécrétion par *F. graminearum* du DON permet à l'hyphe de coloniser le rachis et les grains en développement (Audenaert *et al.*, 2013). Par ailleurs, le DON est reconnu comme un effecteur pouvant notamment interférer dans la synthèse protéique (Kazan *et al.*, 2012).

Parmi les mycotoxines, les fumonisines sont principalement produites par des espèces appartenant au genre *Fusarium*. Leur synthèse est régie par le cluster *FUM* constitué de 17 gènes pour une taille de 50 kpb (Tableau 3). Ce cluster est notamment étudié chez des espèces telles que *Fusarium verticillioides* et *Fusarium proliferatum*. Les fumonisines sont classées selon quatre groupes : A, B, C et P sur la base de leurs divergences de structure (Musser and Plattner, 1997).

Tableau 3. Fonctions des composantes du cluster de biosynthèse des fumonisines (Alexander *et al.*, 2009).

| Gene | Functional category | Mutant phenotype |
|--------------|---|--|
| <i>FUM21</i> | Transcription factor | No fumonisins |
| <i>FUM1</i> | Polyketide synthase | No fumonisins |
| <i>FUM6</i> | Cytochrome P450 monooxygenase and reductase | No fumonisins |
| <i>FUM7</i> | Alcohol dehydrogenase | Tetradehydro-FB |
| <i>FUM8</i> | α- oxoamine synthase | No fumonisins |
| <i>FUM3</i> | Dioxygenase | FB2 and FB4 |
| <i>FUM10</i> | Acyl-CoA synthetase / Acyl- protein synthetase | Hydrolyzed FB3 and hydrolyzed FB4 FB1, FB2, FB3 and FB4 |
| <i>FUM11</i> | Tricarboxylic acid transporter | Half-hydrolyzed FB1, FB2, FB3 and FB4 Keto half-hydrolyzed FB1, FB2, FB3, FB4 |
| <i>FUM2</i> | Cytochrome P450 monooxygenase | FB2 and FB4 |
| <i>FUM20</i> | Unknown | Not determined |
| <i>FUM13</i> | Short chain dehydrogenase / reductase | 3-keto FB3 & 3-keto FB4 |
| <i>FUM14</i> | Nonribosomal peptide synthase (peptidyl and condensation domains) | Hydrolyzed FB3 & hydrolyzed FB4 |
| <i>FUM15</i> | Cytochrome P450 monooxygenase | No effect |
| <i>FUM16</i> | Acyl-CoA synthetase / Acyl- protein synthetase | No effect |
| <i>FUM17</i> | Ceramide synthase | No effect |
| <i>FUM18</i> | Ceramide synthase | No effect |
| <i>FUM19</i> | ABC transporter | Increased ratio FB1:FB3 |

La présence de mutations ponctuelles sur l'une des composantes *FUM* résulte en une capacité de biosynthèse variable pour des souches appartenant à une même espèce (Proctor *et al.*, 2006). Ainsi, pour une espèce considérée, différentes souches peuvent être ou non productrices de fumonisines. Il est alors possible de déterminer des chémotypes spécifiques

sur la base du criblage des gènes *FUM*. De façon analogue aux trichothécènes, les fumonisines constituent des facteurs de virulence indispensables au dialogue moléculaire établi lors du processus infectieux. A titre d'exemple, la mutation $\Delta fum1$ engendre une incapacité de biosynthèse des fumonisines ainsi qu'une diminution de la virulence du pathogène *Fusarium verticillioides*. En l'absence de cette mutation, *F. verticillioides* provoque chez le maïs d'importantes lésions du système foliaire associées à un retard de croissance (Glenn *et al.*, 2008). De plus, l'accumulation de fumonisine B₁ permet au pathogène d'inhiber l'activité d'une exo- β -1,3-glucanase sécrétée par l'hôte suite à la détection du pathogène (Galeana-Sánchez *et al.*, 2017).

La beauvericine est une mycotoxine définie comme un hexadepsipeptide cyclique (Stępień and Waśkiewicz, 2013). Ce métabolite secondaire tient son nom du champignon pathogène *Beauveria bassiana* parasite d'insectes ravageurs. Sa biosynthèse a notamment été caractérisée chez *Fusarium proliferatum* (Tao *et al.*, 2013) et *Fusarium oxysporum* (Moretti *et al.*, 2002). Le cluster de gènes impliqué dans la biosynthèse de la beauvericine est constitué de quatre composantes *Bea1*, *Bea2*, *Bea3* et *Bea4* présentant des patterns de co-régulation (Tableau 4 ; Niehaus *et al.*, 2016).

Tableau 4. Fonctions des composantes du cluster de biosynthèse de la beauvericine (Niehaus *et al.*, 2016).

| Gene | Functional category |
|-------------|---|
| <i>Bea3</i> | ABC transporter |
| <i>Bea2</i> | 2-Ketoisovalerate reductase |
| <i>Bea1</i> | Non-ribosomal peptide synthetase |
| <i>Bea4</i> | Zn(II) ₂ Cys ₆ transcription factor |

En effet, *Bea1* et *Bea2* sont indispensables à la biosynthèse de la beauvericine tandis que *Bea3* et *Bea4* codent pour des protéines assurant un rétrocontrôle négatif de l'expression de ces deux premières composantes (Fumero *et al.*, 2020). Ce métabolite secondaire présente une forte cytotoxicité pour les cellules végétales. *In vitro*, la beauvericine réduit de 80% la viabilité de protoplastes de banane et entraîne un flétrissement des plantules. Cette mycotoxine est notamment accumulée par le pathogène *Fusarium oxysporum* f. sp. *cubense* (races 1 et 4) dans les tissus racinaires, foliaires et les pseudo-troncs des bananiers (Li *et al.*, 2013).

2.2.2. Initiation des mécanismes de signalisation

La biosynthèse des mycotoxines résulte en partie de la perception par les cellules fongiques de stimuli externes associés à des facteurs de stress mécaniques, chimiques, ou encore hormonaux. Les variations de sources de carbones et d'azote, de température ambiante, de pH ou d'activité de l'eau (a_w) entraînent une régulation complexe des clusters de biosynthèse (Camardo Leggieri *et al.*, 2019 ; Perincherry *et al.*, 2019). Pour cela, la transduction des signaux environnementaux est assurée par des facteurs de transcription possédant des domaines zinc finger de types Cys₂-His₂ (Cystéine₂-Histidine₂) ou GATA (Espeso and Peñalva, 1996 ; Michielse *et al.*, 2013 ; Ries *et al.*, 2016). Selon le stimulus perçu, des facteurs de transcription spécifiques sont mobilisés. La littérature fait ainsi référence à des facteurs tels que *CreA* (*Catabolite responsive elements A*) impliqué dans la sélection de sources carbonées énergétiquement favorables pour le champignon (Adnan *et al.*, 2017), *AreA* engagé dans la modulation du métabolisme de l'azote (Tudzynski, 2014) et *PacC* (*loss or reduction in Phosphatase Activity at ACid but not at alkaline pH*) permettant le maintien de l'homéostasie cellulaire à des pH neutres ou alcalins (García-Estrada *et al.*, 2018). Parallèlement, ces facteurs apparaissent comme des régulateurs de la biosynthèse des métabolites secondaires et présentent une influence directe sur la pathogénicité. En effet, la mutation du gène $\Delta CreA$ d'*Aspergillus flavus* conduit à une réduction des potentiels de synthèse d'aflatoxines entraînant une diminution de la capacité de colonisation des tissus hôtes (Fasoyin *et al.*, 2018). De façon analogue, les souches d'*Aspergillus carbonarius* possédant la mutation $\Delta AcpacC$ se caractérisent par une incapacité totale de production d'ochratoxine A à pH neutre. Ces souches mutées présentent également une virulence réduite sur raisin et nectarines (Barda *et al.*, 2020).

Par ailleurs, la régulation du métabolisme secondaire des champignons est étroitement liée aux processus de transition physiologique. Chez les espèces appartenant aux genres *Aspergillus* et *Fusarium*, les événements de sporulation et de synthèse de toxines sont modulés *via* la signalisation des protéines G (Guanine nucleotide-binding proteins). Ces protéines, très conservées chez les eucaryotes, constituent des facteurs clés dans la transduction de signaux externes. Elles sont notamment impliquées dans la régulation de processus physiologiques relatifs à la croissance végétative, à la reproduction asexuée et à la virulence (Brodhagen and Keller, 2006). A titre d'exemple, les souches d'*Aspergillus flavus*

possédant une mutation de la sous-unité α *ΔgpaB* (*G protein alpha subunit B*), présentent une incapacité à former des conidiophores combinée à une inhibition de la production d'aflatoxines (Liu *et al.*, 2018).

De plus, certaines ROS constituent des signaux précurseurs de biosynthèse. Un phénomène de « burst- » oxydatif est observé lors de l'initiation de la sporulation ou de la germination des spores (Zheng *et al.*, 2015). En effet, les mécanismes d'accumulation des ROS interviennent durant les phases de transitions morphologique et métabolique. Lors de ces transitions, certains métabolites secondaires sont accumulés suggérant dès lors de potentielles co-régulations (Reverberi *et al.*, 2010). En effet, il est démontré qu'en condition de stress oxydatif, l'intégrité cellulaire est préservée grâce au contrôle opéré par les mycotoxines (Peng *et al.*, 2010). Par ailleurs, des facteurs tels que des ressources nutritives limitées ou une compétition biotique peuvent également provoquer d'importants changements dans le métabolisme secondaire d'une espèce fongique.

2.3. Toxinogénicité, des potentiels en interaction

2.3.1. Mycotoxines impliquées dans les processus infectieux

Les potentiels toxigènes des espèces fongiques sont le plus souvent étudiés pour leur nocivité avérée chez les mammifères. Bien que leur toxicité sur les cellules végétales soit également reconnue, les mécanismes cellulaires mis en œuvre ne sont pour l'heure que partiellement décrits. A titre d'exemple, le DON interfère notamment dans différentes voies métaboliques des plantes telles que le cycle de Krebs ou la voie de biosynthèse des polyamines (Audenaert *et al.*, 2013). Outre leur influence sur le métabolisme primaire des végétaux, les mycotoxines révèlent d'importantes capacités de modulation des réponses de défense de l'hôte. En effet, au contact du DON, des cellules d'*Arabidopsis thaliana* ayant subi un choc thermique présentent une inhibition du processus de mort cellulaire programmée. Il est donc suggéré que la sécrétion du DON par les espèces appartenant au genre *Fusarium* soit impliquée dans la suppression de l'apoptose lors du processus infectieux (Diamond *et al.*, 2013). Par ailleurs, la surexpression chez *Fusarium avenaceum* du gène *ESYN1* (*Enniatin Synthase 1*) provoque une expansion des nécroses observées sur les tubercules de pomme de terre (Eranthodi *et al.*, 2020).

L'implication des mycotoxines dans la pathogénèse a tout d'abord été suggérée de par leur accumulation élevée dans les tissus infectés. Dans le contexte de la maladie de la tache noire

de l'ananas, les fruits simples naturellement infectés par le pathogène *Fusarium ananatum* présentent des concentrations en fumonisine B₁ (FB₁) et en beauvericine significativement supérieures à celles quantifiées sur des fruits sains (Barral *et al.*, 2020). Ces éléments suggèrent l'implication des mycotoxines dans l'établissement de la sensibilité de l'hôte. De surcroît, les espèces fongiques peuvent sécréter plusieurs mycotoxines de manière synchrone démontrant une importante diversité dans les stratégies de contournement ou de manipulation de l'hôte.

2.3.2. Influence des interactions bactérie-champignon sur la biosynthèse de mycotoxines

La détermination du chémotype d'une espèce pathogène permet en partie de décrypter le dialogue biochimique établi entre les individus constituant le microbiome. Les nombreuses fonctions associées aux mycotoxines suggèrent en effet un rôle écologique clé dans la protection et la conservation de niches écologiques d'intérêt. Notamment, la flore endophyte constitue une source importante d'agents de biocontrôle possédant des capacités de modulation de la biosynthèse ou de dégradation des toxines (Ryan *et al.*, 2008). En effet, les fusaricidines produites par la bactérie endophyte des téosintes *Paenibacillus polymyxa* montrent une activité antifongique contre le pathogène du maïs *Fusarium graminearum*. Cette interaction conduit notamment à l'inhibition de la biosynthèse du DON lors du stockage des grains (Mousa *et al.*, 2015). De façon analogue, certaines bactéries possèdent des capacités de détoxification des mycotoxines. Ceci est notamment observé dans le contexte du FHB induit par le pathogène *Fusarium culmorum* chez le blé et l'orge. La dégradation du DON par la bactérie *Pseudomonas fluorescens* permet de réduire la sévérité des symptômes et de limiter l'accumulation de cette mycotoxine dans les tissus hôtes (Khan and Doohan, 2009). Conjointement, des espèces appartenant aux genres *Sphingomonas*, *Sphingopyxis*, *Exophiala* et *Bacillus* isolées à partir de grains de maïs ont été caractérisées pour leurs potentiels de dégradation ou de détoxification des fumonisines (Vanhoutte *et al.*, 2016). Ces processus reposent notamment sur l'action de carboxylestérases, de transaminases ou de désaminases (Heinl *et al.*, 2010; Heinl *et al.*, 2011). De plus, les capacités de dégradation ou de détoxification des bactéries constituent de nouveaux leviers technologiques pour la maîtrise des risques toxiques des fruits frais ou de leurs dérivés de transformation. *Pseudomonas poae* a notamment montré un potentiel antagoniste contre le phytopathogène *Penicillium*

expansum. Ren *et al.* ont mis en évidence que cette bactérie permet de limiter l'expansion des symptômes de la pourriture verte de la pomme et de diminuer de 79% la concentration en patuline des jus (Ren *et al.*, 2021). Ces potentiels de décontamination de la patuline ont également été démontrés chez des espèces bactériennes telles qu'*Alicyclobacillus acidoterrestris* et *Alicyclobacillus acidocaldarius* respectivement ensemencées dans des jus de pomme et de kiwi (Yuan *et al.*, 2014 ; Gonçalves *et al.*, 2018).

2.3.3. Influence des interactions champignon-champignon sur la biosynthèse de mycotoxines

Les interactions entre espèces fongiques conduisent à des modulations des potentiels toxigènes pouvant être caractérisés selon des tendances de répression ou de surexpression des chémotypes. Ces profils sont complexes et présentent une importante variabilité selon les espèces fongiques considérées et les conditions abiotiques perçues lors de l'interaction (Giorni *et al.*, 2019). Les espèces fongiques peuvent également constituer des ressources d'intérêt pour une gestion agroécologique des pathogènes. Bien qu'étant considérée comme pathogène dans de nombreux pathosystèmes, *Fusarium verticillioides* représente une composante de la flore endophyte du maïs. En outre, cette espèce permet de réduire de façon significative la sévérité de la maladie du charbon du maïs provoquée suite à l'infection par *Ustilago maydis*. *In vitro*, la co-culture de *F. verticillioides* et d'*U. maydis* révèle une réduction significative de la croissance du pathogène résultant de la sécrétion par cette espèce de *Fusarium* d'acide fusarique (Jonkers *et al.*, 2012). Par ailleurs, la co-occurrence de plusieurs pathogènes au sein d'un même hôte peut engendrer des relations de compétition ou de mutualisme conditionnées par la sécrétion de mycotoxines. Comme précédemment exposé, *F. culmorum* est un pathogène majeur du FHB. Chez les céréales, le genre *Alternaria* impose également une forte pression infectieuse de par son implication dans le développement de nécroses sur grains (« smudge kernel ») et sur feuilles (« leaf blight ») (Ramires *et al.*, 2018 ; Turzhanova *et al.*, 2020). La culture *in vitro* sur grains de blé de *F. culmorum* et d'*A. tenuissima* réduit de 95% la production d'alternariol (AOH), d'alternariol monométhyl ether (AME) et d'altenuène (ALT) synthétisés spécifiquement par les espèces appartenant au genre *Alternaria*. A l'inverse, l'accumulation de toxines fusariennes telles que le ZEA (zéaralénone) et le DON est accrue en condition de co-cultures (Müller *et al.*, 2012). Ces modulations suggèrent donc que les mycotoxines peuvent avoir une fonction biologique relative à

l'inhibition de la croissance et des potentiels toxigènes lors de compétitions interspécifiques. De plus, le DON produit par les pathogènes *F. graminearum* et *F. culmorum* engendre chez *Trichoderma atroviride* la répression du gène *nag1* (*N-acetyl-β-D-glucosaminidase 1*) codant pour une chitinase (Lutz *et al.*, 2003). Ceci explique en partie les difficultés rencontrées lors de la détermination d'agents de biocontrôle fongique destinés à la gestion des pathogènes appartenant au *Fusarium* (Pellan *et al.*, 2020).

3. Maladie de la tache noire de l'ananas : un pathosystème pluripartite ?

Sur le marché international des fruits tropicaux, l'ananas constitue l'un des fruits les plus exportés. Pour l'année 2019, les volumes d'exportation d'ananas frais ont été estimés à 3,2 millions de tonnes. Sur cette période, les principaux pays producteurs étaient représentés par le Costa-Rica et les Philippines en réponse à une forte demande du marché chinois. La production d'ananas au Costa-Rica est toutefois spécifiquement destinée aux marchés européen et nord-américain (FAO, 2020). Le commerce d'ananas frais a connu une importante transition à partir des années 2000 suite à l'introduction de l'hybride 'MD-2'. Cette nouvelle variété, développée par la firme américaine Del Monte, a rapidement supplanté le cultivar 'Cayenne Lisse' dominant jusqu'alors le marché mondial. En effet, l'ananas 'MD-2' présente une excellente acceptabilité auprès des consommateurs de par son taux de sucres élevé et sa faible acidité (Bartholomew *et al.*, 2012). Par ailleurs, il présente une bonne résistance au froid lors du stockage et du transport. A l'inverse, le 'Cayenne Lisse' est fortement sujet au développement du brunissement interne, désordre physiologique induit par des températures de stockage comprises entre 0 et 20°C (Luengwilai *et al.*, 2016). Certaines variétés non adaptées au transport par bateau, sont majoritairement destinées aux marchés locaux. L'ananas 'Bouteille' domine ainsi les ventes en frais aux Antilles tandis que la variété 'Queen Victoria' est prédominante dans l'océan Indien. L'ananas 'Queen Victoria', très apprécié pour ses qualités sensorielles, est décrit par les consommateurs selon des descripteurs olfactifs tels que « frais », « fruité » et « sucré » (Leneveu-Jenvrin *et al.*, 2020).

Ainsi, le 'Queen Victoria' constitue le cultivar majoritaire à la Réunion, l'île Maurice et en Afrique du Sud. Parmi ces aires de production, la problématique majeure se rapporte à sa forte sensibilité à la maladie de la tache noire (Barral, 2017 ; Jacobs *et al.*, 2010). Cette pathologie d'origine fongique se caractérise par le développement d'une coloration brunâtre

strictement localisée au fruit simple et sans atteinte de l'épiderme du fruit (Figure 8). L'absence de symptômes externes constitue la particularité de cette maladie et contraint l'évaluation de son incidence au champ ou sur les chaînes de tri.

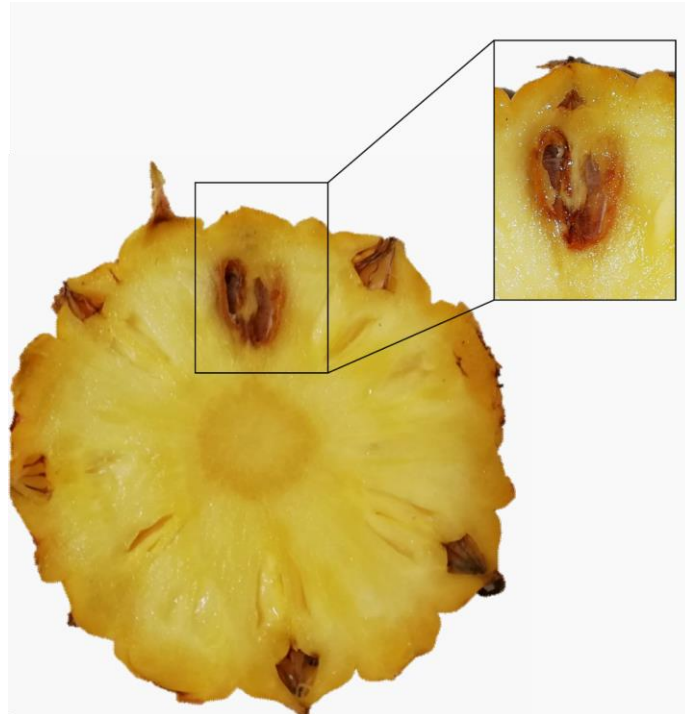


Figure 8. Symptôme caractéristique de la maladie de la tache noire observé sur le cultivar 'Queen Victoria' (photos : M. Vignassa).

3.1. Définition du pathosystème

3.1.1. L'ananas, un hôte singulier

3.1.1.1. Caractéristiques morphologiques

L'ananas (*Ananas comosus* (L.) Merr.) est une plante herbacée pérenne résultant de la germination d'un unique cotylédon (monocotylédone). Il est décrit comme appartenant à la famille des *Bromeliaceae* et à la sous-famille des *Bromelioideae*. L'anatomie d'un plant se définit tout d'abord par la présence d'une infrutescence surplombée à l'apex par une couronne de feuilles. L'infrutescence comprend de nombreux fruits simples organisés selon plusieurs spirales intercalées. L'ananas est parthénocarpique définissant ainsi un développement sans nécessité de fécondation des ovules. Lors de l'anthèse, l'inflorescence est constituée de nombreuses fleurs composées de trois sépales et de trois pétales présentant une coloration violette. Chaque fleur possède six étamines et un pistil qui constituent

respectivement les organes reproducteurs mâle et femelle. L'ensemble de la structure florale est protégé par une bractée et surmonte un ovaire infère. Ce dernier se compose de trois loges carpellaires (locules) contenant les ovules (Figure 9).

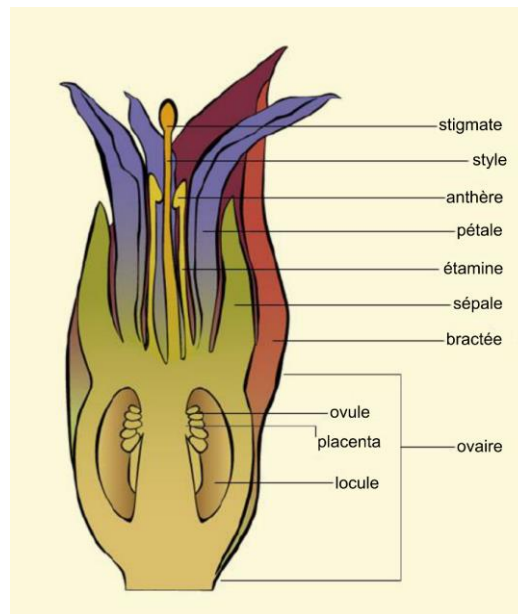


Figure 9. Coupe longitudinale de fleur d'ananas (www.biologievegetale.be/morphologie-vegetale/angiospermes/angiospermes-organographie/fruits/).

Au stade post-anthèse, les parties basales de la bractée et des sépales deviennent charnues. Leur soudure génère une loge contenant les restes de pièces florales. La loge ainsi formée constitue la cavité florale et coiffe la partie supérieure des carpelles. L'ananas est un fruit auto-stérile. De ce fait, la production de graines est majoritairement obtenue dans le cadre des programmes de sélection par le biais de croisements entre différentes variétés.

L'infrutescence est supportée par une tige non ramifiée d'environ 30 centimètres. Cette tige est constituée d'entre-nœuds courts. Le système foliaire se compose de feuilles épaisses disposées en cercles concentriques autour de la tige. Elles se caractérisent par une anatomie concave favorisant une collecte optimale des eaux de pluie. Les feuilles présentent des phénotypes variés permettant une distinction aisée des cultivars. En effet, l'ananas 'MD-2' est caractérisé par des feuilles lisses tandis que le cultivar 'Queen Victoria' possède de nombreuses structures en crochets disposées sur toute la périphérie des feuilles. L'ensemble du système foliaire s'étend sur un diamètre d'un à deux mètres pour une hauteur équivalente.

Le système racinaire est de type traçant et s'établi sur une profondeur n'excédant pas 35 centimètres (Fournier, 2012).

3.1.1.2. Cycle cultural

L'itinéraire technique décrit dans cette section se réfère aux pratiques culturales conventionnelles suivies à la Réunion. L'établissement de ces pratiques résulte d'un travail conjoint entre le CIRAD et la Chambre d'Agriculture de la Réunion (Fournier, 2012). Elles peuvent être appliquées sans distinction du cultivar. Les principales étapes de l'itinéraire technique sont représentées en Figure 10.

La phase de préparation du sol est une étape cruciale lors de la mise en place d'une parcelle. La croissance du système racinaire superficiel nécessite un sol meuble et homogène favorisant le drainage et l'aération. Un sol présentant une forte pierrosité ou compaction restreint le développement racinaire. L'acidité des sols peut également constituer une contrainte culturale. Pour une croissance optimale, le pH du sol doit être compris entre 4,5 et 5,5. Une fois ces paramètres considérés, la préparation du sol est initiée par le broyage du précédent cultural. Cette étape a pour objectif de limiter la prolifération d'agents pathogènes tels que les champignons, les nématodes ou les cochenilles. Pour cela, il est préconisé de procéder au séchage des débris de culture. La matière organique est ensuite enfouie grâce au labour sur une profondeur de 25 à 30 centimètres. Des billons sont alors constitués en veillant à optimiser la surface arable. A ce stade, des apports en urée et en sulfate de potasse sont conseillés (fumure de fond). Le système d'irrigation est ensuite installé. Les billons sont couverts d'un film de polyéthylène noir présentant de nombreux avantages tels que la restriction des adventices, le maintien de l'humidité et de la température des sols ainsi que la limitation des phénomènes de compaction.

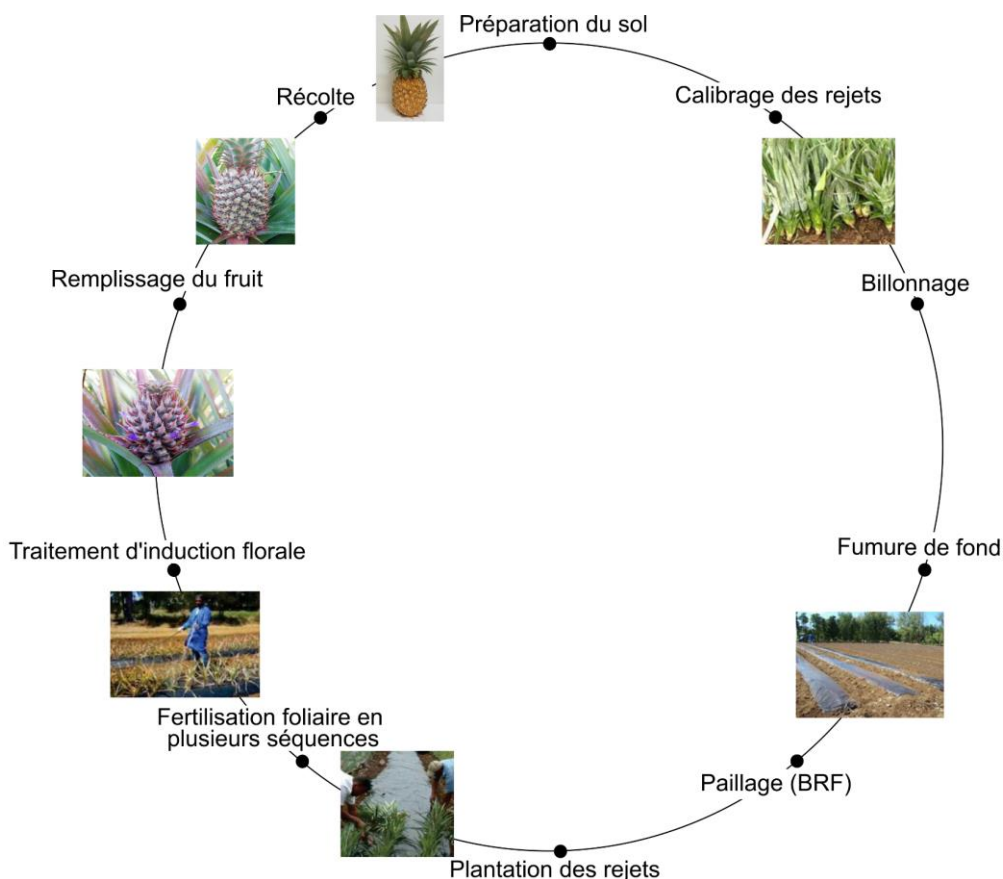


Figure 10. Principales étapes de l'itinéraire technique établi pour la culture de l'ananas à la Réunion. BRF, Bois Raméal Fragmenté; (photos : P. Fournier, M. Darnaudery et M. Vignassa).

La plantation s'effectue à partir de rejets sains et robustes. Les rejets sont générés au niveau du réceptacle supportant l'infrutescence dans les semaines suivant la récolte. Au cours de la phase de plantation, les rejets sont installés de manière intercalée selon une organisation en lignes multiples. Cette étape, effectuée à la main par les planteurs, permet de maximiser la densité de production.

La fertilisation est effectuée durant le développement végétatif qui précède le traitement d'induction florale (TIF). En effet, les apports en azote conditionnent la croissance du plant et *de facto* le poids du fruit à la récolte. Les amendements en potassium déterminent la teneur en sucres et l'acidité, facteurs constituant la qualité finale du fruit. Toutefois, un apport excessif d'azote peut favoriser l'occurrence de la maladie de la tache noire. Pour cette raison, il est donc conseillé de limiter les amendements aux étapes pré-TIF (Darnaudery *et al.*, 2016).

L'anthèse est initiée par une réduction de la photopériode associée à des températures fraîches. Toutefois, les cultivateurs ont largement recours au TIF cinq à sept mois après la

plantation. Cette étape permet de synchroniser la floraison et facilite l'organisation des périodes de récoltes. Le TIF est effectué par pulvérisation d'Ethrel®, composé dont la matière active (éthéphon) est décomposée en éthylène. Le développement des inflorescences est alors observé sous quatre à huit semaines selon la saison et l'altitude de la parcelle considérée. La date optimale de TIF est déterminée sur la base de la corrélation entre le poids des plants lors du TIF et le poids des fruits à la récolte. Le poids du plant est notamment estimé *via* la masse de la feuille adulte ayant achevée sa croissance (feuille D). Par ailleurs, le TIF permet une estimation du calibre des fruits à la récolte. En effet, le nombre de fruits simples constituant une infrutescence est défini dès l'anthèse. Une masse de feuille D comprise entre 45 et 50 grammes est ainsi conseillée pour l'obtention de fruits répondant au calibre export. La date de floraison vraie est ensuite déterminée lorsqu'au moins 50% des inflorescences présentent une corolle épanouie.

Pour l'ananas 'Queen Victoria', la durée du cycle de culture est comprise entre 11 et 20 mois en fonction de la saison de plantation et du contexte pédoclimatique de la parcelle. La définition du stade de récolte doit permettre de répondre aux attentes en termes de qualités gustatives et de résistance à la conservation. Chez le cultivar 'Queen Victoria', la maturité effective est directement corrélée à la coloration externe de l'épiderme du fruit. Les ananas destinés à l'export sont ainsi récoltés selon une coloration jaune étendue sur plus de la moitié de la hauteur du fruit.

3.1.2. La mécanique de l'ombre des genres *Fusarium* et *Talaromyces*

La maladie de la tache noire est étudiée depuis plusieurs décennies. Les connaissances acquises sur ce pathosystème ont donc évolué en concordance avec les avancées technologiques mises à disposition des scientifiques. Dans les années 80, l'expression de la tache noire était ainsi associée aux espèces *Penicillium funiculosum* et *Fusarium moniliforme* (Rohrbach and Pfeiffer, 1976 ; Lim and Rohrbach, 1980). Le développement des approches génomiques a permis d'importantes évolutions dans l'identification et la taxonomie des ascomycètes (Hawksworth and Lücking, 2018). Le séquençage de l'ADN ribosomique comportant les régions SSU (Small-Subunit, 18S), ITS (Internal Transcribed Spacer, ITS1 + 5.8S + ITS2) et LSU (Large-Subunit, 28S) a entraîné la re-classification de l'espèce *Penicillium funiculosum* en *Talaromyces funiculosus* (Samson *et al.*, 2011). De façon analogue, la révision

de la nomenclature taxonomique du complexe d'espèces *Fusarium fujikuroi* a reconsidéré la dénomination de *F. moniliforme* en *F. verticillioides* (Seifert *et al.*, 2003 ; Hawksworth, 2015).

Dans les années 70, les cultures d'ananas du Brésil et d'Hawaï furent soumises à une épidémie de fusariose. Cette pathologie causée par le pathogène *Fusarium guttiforme* engendre des symptômes dont les stades précoces peuvent être confondus avec la tache noire (Ventura *et al.*, 1993 ; Ploetz, 2006). L'observation de symptômes analogues en d'Afrique du Sud a entraîné une nouvelle évaluation des espèces de *Fusarium* pathogènes de l'ananas. Ainsi, des isolats provenant de fruits symptomatiques cultivés au Brésil et en Afrique du Sud ont été comparés par rapprochements phylogénétiques des régions TEF-1 α (Translation Elongation Factor-1 α), histone H3 et β -tubuline. L'analyse des séquences a permis de distinguer *Fusarium guttiforme* d'une nouvelle espèce de *Fusarium*, *Fusarium ananatum* sp. nov. alors respectivement définies comme pathogènes de la fusariose et de la tache noire (Jacobs *et al.*, 2010). La caractérisation de *Fusarium ananatum* a mené à son identification sur des ananas en provenance du Costa-Rica, d'Equateur, du Honduras et de Hawaï (Stępień *et al.*, 2013). Son implication dans l'expression de la tache noire a par la suite été rapportée en Chine, à l'île Maurice ainsi qu'à l'île de la Réunion (Gu *et al.*, 2015 ; Barral, 2017).

3.1.2.1. Processus infectieux

Pour l'heure, la littérature fait uniquement état du processus infectieux du champignon saprophyte *P. funiculosum* (*Talaromyces funiculosus*). La compréhension des différentes phases de progression de la maladie résulte d'une étude menée en Côte-d'Ivoire par Xavier Mourichon (Mourichon, 1997).

La contamination des parcelles par ce pathogène est estimée à la cinquième semaine suivant le TIF et avant l'observation de la floraison vraie. Durant cette période, des températures comprises entre 16 et 21 °C favorisent le développement de l'infection (Rohrbach and Pfeiffer, 1976 ; Rohrbach *et al.*, 2003). La phase initiale d'infection est observée lors de la floraison. Le pathogène se propage dans le style et se développe dans les canaux nectarifères qui constituent un substrat d'intérêt de par leur sécrétion de composés nourriciers (Rohrbach *et al.*, 2003). Au cours de cette phase, un brunissement des zones inter-carpellaires se produit (Figure 11).

Les tissus jeunes se lignifient permettant de restreindre la propagation du pathogène dans les tissus hôtes. Ce cloisonnement engendre une phase de latence chez le champignon.

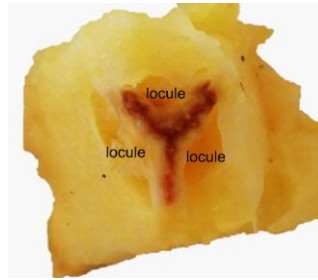


Figure 11. Coupe transversale de fruit simple présentant un brunissement de la zone intercarpellaire (photo : M. Vignassa).

Le pathogène parvient toutefois à contourner les défenses de l'hôte en tirant profit de l'accumulation de sucres et d'acide citrique durant la maturation du fruit. En effet, ces composés constituent une source de carbone pour le métabolisme associé à la croissance du champignon (Mourichon, 1997 ; Khosravi *et al.*, 2015). De plus, la culture *in vitro* de *P. funiculosum* démontre un développement pH-dépendant. En effet, un pH inférieur ou égal à 4 est décrit comme optimal pour la croissance du pathogène par opposition aux pH alcalins. Ces niveaux d'acidité ne sont mesurés chez l'ananas qu'à maturité complète (Mourichon, 1997). Les symptômes de la tache noire ne sont alors visibles qu'une fois la maturation du fruit achevée.

3.1.2.2. Diversité des contaminants fongiques et distribution géographique

Le climat subtropical de la Réunion est idéal pour le développement de maladies cryptogamiques. Avec la progression des terres arables destinées à la culture de l'ananas, la tache noire est devenue une problématique majeure du paysage agricole réunionnais. En 2010, l'ananas représentait 357 hectares d'exploitation, soit 13% des surfaces de cultures fruitières de l'île (Direction de l'alimentation, de l'agriculture et de la forêt de la Réunion, 2014).

De récents travaux menés par le CIRAD ont permis d'identifier les espèces fongiques majoritairement établies au sein de fruits simples naturellement infectés. Cette étude a été effectuée sur dix exploitations réparties au nord-nord-est et au sud-sud-ouest de l'île.

Bien que *F. ananatum* constitue l'unique pathogène décrit comme responsable de la tache noire (Jacobs *et al.*, 2010 ; Gu *et al.*, 2015 ; Barral *et al.*, 2017), quatre espèces ont pu être identifiées : *Fusarium ananatum*, *Fusarium oxysporum*, *Fusarium proliferatum* et *Talaromyces stollii*. *Fusarium ananatum* représente l'espèce prédominante sur l'ensemble des aires de production avec une fréquence relative de 72%. La campagne d'échantillonnage a également permis de caractériser *Talaromyces stollii* comme la seconde espèce majoritaire (21%). *Fusarium oxysporum* et *Fusarium proliferatum* ont été identifiées à de plus faibles fréquences (Barral *et al.*, 2020). D'après ces données, *Talaromyces stollii* est exclusivement représenté sur les exploitations à l'est de l'île, région présentant la pluviométrie la plus élevée. Les espèces du genre *Fusarium* appartiennent à la division des ascomycètes ainsi qu'à la famille des Nectriaceae. *Talaromyces stollii* est un ascomycète saprophyte appartenant à la famille des Trichocomaceae. Par ailleurs, *F. ananatum* et *F. proliferatum* constituent également les espèces les plus fréquemment isolées sur des fruits en provenance des pays suivants : Vietnam, Hawaï, Costa-Rica, Equateur et Honduras. Parmi ces lots d'ananas, des espèces telles que *Fusarium concentricum*, *Fusarium fujikuroi*, *Fusarium incarnatum* et *Fusarium polyphialidicum* ont été identifiées de façon ponctuelle (Stępień *et al.*, 2013).

Des cavités florales d'ananas asymptomatiques correspondant au cultivar résistant 'MD-2' et sensible 'Queen Vitoria' ont été étudiées par microscopie électronique. Cette approche révèle la présence de conidies de forme ellipsoïdale associées à un faible développement mycélien pour les fruits du cultivar 'MD-2' cultivés au Costa-Rica (Barral *et al.*, 2019b). La morphologie de ces conidies a été décrite comme caractéristique du genre *Talaromyces* (Yilmaz *et al.*, 2012). A l'inverse, des conidies fusiformes et un réseau mycélien dense tapissent les cavités florales du cultivar 'Queen Victoria' cultivé à la Réunion et sur l'île Maurice (Barral *et al.*, 2019b). Dans ces conditions, la morphologie des conidies permet de confirmer la présence d'espèces relatives au genre *Fusarium* dans les cavités florales de ce cultivar (Leslie and Summerell, 2006). Ces éléments démontrent qu'une contamination de la cavité florale n'engendre pas nécessairement l'établissement de la maladie.

3.2. Déterminants de la sensibilité de l'ananas 'Queen Victoria'

3.2.1. Caractéristiques morpho-anatomiques

Les structures anatomiques des fruits constituent les premières défenses rencontrées par le pathogène. La comparaison histologique de fruits simples issus des cultivars 'MD-2' et 'Queen Victoria' révèle des dissimilarités de structure et de composition des tissus hôtes (Barral *et al.*, 2019b). En effet, durant les jours suivants l'anthèse, la fusion des trois sépales et de la bractée s'effectue de manière imparfaite chez les fruits du cultivar 'Queen Victoria' (Figure 12).

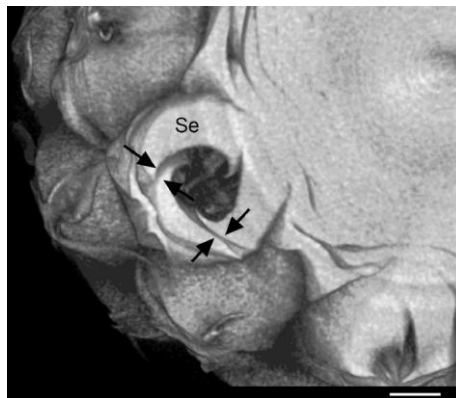


Figure 12. Coupe longitudinale de cavité florale d'ananas 'Queen Victoria' (Barral *et al.*, 2019b). Les flèches noires indiquent les zones de fusion imparfaite entre les sépales; Se, sépale; Echelle = 1 cm.

Des espaces libres pouvant mesurer au-delà de 100 μm sont distingués entre les zones de rencontre des sépales. Cette particularité facilite la pénétration dans les cavités florales de nombreux organismes tels que les fourmis et les cochenilles qui peuvent constituer des vecteurs de spores fongiques.

De plus, l'épiderme des cavités florales présente une couche épaisse et continue de lignine chez le cultivar 'MD-2'. Les cellules épidermiques de ce cultivar disposent également d'une paroi épaisse. Ces structures pariétales se caractérisent par une accumulation de composés phénoliques dont les acides férulique et coumarique sont les plus abondants (Barral *et al.*, 2019b). Ces composés sont notamment impliqués dans la biosynthèse de la lignine ainsi que dans la structuration des parois lignifiées (Lam *et al.*, 2001 ; Dixon and Barros, 2019). A l'inverse, les cavités florales du cultivar 'Queen Victoria' se caractérisent par une lignification faible voire discontinue de l'épiderme. Cette divergence structurale est également observée

au niveau des zones de délimitation des carpelles. En effet, les trois loges carpellaires sont parfaitement soudées chez le cultivar 'MD-2' tandis que la fusion est imparfaite chez le cultivar 'Queen Victoria' dont les nectaires restent ouverts (Figure 13). Ces structures suggèrent ainsi des voies facilitatrices pour les mécanismes de propagation et de colonisation des tissus hôtes par les champignons pathogènes.

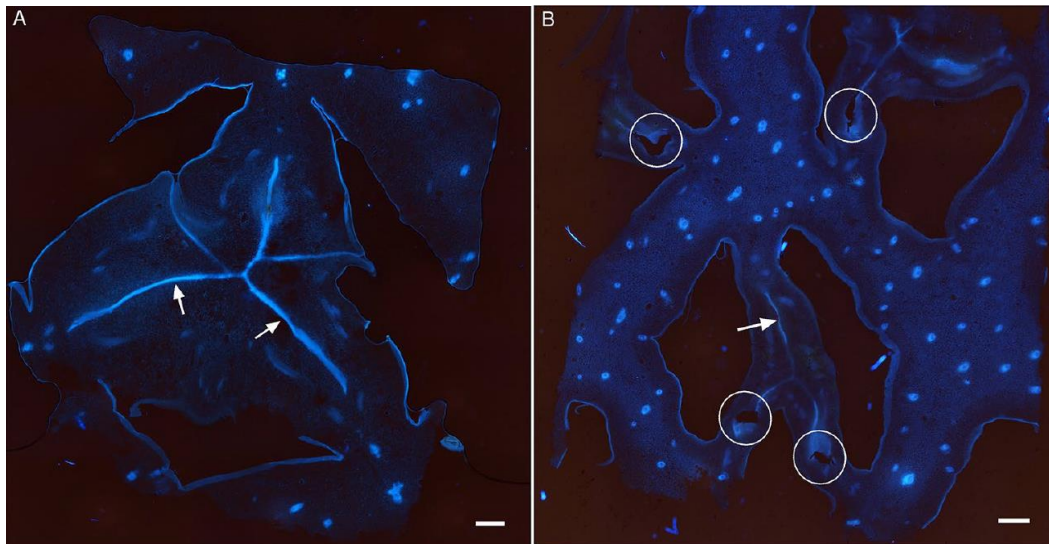


Figure 13. Images d'autofluorescence de coupes transversales de fruits simples d'ananas visualisées par microscope à épifluorescence (Barral *et al.*, 2019b). (A) Cultivar résistant 'MD-2', (B) Cultivar sensible 'Queen Victoria' ; les flèches blanches indiquent la zone de fusion entre les loges carpellaires ; les cercles blancs désignent les nectaires.

Dans le cadre d'inoculations contrôlées, la progression de *F. ananatum* est observée au travers du parenchyme sans que le phloème ou le xylème ne soient atteints (Barral *et al.*, 2019b). La colonisation des tissus par le pathogène s'effectue préférentiellement par l'apoplasme. Il est toutefois possible d'observer une pénétration du milieu intracellulaire de l'hôte pour la ponction de ses ressources nutritives. Le respect de l'intégrité du système vasculaire de l'ananas explique que les symptômes de la tache noire soient restreints aux fruits simples sans atteinte du cœur ou de l'ensemble de l'infrutescence. Au cours du processus infectieux, le suivi histochimique des réponses du cultivar résistant démontre une accumulation d'acide férulique et d'acide coumarique parmi les membranes nectarifères qui parcourent le parenchyme (Barral *et al.*, 2019b). Ces structures parviennent à contenir le processus de colonisation du pathogène durant les six premiers jours de l'interaction. Toutefois, cette modification structurelle est absente chez le 'Queen Victoria', présentant dès lors un

développement mycélien dans tout ou partie de son parenchyme. La colonisation des tissus hôtes s'accompagnent d'importants changements dans la biochimie du fruit simple notamment en termes de composés phénoliques.

3.2.2. Accumulation de composés phénoliques

Suite à l'initiation du dialogue moléculaire entre le champignon pathogène et son hôte, des réponses métaboliques spécifiques sont engagées. L'inoculation de *F. ananatum* induit chez les cultivars sensible et résistant une accumulation significative de coumaroylisocitrate et de caffeoylisocitrate (Barral *et al.*, 2019a). Chez les plantes, ces composés constituent des précurseurs de la lignine et sont impliqués dans les mécanismes de réponses aux stress (Vanholme *et al.*, 2010 ; Varbanova *et al.*, 2011). *In vitro*, les acides coumarique, caffeoylquinique et férulique présentent des potentiels antifongiques sur la croissance et la toxigenèse de *F. ananatum* (Barral *et al.*, 2017). Les mycotoxines produites par le pathogène sont alors quantifiées à des concentrations analogues à celles déterminées pour des fruits naturellement infectés par le pathogène (Barral *et al.*, 2020). Toutefois, les niveaux d'acides hydroxycinnamiques décrivent une élévation plus précoce et pour des concentrations plus élevées chez le cultivar résistant par opposition au cultivar 'Queen Victoria' (Barral *et al.*, 2019a). Ces profils d'accumulation d'acides hydroxycinnamiques sont également décrits dans le contexte de la tavelure du pommier causé par le champignon pathogène *Venturia inaequalis* (Mikulič Petkovšek *et al.*, 2009). Par ailleurs, l'accumulation constitutive de composés phénoliques des fruits sains au cours de la maturation révèle d'importants contrastes entre les cultivars 'MD-2' et 'Queen Victoria'. En effet, les concentrations basales en coumaroylisocitrate et en acides hydroxybenzoïques sont respectivement cinq et quatre fois supérieures chez le cultivar résistant par opposition au cultivar sensible (Barral *et al.*, 2019a). Ainsi, ces éléments suggèrent que la sensibilité de l'ananas 'Queen Victoria' puisse être en partie conditionnée par de faibles niveaux de défenses constitutives en termes de profils métaboliques.

3.2.3. Influence des facteurs abiotiques

La compréhension des épidémies peut s'avérer complexe dans des régions subtropicales telles que la Réunion dont le climat est défini comme tropical humide. Cette île se caractérise notamment par une grande diversité de microclimats expliquée par le contexte insulaire et le relief. La culture de l'ananas est influencée par deux saisons : la saison des pluies ou « saison cyclonique » de décembre à avril, caractérisée par une forte pluviométrie et des températures comprises entre 23 et 30°C (minima et maxima moyens) en zone côtière, et la saison sèche ou « hiver austral » de mai à novembre associée à une faible pluviométrie, des températures oscillant entre 18 et 27°C (minima et maxima moyens) en zone côtière et des vents soumis au flux d'alizés (Météo-France Réunion).

L'hiver austral constitue la saison pour laquelle les niveaux d'infection de la tache noire sont les plus élevés. Ces profils d'incidence sont corrélés aux conditions climatiques au cours des semaines suivant l'induction florale (Mourichon *et al.*, 1987). En effet, la combinaison d'une forte pluviométrie et de températures comprises entre 16 et 20 °C favorise la décharge de l'inoculum (Mourichon *et al.*, 1983). Parmi les aires de productions hawaïennes, les périodes clés de la contamination ont été déterminées pour plusieurs cultivars dont le 'Cayenne Lisse'. L'occurrence de la tache noire a ainsi été corrélée au cumul des heures fraîches (16-21°C) et aux précipitations au cours des quatre semaines suivant l'induction florale (Rohrbach and Taniguchi, 1984). A l'inverse, la faible incidence de la tache noire sur les ananas 'Queen Victoria' cultivés en Côte d'Ivoire démontre que des températures élevées permettent de prévenir ces contaminations (Teisson, 2002). En 2015, la présence d'espèces appartenant aux genres *Penicillium* et *Fusarium* a pu être rapportée de la plantation à la récolte sur des parcelles d'ananas localisées à l'est et au sud-ouest de la Réunion (Fournier *et al.*, 2015). Toutefois, cette étude démontre que l'occurrence de la tache noire n'est pas exclusivement conditionnée par les conditions climatiques lors des phases de contamination. En effet, l'établissement d'un modèle pluviothermique basé sur la pluviométrie et les températures moyennes montre que l'expression des symptômes est associée aux conditions climatiques intervenant jusqu'à la récolte. Ce modèle a également mis en évidence que l'incidence de la tache noire est positivement corrélée à l'altitude. En altitude, les paramètres météorologiques relevés à la Réunion sont en effet éloignés des conditions de cultures tropicales.

4. Problématique scientifique et objectifs de travail

Les préoccupations écologiques actuelles illustrent la nécessité de compréhension des interactions hôte-pathogène touchant les cultures d'intérêt agronomique afin de définir de nouveaux leviers de résistance efficaces et durables. L'étude bibliographique précédente démontre la complexité de ces relations plante – champignon pathogène et des nombreux paramètres qui les régissent. Particulièrement, la culture de l'ananas est confrontée depuis plusieurs décennies à la problématique de la tache noire. A la Réunion, cette pathologie fongique constitue une contrainte économique majeure entraînant chaque année le refus de lots destinés à l'export vers le marché métropolitain ou au marché local. Les ananas symptomatiques génèrent également de nombreux questionnements de la part des consommateurs locaux quant aux potentiels risques sanitaires encourus. Par ailleurs, l'ananas 'Queen Victoria' est considéré comme une production fruitière faisant partie intégrante du patrimoine de l'île. Les réunionnais ne souhaitent pas le voir disparaître au profit d'une variété résistante telle que le 'MD-2'. C'est dans ce contexte que ces travaux de thèse ont été réalisés au sein de l'Unité Mixte de Recherche QualiSud.

Les recherches menées au cours de mon doctorat ont eu pour objectif de caractériser les déterminants de la sensibilité de l'ananas 'Queen Victoria' à la maladie de la tache noire. Pour ce faire, une approche globale du processus infectieux a été entreprise sur chacune des composantes du pathosystème. Ces connaissances constitueront de nouveaux leviers pour la prévention et la gestion de la maladie de la tache noire tout en assurant la pérennité et la qualité des productions d'ananas 'Queen Victoria' à la Réunion.

Cette démarche a ainsi été structurée sur la base de trois questions de recherche :

- i) Quelle est l'influence des facteurs environnementaux sur la pression infectieuse ?
- ii) Quelle communication biochimique est établie au cours des interactions entre les pathogènes ?
- iii) Quelles sont les réponses génétiques permettant d'expliquer la sensibilité de l'ananas 'Queen Victoria' ?

Ce travail de thèse ambitionne de répondre à ces trois questions de recherche et s'articule selon les chapitres suivants :

Objectif 1 : Evaluation contexte-spécifique de la pression infectieuse au champ.

Cet objectif tend à déterminer l'influence des facteurs environnementaux sur la flore fongique de l'ananas 'Queen Victoria' en corrélation avec l'occurrence de la tache noire. La première phase de cette étude a porté sur la caractérisation des mycobiomes de fruits simples sains ou infectés *via* des approches de microbiologie classique et de génétique d'identification. Les abondances relatives des espèces fongiques identifiées ont ensuite été contextualisées selon les paramètres biotiques et abiotiques constituant l'environnement des parcelles expérimentales. Cet objectif correspond au premier chapitre de cette thèse et a permis la rédaction d'un premier article intitulé « ***Pineapple mycobiome related to Fruitlet Core Rot occurrence and the influence of fungal species dispersion patterns*** » publié en février 2021 dans la revue *Journal of Fungi*.

Objectif 2 : Caractérisation du dialogue inter-pathogènes à l'échelle du métabolisme secondaire.

Le second objectif de cette thèse tend à évaluer l'impact de la co-occurrence des agents pathogènes sur la structuration du mycobiome et la modulation de la virulence. Cette étude a tout d'abord permis de définir la nature des interactions intervenant entre les pathogènes de la tache noire. Pour ce faire, une approche par co-cultures a été mise en œuvre sur une cinétique de 10 jours visant à déterminer l'influence des confrontations inter-pathogènes sur leur capacité de colonisation et leur potentiel toxigène. Cet objectif correspond au second chapitre de ce manuscrit et a permis la rédaction d'un deuxième article intitulé « ***Interactions between fungal pathogens of pineapple Fruitlet Core Rot modulate their mycotoxigenic potential*** » soumis le 01 avril 2021 dans la revue *Toxins*.

Objectif 3 : Détermination des facteurs génétiques impliqués dans la sensibilité de l'ananas 'Queen Victoria' à la maladie de la tache noire.

Le troisième objectif de ce manuscrit interroge le rôle du génotype dans la mise œuvre des réponses de défense au cours des stades précoces de l'interaction ananas – *F. ananatum*.

Dans un premier temps, cet axe de recherche a été basé sur la sélection de huit gènes candidats impliqués dans les voies de signalisation de l'acide salicylique, de l'acide jasmonique et de l'éthylène. L'expression relative de ces facteurs d'intérêt a ensuite été étudiée par RT-qPCR sur la base d'une comparaison variétale entre le cultivar résistant 'MD-2' et le cultivar sensible 'Queen Victoria'. Cet objectif est développé dans le troisième chapitre de ce manuscrit de thèse et a permis la rédaction d'un troisième article intitulé « **Early genetic markers of 'Queen Victoria' pineapple susceptibility to Fruitlet Core Rot** » qui sera soumis dans la revue *Molecular Plant-Microbe Interactions (MPMI)*.

Enfin, le dernier chapitre de ce manuscrit sera destiné à la discussion conjointe de l'ensemble des résultats obtenus au cours de ce travail de thèse. De futures questions de recherche portant sur le déterminisme des facteurs de résistance et de sensibilité seront également proposées.

CHAPITRE I

Approche contexte-spécifique de
l'épidémiologie de la tache noire à la
Réunion

**Article 1 - Pineapple mycobiome related to Fruitlet
Core Rot occurrence and the influence of fungal
species dispersion patterns**

Au cours d'une récente étude portant sur l'épidémiologie de la tache noire à la Réunion, la distribution des profils de prévalence des espèces fongiques appartenant aux genres *Fusarium* et *Talaromyces* a été établie sur plusieurs aires de production. L'agent pathogène *Fusarium ananatum* a été défini comme l'espèce majoritaire sur l'ensemble des exploitations agricoles suivi par *Talaromyces stollii*, toutefois exclusivement identifiée dans les régions nord-est et sud-sud-est de l'île (Barral *et al.*, 2020). Ces données ont suggéré une influence directe des conditions pédoclimatiques et des pratiques culturales sur la constitution du mycobiome des fruits et *de facto* sur l'occurrence de la maladie de la tache noire.

Le présent chapitre repose sur l'article intitulé « **Pineapple mycobiome related to Fruitlet Core Rot occurrence and the influence of fungal species dispersion patterns** » publié dans *Journal of Fungi*. Au cours de cette étude, les mycobiomes de fruits simples sains et présentant les symptômes caractéristiques de la tache noire ont été caractérisés. L'influence du contexte parcellaire (espèces cultivées à proximité, orientation de billons) et des conditions météorologiques sur la composition des mycobiomes a été étudiée. Les résultats obtenus ont montré d'importantes similarités dans la composition des mycobiomes de fruits simples sains et symptomatiques. L'identification de 344 isolats appartenant à 49 espèces a permis de décrire les espèces *Fusarium proliferatum*, *Fusarium ananatum*, *Fusarium oxysporum*, *Talaromyces stollii* et *Talaromyces amestolkiae* comme majoritaires sur la base de leur abondance relative évaluée après culture et isolement. La répartition de ces espèces a pu être positivement corrélée aux profils d'expression de la tache noire observés sur les parcelles expérimentales. Ainsi, les données obtenues ont permis de mettre en évidence le rôle de cinq nouvelles espèces dans le pathosystème de la tache noire. Par ailleurs, la contextualisation des profils de dispersion des agents pathogènes a montré pour la première fois l'implication d'un flux d'inoculum aérien potentiellement conditionné par des contaminations croisées entre les différentes espèces végétales cultivées à la Réunion.

Article

Pineapple Mycobiome Related to Fruitlet Core Rot Occurrence and the Influence of Fungal Species Dispersion Patterns

Manon Vignassa ^{1,2,*}, Jean-Christophe Meile ^{1,2}, Frédéric Chiroleu ³, Christian Soria ^{1,2},
Charlène Leneveu-Jenvrin ⁴, Sabine Schorr-Galindo ² and Marc Chillet ^{1,2}

¹ Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), UMR Qua-lisud, F-97410 Saint-Pierre, 97410 Réunion, France; meile@cirad.fr (J.-C.M.); christian.soria@cirad.fr (C.S.); marc.chillet@cirad.fr (M.C.)

² Unité Mixte de Recherche Qualisud, Université de Montpellier, Avignon Université, Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Institut Agro, Institut de Recherche pour le Développement, Université de La Réunion, Montpellier, France; sabine.galindo@umontpellier.fr

³ Centre de coopération internationale en recherche agronomique pour le développement CIRAD, UMR PVBMT, 97410 Saint-Pierre, F-97410 La Réunion, France; frederic.chiroleu@cirad.fr

⁴ Unité Mixte de Recherche Qualisud, Université de La Réunion, Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Université de Montpellier, Institut Agro, Avignon Université, Sainte-Clotilde, France; charlene.leneveujenvrin@yahoo.fr

* Correspondence: manon.vignassa@cirad.fr; Tel.: +262-2-62-49-28-62

Abstract: Fruitlet Core Rot (FCR) is a fungal disease that negatively impacts the quality of pineapple, in particular the ‘Queen Victoria’ cultivar. The main FCR causal agent has been identified as *Fusarium ananatum*. This study focused on the correlation between FCR disease occurrence, fungal diversity, and environmental factors. FCR incidence and fungal species repartition patterns were spatially contextualized with specific surrounding parameters of the experimental plots. The mycobiome composition of healthy and diseased fruitlets was compared in order to search for potential fungal markers. A total of 240 pineapple fruits were sampled, and 344 fungal isolates were identified as belonging to 49 species among 17 genera. FCR symptom distribution revealed a significant gradient that correlated to that of the most abundant fungal species. The association of wind direction and the position of proximal cultivated crops sharing pathogens constituted an elevated risk of FCR incidence. Five highly represented species were assayed by Koch’s postulates, and their pathogenicity was confirmed. These novel pathogens belonging to *Fusarium fujikuroi* and *Talaromyces purpureogenus* species complexes were identified, unravelling the complexity of the FCR pathosystem and the difficulty of apprehending the pathogenesis over the last several decades. This study revealed that FCR is an airborne disease characterized by a multi-partite pathosystem.

Keywords: *Fusarium*; pathogenic fungi; pineapple disease; *Talaromyces*



Citation: Vignassa, M.; Meile, J.-C.; Chiroleu, F.; Soria, C.; Leneveu-Jenvrin, C.; Schorr-Galindo, S.; Chillet, M. Pineapple Mycobiome Related to Fruitlet Core Rot Occurrence and the Influence of Fungal Species Dispersion Patterns. *J. Fungi* **2021**, *7*, 175. <https://doi.org/10.3390/jof7030175>

Academic Editor: Lei Cai

Received: 28 January 2021

Accepted: 24 February 2021

Published: 28 February 2021

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Ananas comosus has become a highly appreciated fruit and is considered the best tropical fruit in terms of world exportation volumes, with 3.2 million tons in 2019 [1]. Pineapple production is affected by numerous pests and diseases, including Fruitlet Core Rot (FCR). The infection leads to brown discoloration of the flesh, impacting fruit acceptability by the consumers. FCR occurrence in the field of tropical and subtropical areas impacts pineapple post-harvest quality and both local and export markets. Current strategies focus on the creation of new cultivars trying to combine desired sensorial qualities and an optimal level of resistance to pathogens, as with the ‘MD-2’ cultivar [2]. Nevertheless, the ‘Queen Victoria’ cultivar still remains a favorite, especially for its flavor, in the Indian Ocean, as well as in Europe and some parts of Asia, despite its high susceptibility to a broad spectrum of pathogens [3]. Disease incidence and severity have increased in the producing regions, resulting in significant fruit quality depreciation and thus economic concerns [4]. The FCR

pathosystem was originally considered as the relation between *Ananas comosus* var. *comosus* and the fungus *Fusarium verticillioides* (syn. *Fusarium moniliforme*) associated with *Penicillium funiculosum* [5–7]. However, the evolution of molecular tools led to the identification of *Fusarium ananatum* and *Talaromyces funiculosus* as causal agents of FCR in China, South Africa, Mauritius, and Reunion Island [4,8–11]. Contamination by soil-borne pathogens (such as *P. funiculosum*) occurs at pre-flowering stages, and the fungus remains latent in a blossom cup. During fruit development, causal agents spread into septal nectaries, but propagation is restricted by the lignification of young plant tissues. The accumulation of sugar following fruit ripening finally enables the fungus to counterbalance the plant defenses and then colonize host tissues [12]. These events trigger the browning of the flesh, whose expansion is limited to the fruitlet, without reaching the core or the skin of the pineapple fruit. Those symptoms are defined as ‘black spots’, thus constituting a typical marker of FCR-infected fruitlets. Nevertheless, the disease incidence could not be directly evaluated during pineapple cultivation or after harvesting due to the absence of external symptoms. The complexity of the FCR pathosystem relies on the presence of both diseased and healthy fruitlets within the same pineapple fruit, thus suggesting that pathogenesis could be driven by a specific microbiota. Thus far, the critical biotic parameters that mediate pineapple immunity or susceptibility remain unknown.

Plant diseases are closely related to numerous biotic and abiotic factors. Fungal species can be found in association with various plant species leading to a large-scale distribution and high levels of diversity according to their habitat [13–15]. Considering the wide range of pineapple crop systems and abiotic environments, fungi identified as belonging to *Trichoderma* spp. have been isolated in higher relative abundances in leaf and leaf sheath tissues when compared to roots and rhizospheric areas. By contrast, these plant sections are characterized by a microbiota, at least 70% of which is composed of bacterial species [16]. Nonetheless, there has been no study about fungal taxa richness and diversity associated with pineapple. Among abiotic factors, previous work has shown a link between FCR incidence and specific climatic conditions, which allowed for the elucidation of a predictive model based on a pluviothermic index. Predictions showed that, in subtropical regions (such as Reunion Island), the combination of a high altitude, high rainfall and cool temperatures (16 °C to 27 °C) led to an elevated risk of harvesting fruits with FCR symptoms [17,18]. In spite of this, the impact of environmental factors on the fruit mycobiome and pathogen dispersion patterns remains a ‘black box’ in the fate of this plant–pathogen interaction. Further studies on inoculum availability have reported the prevalence of disease severity related to the spatial distribution pattern on mango and banana crops [19,20]. Current ecological concerns have induced the development of alternative strategies for managing the spreading of fungal pathogens. To this end, multi-scale assays are conducted in order to fit with environmental changes and their impact on microbial epidemics [21,22]. The few studies dealing with short-distance dispersal patterns have mostly focused on vineyard [23], mango [19], and apple orchards [24]. Those data demonstrated the impact of soil and row management on conidia dispersion dynamics. One of the relevant aspects of estimating the infection pressure is the relation between the pineapple plot and its surrounding environment, notably the concomitant crops. Indeed, fungal isolates belonging to *Fusarium* genus are able to infect a broad spectrum of hosts such as fruits, small grain cereals, and horticultural plants [25–27].

Based on these assumptions, the main goal of the present study was to characterize the fungal flora of healthy and naturally infected pineapple fruitlets. From the data obtained, fungal species distribution was linked to the proximal environment of the pineapple plots. We also investigated the potential co-occurrence between fungal species as potential key factors for disease development. Thanks to a context-specific approach, we report here for the first time the contribution of five new pathogenic fungal species belonging to *Fusarium* and *Talaromyces* genera in FCR incidence in pineapple cv. ‘Queen Victoria’.

2. Materials and Methods

2.1. Plant Material and Agricultural Practices

The experiment was conducted on the ‘Queen Victoria’ pineapple cultivar in accordance with the conventional cultural practices locally applied [28]. The plots were located in the southwest of Reunion Island at the Experimental Research Station of CIRAD Bassin-Plat, Saint-Pierre, located at an altitude of 150 meters above sea level (21°19′21″ S, 55°29′26″ E). The surface was divided into 2 plots with perpendicular row directions. Thereby, row orientations were North-West/South-East (N-W/S-E) and North-East/South-West (N-E/S-W) for Plot 1 and Plot 2, respectively (Figure 1). Each of the 2 experimental plots were subdivided into 3 sections (2 rows per section) along an N-E/S-W axis corresponding to rows adjacent to the mango orchard (1), central rows (2), and rows proximal to jackfruit trees (3). Each section was subdivided into 4 quadrats of 4 m long ($n = 24$). Meteorological data from flower induction treatment (FIT) in April 2018 to harvest in November 2018 were considered [29]. The wind data collection was performed by the Météo-France weather station located in Pierrefonds [30], Saint-Pierre, approximately 6 km away from the plots (Table 1). A total of 240 fruits were harvested at the C1 stage, corresponding to 1/4 yellow fruit according to the shell’s color. The ripening was completed at 19 °C in a cold room until the C4 stage, when pineapples became entirely yellow.

2.2. FCR Symptom Occurrence and Fruitlets Sampling

Ten fruits per quadrat were collected. Each fruit was cut, and the number of black spots (infected fruitlets) was counted and recorded. FCR occurrence per fruit was then calculated for each section of each of the 2 plots.

Fruitlets sampling was performed on 96 fruits randomly chosen (4 fruits per quadrat). For each pineapple fruit, 1 healthy-looking fruitlet (HF) and 1 naturally infected fruitlet (IF) were sampled with sterile equipment for a total of 192 fruitlets.



Figure 1. Environmental context of pineapple plots located in Reunion Island. Plot 1: North-West/South-East-directed rows; Plot 2: South-West/North-East-directed rows. (1) Plot sections proximal to the mango orchard, (2) central section of the plots, and (3) plot sections near the jackfruit trees. Quadrats are shown as yellow rectangles. White asterisks indicate coring points.

Table 1. Meteorological factors recorded from FIT (Flower Induction Treatment) in April 2018 to harvest in November 2018. Source of satellite image: <https://www.geoportail.gouv.fr/> (accessed on 28 January 2021).

| | Min Temp (°C) | Average Temp (°C) | Max Temp (°C) | Average Rainfall (mm) | Number of Day(s) with Rain | Wind Direction | Max Gust Speed (km h ⁻¹) |
|-----------|---------------|-------------------|---------------|-----------------------|----------------------------|-----------------|--------------------------------------|
| April | 20.12 | 23.19 | 27.85 | 15.78 * | 10 | south | 116 * |
| May | 18.42 | 22.20 | 27.48 | 0.66 | 5 | east-south-east | 85 |
| June | 17.10 | 20.68 | 25.72 | 2.47 | 8 | south-east | 73 |
| July | 15.53 | 19.50 | 24.64 | 4.46 | 12 | south-east | 80 |
| August | 17.07 | 21.10 | 26.60 | 0.00 | 0 | south-east | 75 |
| September | 16.64 | 21.15 | 27.60 | 0.15 | 1 | south-east | 80 |
| October | 17.65 | 21.75 | 27.79 | 3.85 | 8 | east-south-east | 88 |
| November | 18.98 | 23.49 | 28.66 | 0.03 | 1 | south-east | 69 |

(*) The tropical cyclone Fakir passed near the eastern coast of Reunion Island from 24 April to 26 April 2018.

2.3. Isolation of Cultivable Fungal Flora from Fruitlet and Soil Samples

2.3.1. Fruitlet Tissues

The pineapple fruitlets collected (Section 2.2.) were deposited on Sabouraud glucose agar Petri dish (Biokar diagnostic, Solabia, Allonne, France) supplemented with 100 mg L⁻¹ chloramphenicol at 27 °C in the dark. Fungal colony growth and isolation were processed daily following similar conditions until 5 days of incubation. Pure strains isolated were observed for morphological (colony aspect and color) and microscopic (macroconidia and microconidia) characterizations (Zeiss, Germany, model Axiostar Plus, magnification $\times 100$). These observations enabled the taxonomic assignation for each isolate at the genus level. Consequently, a conidia solution was prepared by adding 2 mL of SPW (Saline Peptone Water, Condalab, Torrejón de Ardoz, Madrid, Spain) to the Petri dish and gently scratching the mycelium surface with a sterile spreader. From the conidia solution, 1 mL was transferred into sterile cryovials with the addition of an equal volume of 40% glycerol (Sigma-Aldrich, Darmstadt, Germany) and stored at -80 °C. Another volume of 500 μ L was recovered for the molecular identification procedure and stored at -20 °C.

2.3.2. Soil

Soil sampling was conducted at 3 points 10 m apart on the 2 opposite junctions between the pineapple field and the surrounding crops: The jackfruit trees (North-East) and the mango orchard (South-West) (Figure 1). For each coring point (white asterisks), 1 g of a 5 cm soil layer was suspended in a sterile tube containing 9 mL of sterile physiological saline water with 9 g of NaCl supplemented with 1% Tween[®] 80 (Merck, Darmstadt, Germany). Soil suspensions were then placed into a shaking incubator for 30 min at 25 °C and 200 rpm. Two technical repetitions per coring point were performed. For microbial assays, serial decimal dilutions were conducted to 10^{-6} in physiological saline water supplemented with 1% Tween[®] 80. Fungi were isolated on PDA (Potato Dextrose Agar, BD Difco[™], Le Pont-de-Claix, France) supplemented with cycloheximide (10 mg L⁻¹, Merck, Darmstadt, Germany) and on Sabouraud glucose agar supplemented with Chloramphenicol 100 mg L⁻¹, after incubation for 5 days at 27 °C and 4 days at 30 °C, respectively. Fungal colonies were removed with a sterile scalpel and transferred into 1.5 mL sterile tubes with 500 μ L of SPW. Isolates were finally stored at -20 °C prior to molecular procedures.

2.4. Molecular Characterization of Cultivable Fungal Flora from Fruitlets and Soil Samples

According to the morphological analysis, genus-specific DNA primers were used to infer phylogenetic relationships between closely related genotypes of strains isolated from fruitlets.

PCR amplification of fungal DNA was performed on conidia solutions with no DNA extraction procedure. For *Fusarium* sp. genus, the EF1 α (TEF-1 α) reference gene region was amplified by PCR. For *Aspergillus* sp., *Penicillium* sp., and *Talaromyces* sp. genus, the β -tubulin gene region was targeted for PCR amplification. Afterward, when no amplification could be obtained with those specific primers, an amplification of the Internal Transcribed

Spacer (ITS) region was conducted with the ITS1F/ITS4 primer pair. The list of the primers used in this study is given in Table 2. The identification of soil fungal isolates was performed by PCR amplification of the Internal Transcribed Spacer (ITS) region.

Table 2. PCR primers sequences (forward and reverse) used for fungal strains identification.

| Sequence ID of Primers Pair | Target Locus | Sequence Forward (5'→3') | Sequence Reverse (5'→3') | Product Length (bp) | References |
|-----------------------------|--|--|------------------------------|---------------------|------------|
| ef1/ef2 | Translation Elongation Factor-1 α | ATGGGTAAGG AAGACAAGAC | GGAAGTACCA GTGATCATGTT | 380–680 | [31–33] |
| Bt2a/Bt2b | β -tubulin | GGTAACCAAAT CGGTGCTGCTTTC | ACCCTCAGTGTA GTGACCCTTGCC | 250–500 | [34] |
| ITS1F/ITS2 | Internal Transcribed Spacer 1 | CTTGTCATTT AGAGGAAGTAA | GCTGCGTTCT TCATCGATGC | 145–695 | [35,36] |
| ITS1F/ITS4 | | | TCCTCCGCT TATTGATATGC | | |
| GC-ITS1F/ITS4 | Entire Internal Transcribed Spacer | CGCCCCCGCGCGCGG CGGGCGGGGCGGGGG CACGGGGGGCTTGTT CATTAGAGGAAGTAA | TCCTCCGCTT ATTGATATGC | 600–800 | |

PCR amplification reactions were performed in a final volume of 50 μ L containing 0.3 μ M of each primer, all the deoxyribonucleotide triphosphates (dNTPs) at 0.2 mM, 2 mM MgCl₂, 10 μ L of 5x GoTaq Flexi buffer, 1.25 U GoTaq[®] G2 Flexi DNA polymerase (Promega, Charbonnières-les-Bains, France), and 2 μ L of conidia solution. Conditions for the PCR amplification of the TEF-1 α region were an initial denaturation at 95 °C for 3 min, 33 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension stage of 5 min at 72 °C. Similarly, PCR amplification reactions for the β -tubulin region were established as follows: An initial denaturation at 95 °C for 2 min, 30 cycles at 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR amplification reactions for the ITS region were carried out as follows: An initial denaturation at 95 °C for 2 min, 40 cycles at 95 °C for 15 s, 53 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 5 min. The PCR reactions were performed in a thermocycler (Veriti, Applied Biosystems, United Kingdom). PCR products were analyzed with the Qiaxcel[®] Advanced System (Qiagen, Hilden, Germany) using size markers 250 bp–4 kb.

The PCR products were sent to Macrogen (Amsterdam, The Netherlands) for purification and sequencing. The DNA sequences obtained were aligned with SnapGene v5.0 software, and identification was performed using a BLASTn similarity search. Sequences having a percentage of identity of at least 98% and 95% for fruit and soil isolates, respectively, and those with the lowest E-values were considered as belonging to the same species.

2.5. Koch's Postulates

2.5.1. Controlled Inoculations and Plant Material

Among fungal strains identified on infected fruitlets, 5 highly represented species were selected for pathogenicity assays. For each of the 5 species, 2–3 isolates were randomly tested according to the following correspondence: *Fusarium oxysporum* strains BP369 and BP460; *Fusarium proliferatum* strains BP114, BP429, and BP436; *Fusarium sacchari* strains BP138 and BP575; *Talaromyces stollii* strains BP054, BP185, and BP462; *Talaromyces amestolkiae* strains BP002, BP257, and BP605. First, strains of interest were grown on PDA for 7 days at 27 °C in the dark. For each isolate, an inoculum solution was prepared with sterile water as described in Section 2.3.1, and a final concentration was normalized at 10⁵ conidia per mL. Inoculations were performed on cv. 'Queen Victoria' pineapple fruits were harvested at the

C1 stage and grown according to locally recommended conventional agricultural practices. For each of the 13 tested strains, 3 fruits were inoculated. For each fruit, 3 fruitlets were inoculated by injecting 25 µL of inoculum in a blossom cup from the upper, median, or basal fruits parts. Inoculations were also conducted with *Fusarium ananatum* strain BP383 as a positive control and with sterile water as a negative control (H₂O), and a 2nd class of negative control fruits (Mock) was not inoculated for comparison to the initial fungal load of fruitlets. Fruits were then incubated for 7 days at 19 °C in a cold room. Thus, 16 different conditions (13 tested strains + 1 positive and 2 negative controls) were assayed on a total of 144 fruitlets from 48 pineapple fruits.

2.5.2. Fruit Sampling

After incubation, inoculated fruitlets were sampled. The 3 infected or control fruitlets of each fruit were pooled and placed in a sealed sterile lab blender bag and mixed with 10 mL of SPW in a stomacher for 2 min at maximal speed. Prior to storage at −80 °C, 5 mL of lysate were recovered and supplemented with an equal volume of 40% glycerol.

2.5.3. DNA Extraction

For each fruit sample and each pure fungal strain used for inoculation, 2 mL of lysate were collected in 2 mL sterile tubes and centrifuged at 14,000 × *g* for 2 min. DNA extractions were performed on the biomass pellets with the FastDNA SPIN kit and the FastPrep-24 Instrument (MP Biomedicals®, Illkirch, France) using Lysing Matrix A and Lysis Buffer CLS-Y in accordance with the manufacturer's instructions.

2.5.4. PCR-Denaturing Gradient Gel Electrophoresis (DGGE)

PCR amplifications were performed using the GC-ITS1F and ITS2 DNA primers (Table 2). A 40 bp GC-clamp was added to the 5' end of the ITS1F primer in order to ensure that the DNA fragment remains partially double-stranded and that the region screened was in the lowest melting domain [37]. This method was limited by the fragment length, which should not exceed 500 bp. In spite of this, a large fragment length resulted in a poor gel resolution complicating the recovery of DNA bands. For these reasons, PCR-DGGE was performed on the ITS1 region (~200 bp), conferring a good resolution but discrimination of fungal strains only at genus level. PCR reaction was performed in a final volume of 50 µL containing 0.6 µM of each primer, all the deoxyribonucleotide triphosphates (dNTPs) at 200 µM, 2 mM of MgCl₂, 10 µL of 5× GoTaq Flexi buffer, 1.25 U of GoTaq® G2 Flexi DNA polymerase, and 1 µL of extracted DNA. PCR amplification reactions were carried out as follows: An initial denaturation at 95 °C for 2 min, 40 cycles at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 20 s, and a final extension at 72 °C for 5 min. The PCR reactions were performed in a thermocycler (Veriti, Applied Biosystems, United Kingdom). PCR products were then analyzed as previously described.

The PCR products were separated by DGGE using a Cleaver Scientific system (Cleaver Scientific, Rugby, Warwickshire, United Kingdom). Briefly, 30 µL of PCR amplicons were loaded onto 8% (*w/v*) polyacrylamide gels (acrylamide: N,N-methylene bis-acrylamide, 37.5:1, Sigma-Aldrich, Darmstadt, Germany) in a 1× TAE buffer (40 mM Tris-HCl pH 7.4, 20 mM sodium acetate, 1.0 mM Na₂-EDTA). Electrophoresis was performed at 60 °C using a denaturing gradient ranging from 20% to 60% (100% denaturant corresponding to 7 M urea and 40% *v/v* formamide, Carlo Erba Reagents, Val-de-Reuil, France). The gels were run at 20 V for 10 min and then at 75 V for 16 h. After electrophoresis, the gels were stained for 1 h with ethidium bromide solution (50 µg mL^{−1} in 1× TAE), rinsed for 1 h in distilled water, and then visualized with a UV transilluminator (Bio-Rad®, Marnes-la-Coquette, France). Bands of interest were excised from the DGGE gels with a sterile scalpel as previously described [38]. Briefly, DNA of each band was then eluted in 100 µL of TE buffer (10 mM TrisHCl; 1 mM EDTA; pH 7.4, Sigma-Aldrich, Darmstadt, Germany) at 4 °C overnight. DNA was precipitated by adding 10 µL of sodium acetate (3 M, pH 5), 1 µL of glycogen (Molecular Grade, Roche Diagnostics, Meylan, France), and 300 µL of 100%

ethanol and centrifuged at $15,000\times g$ for 30 min at 4 °C. The supernatant was discarded, DNA pellets were washed with 500 μ L of 70% ethanol, and, after 5 min of centrifugation, the DNA pellets were air-dried for 1 h. Finally, the DNA was re-suspended in 20 μ L of TE buffer (10 mM TrisHCl; 1 mM EDTA; pH 7.4) and stored at -20 °C. PCR amplification reaction was performed on purified DNA with the ITS1F/ITS2 primer set. Reactions were carried out as follows: An initial denaturation at 95 °C for 2 min, 40 cycles at 95 °C for 15 s, 53 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 5 min. PCR products were analyzed and sequenced as previously described in Section 2.4.

2.6. Computational Analysis

2.6.1. Phylogenetic Analysis

The determination of phylogenetic relationships between fungal strains was conducted by considering each genomic region. Multiple alignments of the nucleotide sequences were performed with the MEGAX computer program and the MUSCLE automatic alignments method [39,40]. Subsequently, the jModelTest v2.1.7 program was used to select the nucleotide substitution model for each sequence set [41]. The maximum-Likelihood analysis was inferred with the PhyML v3.0 program implemented with 'ape' R package according to the best fitting model [42,43]. Branch supports were tested with 100 bootstrap replications. Trees were then visualized and edited with the the FigTree v1.4.4 program.

2.6.2. DGGE Band Pattern

Electrophoretic profiles were analyzed with CLIQS 1D Pro software (TotalLab, Newcastle-Upon-Tyne, United Kingdom). In the DGGE gel, for each sample (lane), the DNA band presence and its relative intensity were recorded. The Unweighted Pair-Group Method using Arithmetic Average (UPGMA) and Pearson coefficient correlation were used to build the dendrogram.

2.6.3. Statistical Analysis

Data were analyzed with the R statistical language v4.0.3 (R Core Team, 2020). The effect of row direction and proximal crops on the mean number of infected-fruitlets per fruit was tested with a Deviance test on a generalized linear model with Poisson distribution, and a Tukey's multiple comparison test. Principal Component Analysis (PCA) was computed with 'FactoMineR' on the centered-fungal species abundances with no scaling [44]. The 6 combinations between proximal crop and plot were considered as individuals. Results of PCA were then visualized with the 'factoextra' package [45]. Correlation between variables and each principal component of the factorial plane was tested at $p < 0.05$. Hierarchical clustering of species abundances in healthy and infected tissues was performed according to the Raup–Crick dissimilarity method from the 'vegan' package [46]. Species co-occurrence was computed with the 'cooccur' package based on a probabilistic model of calculations between expected and observed pairwise frequencies [47,48]. Correlation between species co-occurrence and fruitlet phenotype was assessed by a Mantel test on distance matrixes of each dataset.

3. Results

3.1. Fungal Flora of Healthy and Naturally Infected Fruitlets

The exploration of the mycobiome resulted in the identification of 170 isolates from healthy-looking fruitlets (HF), and 174 isolates from infected samples (IF) exhibiting black spots. In all, the 344 isolates belonged to 17 genera and represented a total of 49 different species. The relative abundance of the isolated fungal species identified five highly represented genera that corresponded to *Fusarium*, *Talaromyces*, *Aspergillus*, *Phialemoniopsis*, and *Trichoderma*, together supporting 90% (153 isolates) and 94% (164 isolates) of the cultivatable fungal flora of HF and IF, respectively (Table 3). The remaining isolates belonged to 12 genera (*Pestalotiopsis*, *Lasiodiplodia*, *Penicillium*, *Clonostachys*, *Diaporthe*, *Epicoccum*, *Bionectria*, *Cosmospora*, *Curvularia*, *Davidiella*, *Glomus*, and *Rhizopus*) with low relative abundances

ranging from 0.58% to 2.35%. Although these were sparse isolates, *Lasiodiplodia*, *Bionectria*, *Cosmospora*, *Davidiella*, and *Glomus* were exclusively recorded in HF. Contrariwise, isolates corresponding to *Curvularia* and *Rhizopus* could only be observed in IF. The contextualization of predominant species among studied conditions revealed a decrease in relative abundances for *F. proliferatum* and *T. amestolkiae*, from 16.5% and 7.6% in HF to 12.6% and 5.2% in IF samples, respectively. By contrast, the other highly represented species were characterized by an increase in their relative abundances in the IF samples. This trend was notably observed for *T. stollii* and *P. curvata*, which extended from 4.7% and 3.5% in HF to 9.2% and 6.9% in IF, respectively (Figure 2). Particularly, only one *Trichoderma* species (*T. paraviridescens*) was isolated, showing a low relative abundance (0.6%) in HF, while this genus was supported by five species in IF (*T. asperellum*, *T. erinaceum*, *T. harzianum*, *T. paraviridescens*, and *T. trixiae*) contributing to 4.6% of the identified fungal flora. Although *F. ananatum* was the most frequently described FCR-associated pathogen [10,11,49], its occurrence and relative abundances were similar in HF and IF, with 11.2% and 10.3%, respectively. Interestingly, *F. proliferatum*, *F. ananatum*, *F. fujikuroi*, *F. circinatum*, *F. oxysporum*, *F. sacchari*, and *F. verticillioides* all belonged to the *Fusarium fujikuroi* species complex (FFSC) [49], which represented 43.5% and 37.9% of HF and IF mycobiomes, respectively. Similarly, the *Talaromyces purpureogenus* species complex was also highly represented following the identification of *T. stollii*, *T. amestolkiae*, and *T. purpureogenus*, together gathering 15.3% of isolates in HF and 17.2% in IF. Thus, those two species complexes together contributed to 58.8% and 55.2% of the cultivatable fungal flora of HF and IF, respectively.

Table 3. Identification of fungal species and their respective occurrence in healthy and naturally infected fruitlets.

| Genus | Species | Occurrence | | Top Match GenBank Accession Number * (Frequency in Data) |
|------------------------|------------------------|---------------------------|----------------------------|--|
| | | Healthy Fruitlet (n = 96) | Infected Fruitlet (n = 96) | |
| <i>Fusarium</i> | <i>proliferatum</i> | 28 | 22 | KF993985.1 (12) |
| <i>Fusarium</i> | <i>ananatum</i> | 19 | 18 | MT010996.1 (36) |
| <i>Talaromyces</i> | <i>stollii</i> | 8 | 16 | JX315634.1 (24) |
| <i>Fusarium</i> | <i>verticillioides</i> | 12 | 12 | MT594370.1 (10) |
| <i>Talaromyces</i> | <i>amestolkiae</i> | 13 | 9 | KJ413360.1 (13) |
| <i>Fusarium</i> | <i>equiseti</i> | 10 | 11 | MN589630.1 (4) |
| <i>Phialemoniopsis</i> | <i>curvata</i> | 6 | 12 | AB278180.1 (15) |
| <i>Fusarium</i> | <i>oxysporum</i> | 9 | 9 | CP053267.1 (4) |
| <i>Fusarium</i> | <i>sp.</i> | 10 | 5 | JF740861.1 (5) |
| <i>Fusarium</i> | <i>solani</i> | 5 | 8 | MT594367.1 (3)/MK968891.1 (3) |
| <i>Talaromyces</i> | <i>purpureogenus</i> | 5 | 5 | KJ528885.1 (3)/MF476006.1 (3) |
| <i>Aspergillus</i> | <i>flavus</i> | 2 | 6 | MN955851.1 (3) |
| <i>Fusarium</i> | <i>incarnatum</i> | 3 | 5 | MK752398.1 (2)/MN882829.1 (2) |
| <i>Fusarium</i> | <i>fujikuroi</i> | 6 | 1 | MF281281.2 (4) |
| <i>Aspergillus</i> | <i>niger</i> | 3 | 4 | KY357318.1 (2)/MN788116.1 (2) |
| <i>Fusarium</i> | <i>chlamydosporum</i> | 3 | 1 | KJ125830.1 (3) |
| <i>Talaromyces</i> | <i>funiculosus</i> | 2 | 2 | AB893941.1 (3) |
| <i>Penicillium</i> | <i>sp.</i> | 3 | 1 | EU330619.1 (2) |
| <i>Pestalotiopsis</i> | <i>trachicarpicola</i> | 2 | 2 | MN295594.1 (4) |
| <i>Fusarium</i> | <i>sacchari</i> | 0 | 3 | MN193868.1 (2) |
| <i>Trichoderma</i> | <i>erinaceum</i> | 0 | 2 | MK109820.1 (2) |
| <i>Fusarium</i> | <i>napiforme</i> | 1 | 1 | MH862670.1 (2) |
| <i>Trichoderma</i> | <i>paraviridescens</i> | 1 | 1 | MF782827.1 (1)/MK418756.1 (1) |
| <i>Epicoccum</i> | <i>sorghinum</i> | 1 | 1 | MF782827.1 (1)/MK418756.1 (1) |
| <i>Trichoderma</i> | <i>sp.</i> | 0 | 2 | KX449479.1 (1)/MK870964.1 (1) |
| <i>Aspergillus</i> | <i>awamori</i> | 2 | 0 | KY416558.1 (2) |
| <i>Fusarium</i> | <i>falciforme</i> | 2 | 0 | MT251175.1 (2) |
| <i>Lasiodiplodia</i> | <i>mahajangana</i> | 2 | 0 | MH057188.1 (2) |
| <i>Trichoderma</i> | <i>asperellum</i> | 0 | 1 | KX538815.1 (1) |

Table 3. Cont.

| Genus | Species | Occurrence | | Top Match GenBank Accession Number * (Frequency in Data) |
|-----------------------|------------------------|------------------------------|-------------------------------|---|
| | | Healthy Fruitlet (n = 96) | Infected Fruitlet (n = 96) | |
| <i>Fusarium</i> | <i>circinatum</i> | 0 | 1 | MK334369.1 (1) |
| <i>Fusarium</i> | <i>cortaderiae</i> | 0 | 1 | AH012626.2 (1) |
| <i>Aspergillus</i> | <i>fumigatus</i> | 0 | 1 | MH844690.1 (1) |
| <i>Fusarium</i> | <i>graminearum</i> | 0 | 1 | MK460853.1 (1) |
| <i>Trichoderma</i> | <i>harzianum</i> | 0 | 1 | JN116710.1 (1) |
| <i>Diaporthe</i> | <i>kongii</i> | 0 | 1 | KR024740.1 (1) |
| <i>Curvularia</i> | <i>lunata</i> | 0 | 1 | MN971669.1 (1) |
| <i>Diaporthe</i> | <i>masirevicii</i> | 0 | 1 | MF668289.1 (1) |
| <i>Aspergillus</i> | <i>novoparasiticus</i> | 0 | 1 | MH279415.1 (1) |
| <i>Aspergillus</i> | <i>oryzae</i> | 0 | 1 | MN648727.1 (1) |
| <i>Rhizopus</i> | <i>stolonifer</i> | 0 | 1 | MF461025.1 (1) |
| <i>Trichoderma</i> | <i>trixiae</i> | 0 | 1 | MN889512.1 (1) |
| <i>Pestalotiopsis</i> | <i>vismiae</i> | 0 | 1 | KP747694.1 (1) |
| <i>Clonostachys</i> | <i>wenpingii</i> | 0 | 1 | NR_119651.1 (1) |
| <i>Cosmospora</i> | <i>butyri</i> | 1 | 0 | KU204560.1 (1) |
| <i>Lasiodiplodia</i> | <i>citricola</i> | 1 | 0 | KU530119.1 (1) |
| <i>Glomus</i> | <i>clarum</i> | 1 | 0 | AY035654.1 (1) |
| <i>Fusarium</i> | <i>dlamini</i> | 1 | 0 | MN173109.1 (1) |
| <i>Fusarium</i> | <i>ficicrescens</i> | 1 | 0 | KP662895.1 (1) |
| <i>Bionectria</i> | <i>ochroleuca</i> | 1 | 0 | EU552110.1 (1) |
| <i>Clonostachys</i> | <i>rosea</i> | 1 | 0 | MH047188.1 (1) |
| <i>Clonostachys</i> | <i>sp.</i> | 1 | 0 | MH681594.1 (1) |
| <i>Davidiella</i> | <i>sp.</i> | 1 | 0 | KX621979.1 (1) |
| <i>Diaporthe</i> | <i>sp.</i> | 1 | 0 | MH220834.1 (1) |
| <i>Lasiodiplodia</i> | <i>theobromae</i> | 1 | 0 | KR260829.1 (1) |
| <i>Aspergillus</i> | <i>welwitschiae</i> | 1 | 0 | MH374611.1 (1) |

(*) The listed accession numbers correspond to the most frequent top match for each species.

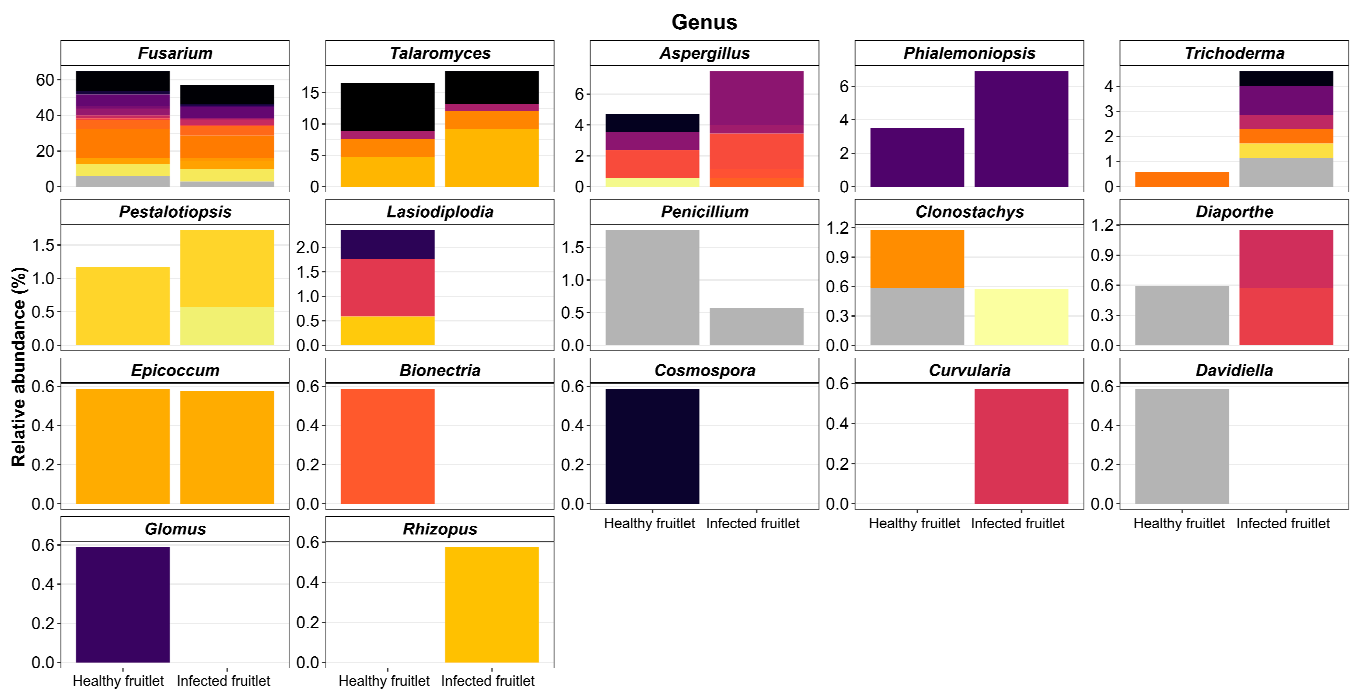


Figure 2. Cont.

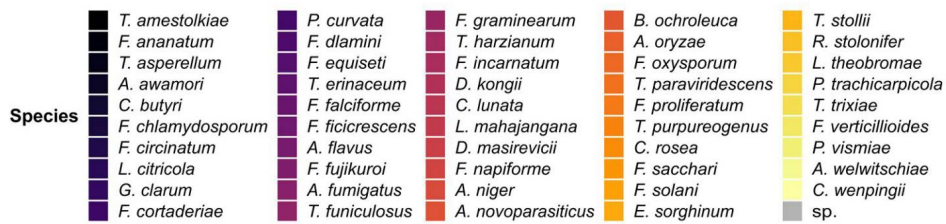


Figure 2. Relative abundances of fungal isolates sampled from healthy and infected pineapple fruitlets at genus and species taxonomic levels.

3.2. Correlation between the Diversity of Fruitlet Mycobiomes and FCR Incidence

To determine the prevalence of FCR over the experimental plots, the number of IF per fruit was reported on a total of 240 pineapple fruits. The deviance test evidenced significant interaction between plot orientation and adjacent crops ($p < 0.0001$) on the average of IF per pineapple fruit. Considering Plot 1, the mean IF counts per pineapple fruit (mean \pm sd) were 6.65 ± 4.36 for the row section proximal to the mango orchard, 4.92 ± 4.16 for rows localized in the center of the plot, and 1.57 ± 1.92 for rows near the jackfruits trees. Similar patterns were observed over rows of Plot 2, however, showing the highest infestation level with a mean of 8.55 ± 4.80 IF per pineapple fruit grown near the mango orchard, 5.82 ± 4.32 IF for pineapples in the plot center, and 4.75 ± 2.81 IF for fruits proximal to the jackfruit trees (Figure 3). Among Plot 1, the main significant differences in the average IF number were observed between mango orchard and jackfruit trees sections ($p = 3.03 \times 10^{-7}$) and between center and jackfruit trees sections ($p = 5.88 \times 10^{-5}$). Considering Plot 2, the average IF number per fruit were significantly different between mango orchard and jackfruit trees sections ($p = 0.0002$) and between center and mango orchard sections ($p = 0.013$). Moreover, the comparison data in jackfruit sections also evidenced a significant rise of the average IF number per fruit in Plot 2 compared to Plot 1 ($p = 0.0001$).

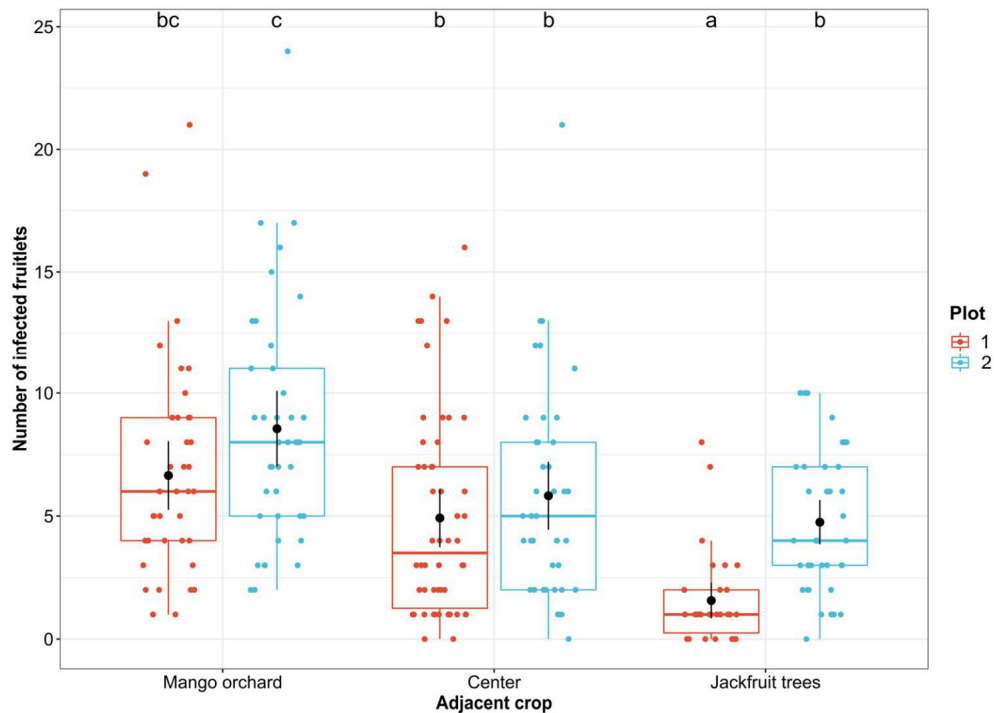


Figure 3. Scatter and box plots of numbers of FCR-infected fruitlets (IF) per pineapple fruit in the function of the adjacent crop according to plot orientation (Plot 1: Red, Plot 2: Blue). Black dots with bars show mean numbers of IF per pineapple with asymptotic confidence intervals at 95%. Letters indicate significant average differences between plot \times sections ($n = 40$) according to Tukey’s multiple comparison test ($p \leq 0.013$).

Considering the distribution profile of FCR symptoms, the distribution of the identified fungal species over the plots was investigated to search for a correlation. The first factorial axis (Dim1) of the PCA computed on the abundance of the 344 identified isolates according to plot sections explained 55.6% of the variance (Figure 4). Jackfruit trees and mango orchard sections of Plot 1 contributed to Dim1 with 48.9% and 22.8%, respectively.

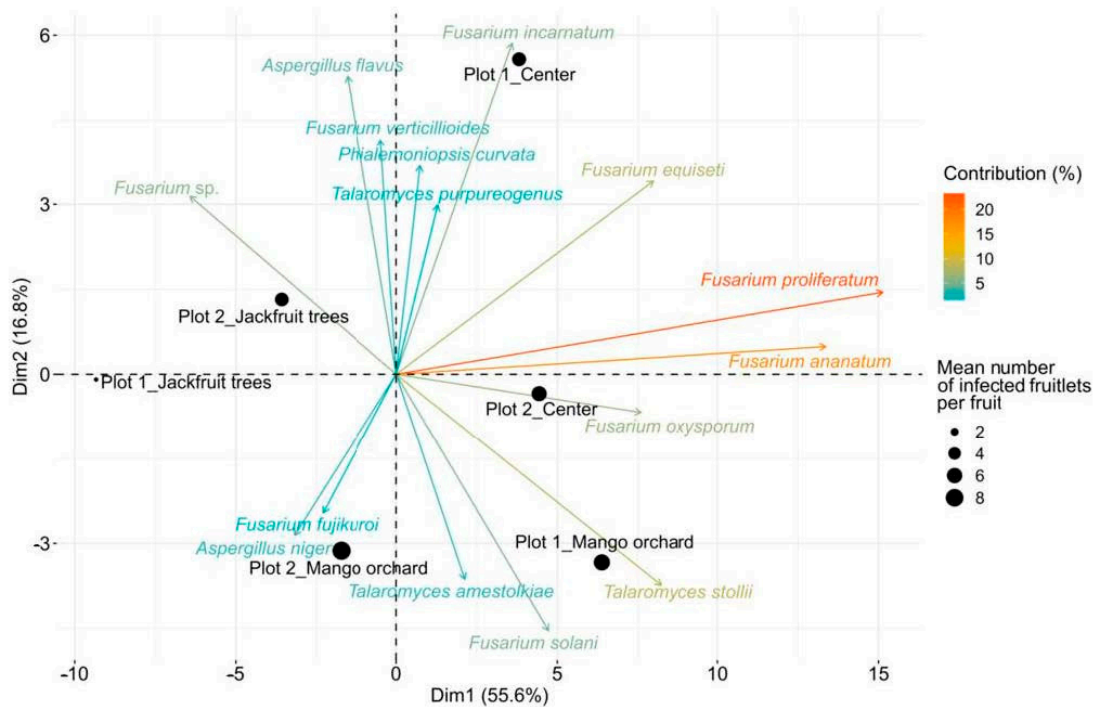


Figure 4. Principal component analysis (PCA) of combinations between proximal crop and plot direction. Only the 15 most contributory fungal species are shown (contributions ranging from 23.60% to 1.08%). Plot 1: North-West/South-East-directed rows; Plot 2: South-West/North-East-directed rows; mango orchard or jackfruit trees as adjacent crops, respectively; center: No adjacent crops. The size of each dot is proportional to the average number of infected fruitlets per fruit for each section of plots.

Symmetrically, Dim1 was supported by *F. proliferatum*, *F. ananatum*, *T. stollii*, *F. equiseti*, and *F. oxysporum* with respective contributions of 30.4%, 23.7%, 9.02%, 8.4%, and 7.68%. Correlation between variables and the first component evidenced that *F. ananatum* ($R = 0.99$) and *F. proliferatum* ($R = 0.96$) were positively correlated to PC1 at $p = 0.0002$ and $p = 0.002$, respectively. Thus, the mango orchard section of Plot 1 was associated with elevated abundances of *F. proliferatum*, *F. ananatum*, *T. stollii*, *F. equiseti*, and *F. oxysporum* by opposition to the jackfruit trees section of the same plot. Additionally, 16.8% of the variance was explained by the Dim2. The construction of PCA factorial plane established that the center section of Plot 1 (57.71%) and mango orchard proximal rows of Plot 2 (18.1%) were the main contributive individuals of Dim2 in association with *F. incarnatum* (15.0%), *A. flavus* (12.2%), *F. solani* (9.0%), and *F. verticillioides* (7.5%). Moreover, *A. flavus* ($R = 0.95$), *F. incarnatum* ($R = 0.84$), *F. dlamini* ($R = 0.83$), *Diaporthe* sp. ($R = 0.83$), *D. masirevicii* ($R = 0.83$), *D. kongii* ($R = 0.83$), *C. butyri* ($R = 0.83$), *C. wenpingii* ($R = 0.83$), *A. fumigatus* ($R = 0.83$), and *F. verticillioides* ($R = 0.82$) were the main variables positively correlated to Dim2 at $p \leq 0.04$. This demonstrates that center and mango orchard sections of Plot 1 were characterized by different structuration in fungal communities even if their average number of IF per fruit were not significantly different. In addition, the jackfruit trees section of Plot 1 that presented the lowest FCR incidence also demonstrated the poorest fungal diversity and abundances. Considering that the most contributive fungal species were also among the most abundant in fruitlet samples, this suggested that fungal species repartition was

closely linked with FCR occurrence inside the plot sections. Consequently, relations between host tissues and fungal species prevalence were tested through the establishment of hierarchical clustering based on isolate counts in the 24 quadrats (Figure 5). Two distinct groups could be observed: Group 1 highlighted co-occurrence depicting similar profiles of highly represented fungal species, corresponding to identification in at least 25% of each sample set.

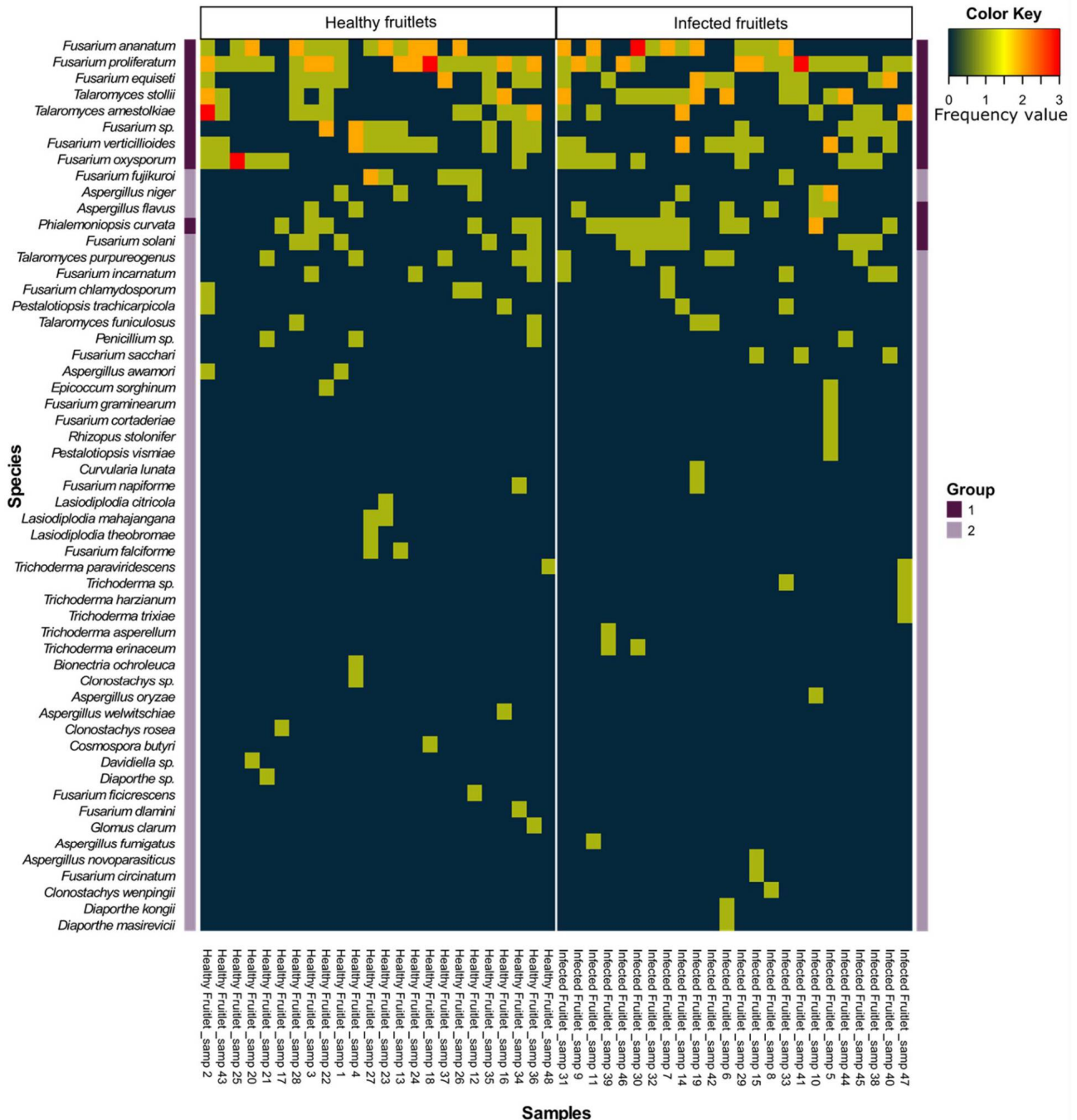


Figure 5. Heat map representation of fungal species frequencies in healthy and infected fruitlets of each quadrat. Group 1 corresponds to species identified in at least 25% of healthy and infected fruitlet sets, and Group 2 shows species identified in less than 25% of healthy and infected fruitlet sets.

HF ($n = 24$) seems mainly characterized by *F. ananatum*, *F. proliferatum*, *F. equiseti*, *T. stollii*, *T. amestolkiae*, *F. verticillioides*, *F. oxysporum*, and *P. curvata*, together gathering 61.8%

of the fungal flora. Interestingly, IF clustering ($n = 24$) shared the same species in addition to *A. flavus* and *F. solani*, which all contributed to 70.7% of the overall mycobiome diversity. In both types of fruitlet sets, a second group (Group 2) contained low abundance species identified in a limited number of quadrats and were thus considered as non-prevalent species for the rest of the analyses. Fungal communities were thus explored according to a combinatorial approach. Pairwise effect sizes were computed and revealed positive co-occurrence patterns in both HF and IF (only significant species combinations were displayed). Both matrixes showed 19 and 22 positive pair profiles for HF and IF fungal species datasets, respectively (Figure 6). Positive associations evidenced species pairs with significantly greater and large frequency than predicted by the model. By contrast, random associations were defined by observed values nearly equal to expectations and with no significant difference.

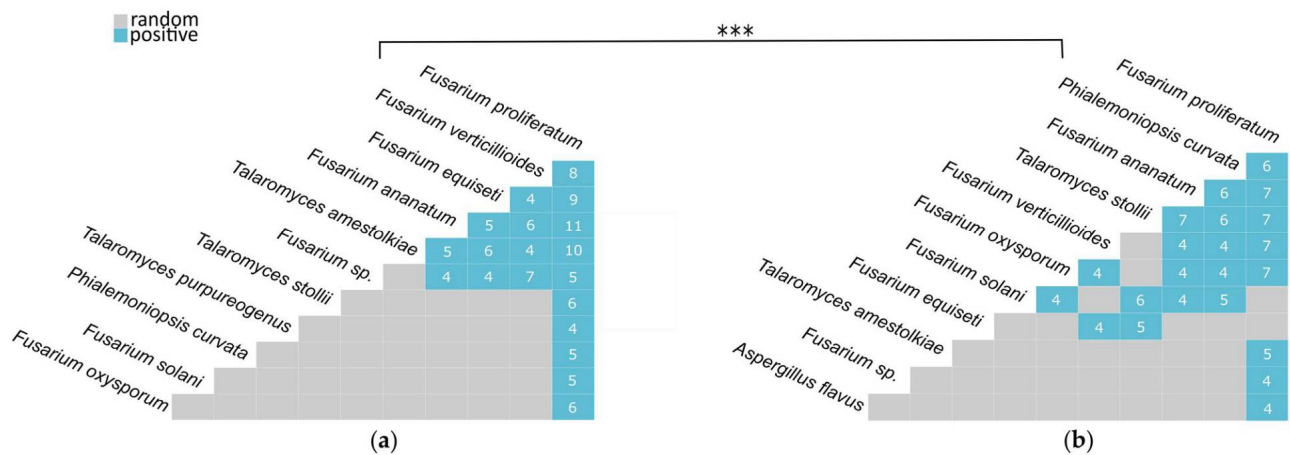


Figure 6. Matrixes of fungal species co-occurrences determined in healthy (a) and infected fruitlets (b) based on a pairwise approach. Significant positive associations are indicated in blue with the corresponding number of observations. Random associations are indicated in grey. The correlation between matrixes was assayed by the Mantel test (***) significance at $p \leq 0.001$).

F. proliferatum was found in a positive combination with all individuals belonging to Group 1. Despite being the most prevalent species in the dataset, *F. proliferatum* had the highest number of pairwise associations in both tissues by positively co-occurring with 10 species (69 observations) in HF and with 8 species in IF (47 observations). Alternatively, co-occurrence patterns of several species were contrasted by presenting fewer combinations in IF samples than in HF samples. This trend was reported for *F. equiseti* and *T. amestolkiae*, which were represented 28 and 25 times in associations with 5 and 4 species in HF but only combined 9 and 5 times with 2 and 1 species in IF, respectively. On the contrary, 5 species displayed more positive combinations in the IF set than in the HF set. As an example, *P. curvata* was described to be positively associated only with *F. proliferatum* in HF (5 observations), while a combination with 6 species (31 observations) was highlighted in IF samples. This profile was also observed, to a lesser extent, for *T. stollii* (HF: 1 association observed 6 times; IF: 5 associations observed 31 times), *F. solani* (HF: 1 association observed 5 times; IF: 4 associations observed 19 times), and *F. oxysporum* (HF: 1 association observed 6 times; IF: 5 associations observed 23 times). Association patterns suggested that the fungal flora associated with HF and IF was structured. However, the Mantel test processed on distance matrixes demonstrated a significant relationship between the mycobiomes of the HF and IF samples ($p = 0.0001$). This revealed that species associations did not depend on the fruitlet phenotype (healthy or infected) when considered through a pairwise approach.

Among plants pathogenic species, strains may exhibit a contrasting level of virulence, partly due to a genetic polymorphism inherited from co-evolution. TEF-1 α and ITS gene sequence datasets represented, respectively, 101 and 98 *Fusarium* DNA sequences, with at least 98% of identity according to the reference nucleotide sequence from NCBI (Table S1). These loci were submitted to phylogenetic analyses following the Maximum Likelihood

(ML) approach, with a bootstrap of 100 replications. Over the TEF-1 α region, 6 clades related to the 6 different species represented in this sequence dataset were discriminated without the distinction of strains isolated from HF or IF samples, thus describing a high level of homology (Figure 7).

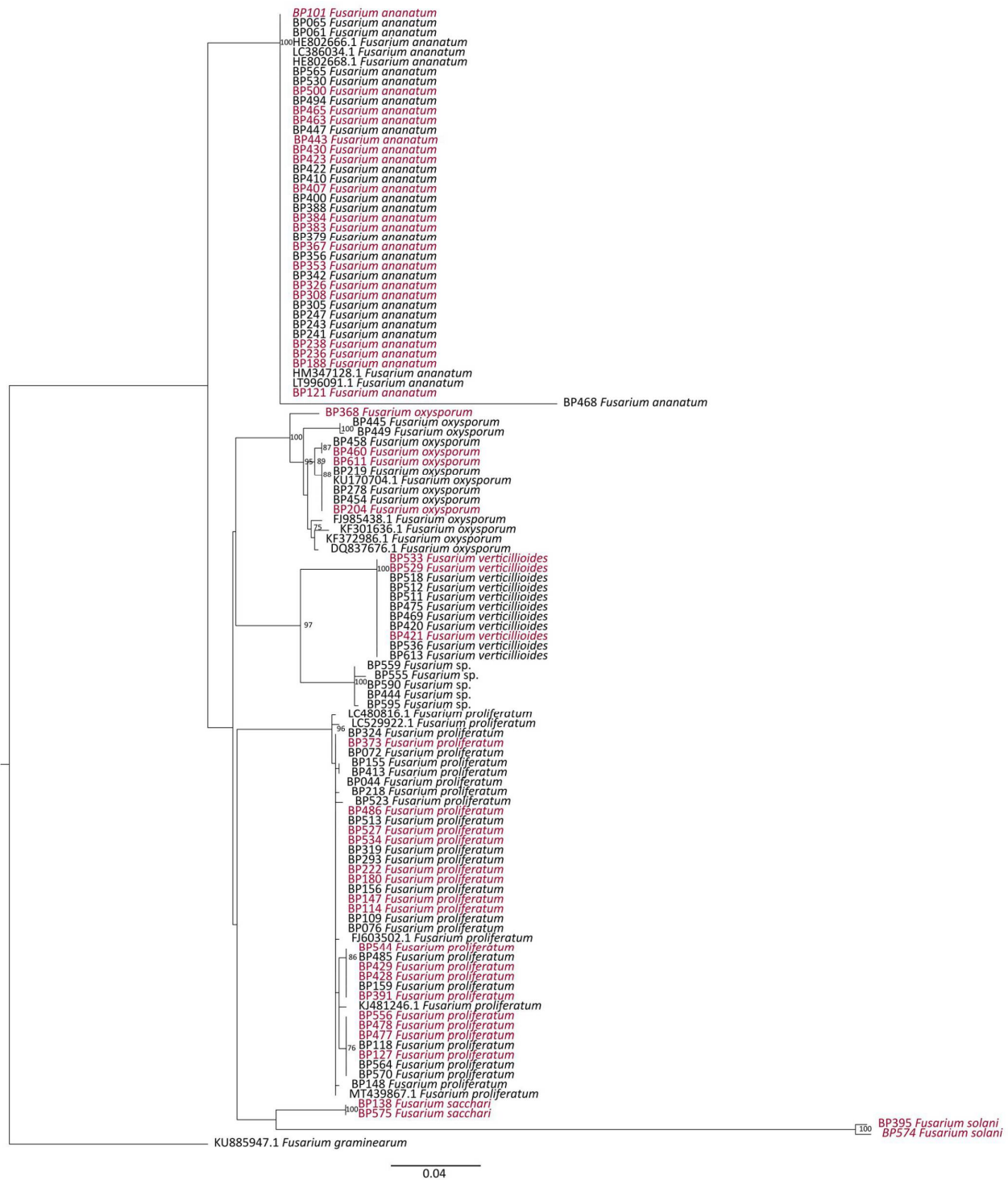


Figure 7. Phylogenetic tree of *Fusarium* species computed by Maximum Likelihood (ML) analysis (TIM2ef+G as the best fit model) and based on a TEF-1 α sequences dataset. Only ML bootstrap branches that support values greater than 70% are shown. Strains isolated from infected (IF) and healthy fruitlets (HF) are indicated in red and black, respectively. The tree is rooted with *F. graminearum* accession KU885947.1.

The same method was performed over the 61 *Talaromyces* strains. All of the 48 isolates characterized under the β -tubulin region corresponded to *T. amestolkiae* and *T. stollii*. As for the *Fusarium* strains, 2 clades were distinguished in concordance with the represented species (Figure 8). Strains were closely related inside clades, regardless of the phenotype (infected or healthy) of the fruitlets. This indicated that species virulence and pathogenicity were not linked to a polymorphism of TEF-1 α , ITS, or β -tubulin regions. Moreover, sequence analysis on TEF-1 α and β -tubulin loci enabled a higher taxonomic resolution when compared to ITS (Figures S1 and S2).

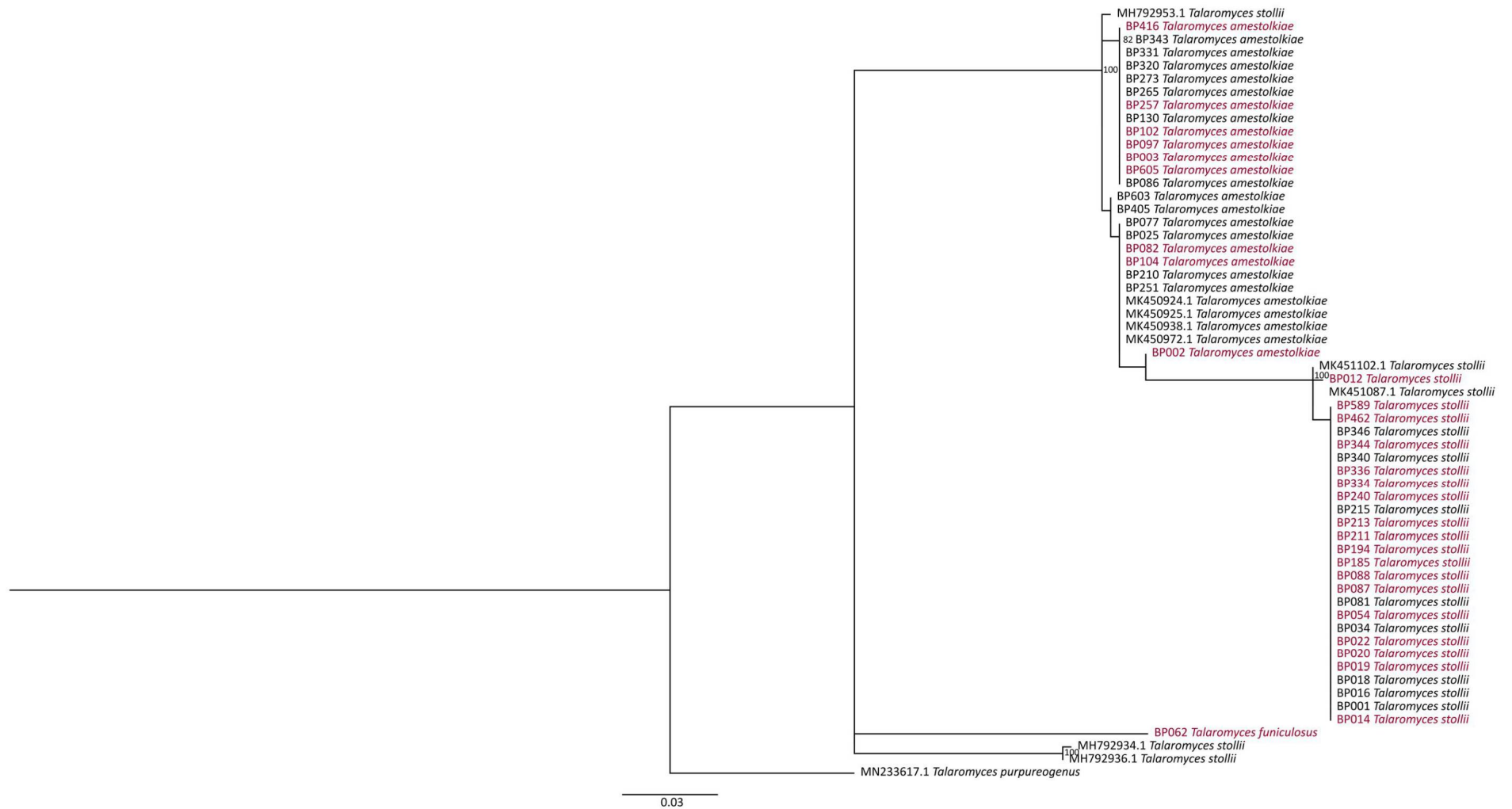


Figure 8. Phylogenetic tree of *Talaromyces* species computed by Maximum Likelihood (ML) analysis (TPM2+G as the best fit model) and based on a β -tubulin sequences dataset. Only ML bootstrap branches that support values greater than 70% are shown. Strains isolated from infected (IF) and healthy fruitlets (HF) are indicated in red and black, respectively. The tree is rooted with *T. purpureogenus* accession MN233617.1.

3.3. Potential Sources of Inoculum and Dispersion Patterns

The distribution of fungal species over the experimental plots suggested the presence of an important inoculum source. Fungal flora of the soil at the interface between pineapple and jackfruit trees or the mango orchard was studied in order to elucidate its origin. Along the two junctions, 26 (mango orchard) and 33 (jackfruit trees) isolates were identified as belonging to 27 genera and 35 species. Most of the identified species belonged to the Trichocomaceae (32.2%) and Nectriaceae (10.2%) families (Figure 9a). The Trichocomaceae family was represented by *Talaromyces* (teleomorph of *Penicillium*), *Penicillium*, and *Aspergillus* species, which were detected in lower abundances in the soil related to jackfruit trees (19.2%) than in the mango orchard soil (45.6%), respectively.

Talaromyces was strictly represented by *T. purpureogenus*, while 8 *Penicillium* species were isolated: *P. janthinellum*, *P. sumatraense*, *P. citrinum*, *P. guanacastense*, *P. multicolor*, *P. ochrochloron*, *P. sclerotigenum*, and *P. spinulosum*. This family was also supported by 4 *Aspergillus* species: *A. sclerotiorum*, *A. subramanianii*, *A. flavipes*, and *A. tardicrescens*. Moreover, the Nectriaceae family was only represented by *Fusarium* species. The fungal isolates that belonged to *Fusarium* genus were detected at higher frequencies close to the jackfruit trees (15.4%) when compared to the mango orchard interface (6.1%) (Figure 9b, Table S2). Considering the diversity, two distinct species were identified in each junction: *F. equiseti*/*F. polyphialidicum* at the jackfruit trees and *F. oxysporum*/*F. solani* at the mango orchard interface. Remarkably, among all fungal soil isolates, no *F. anatum* was identified. Subsequent isolates belonged to the *Cladosporium*, *Plectosphaerella*, *Trichoderma*, *Diaporthe*, *Mortierella*, *Xepicula*, *Bartalinia*, *Rousoella*, *Acrostalagmus*, *Cystofilobasidiales*, *Pestalotiopsis*, *Phoma*, *Pleosporales*, *Pyrenochaeta*, *Heterocephalum*, *Edenia*, *Purpureocillium*, *Aureobasidium*, *Fennellia*, *Lecanicillium*, *Robillarda*, *Peniophora*, and *Leptosphaeria* genera and were found with relative abundances ranging from 3% to 11.5%. Several contrasted profiles were noticed with the strict characterization of *Lecanicillium*, *Heterocephalum*, *Aureobasidium*, *Edenia*, *Fennellia*, *Leptosphaeria*, *Peniophora*, *Purpureocillium*, *Robillarda*, and *Talaromyces* genera in samples from the soil interface with the mango orchard. In contrast, *Diaporthe*, *Acrostalagmus*, *Cystofilobasidiales*, *Pestalotiopsis*, *Phoma*, *Plectosphaerella*, *Pleosporales*, *Pyrenochaeta*, *Rousoella*, and *Xepicula* genera were exclusively detected in the jackfruit tree junctions.

To evaluate the influence of abiotic factors on the fungal species spreading, meteorological parameters were obtained for the seven-month period from the flower induction treatment to harvest. Average temperatures ranged from 19.5 °C to 23.49 °C, with a low average rainfall below 5 mm. Data also demonstrated that the major wind direction was south-east during anthesis and fruit development stages, with a gust speed between 69 and 88 km h⁻¹ (Table 1). The dynamics of these factors typically described the setting of the austral winter.

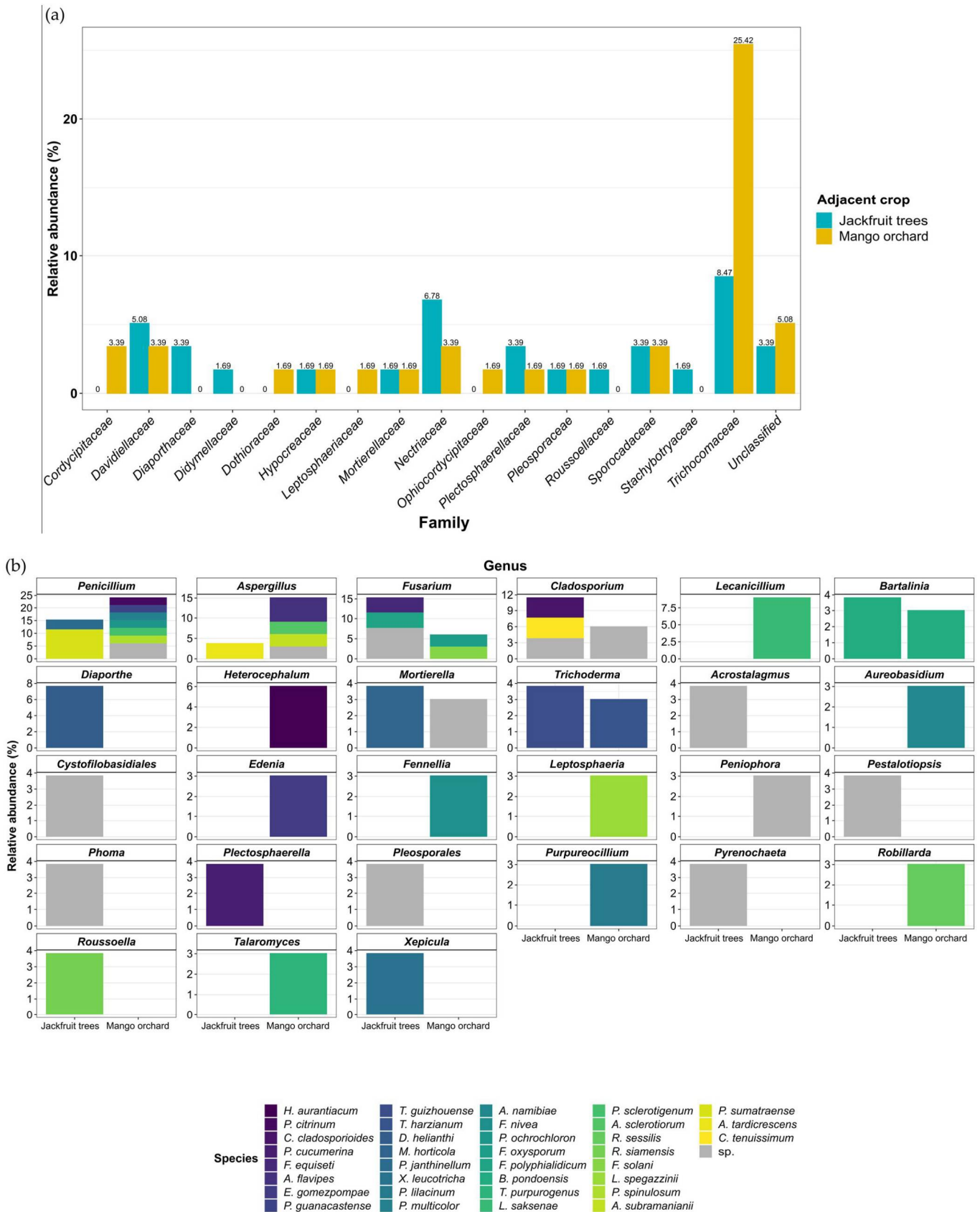


Figure 9. Relative abundances of fungal isolates identified from soil sampled on either side of the pineapple plots (jackfruit trees or mango orchard) at (a) family, (b) genus, and species taxonomic levels.

3.4. Koch's Postulates for the Determination of New FCR Pathogens

In IF samples, species belonging to the *Fusarium fujikuroi* species complex were represented with high frequencies and concomitantly with two *Talaromyces* species related to the *Talaromyces purpureogenus* species complex. Koch's postulates were verified for all of the following species: *F. proliferatum*, *F. sacchari*, *F. oxysporum*, *T. stollii*, and *T. amestolkiae*, in order to test for their pathogenicity.

After 7 days of incubation on PDA Petri dishes, all species presented fast growth rates with a mean diameter (av. \pm sd) reaching 7.25 cm, 7.77 ± 0.69 cm, 7.75 ± 0.70 cm, and 7.90 ± 0.19 cm for *F. ananatum*, *F. oxysporum*, *F. proliferatum*, and *F. sacchari*, respectively (Figure 10a1–h1). *T. stollii* and *T. amestolkiae* showed a slightly slower growth rate with respective mean diameters of 6.66 ± 0.08 cm and 6.68 ± 0.90 cm (Figure 10i1–n1). *Fusarium* colonies were circular with a thick and aerial white mycelium, except for strain BP575 (*F. sacchari*), which showed a short aerial mycelium. Species can be distinguished by their coloration when observed from a bottom range from orange (*F. sacchari*), to violet (*F. oxysporum*), to ochre/brown (*F. proliferatum*).

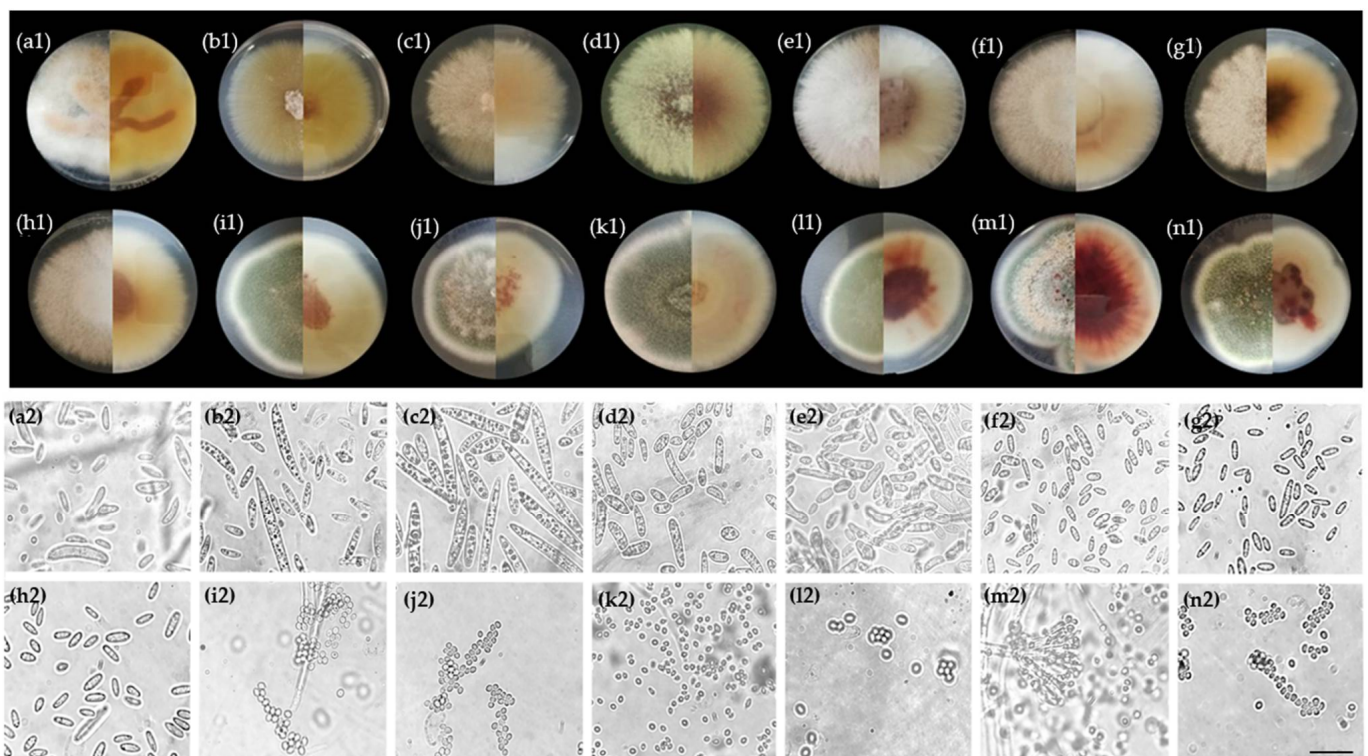


Figure 10. Morphological observations of fungal species isolated from pineapple-infected fruitlets and assayed in Koch's postulates. (1) Colonies were incubated on potato dextrose agar (PDA) for 7 days at 27 °C in the dark. Each colony is shown from top (left) to bottom (right). (2) Conidia. *Fusarium sacchari* strains (a) BP138 and (b) BP575; *Fusarium ananatum* strain (c) BP383; *Fusarium oxysporum* strains (d) BP369 and (e) BP460; *Fusarium proliferatum* strains (f) BP114, (g) BP429, and (h) BP436; *Talaromyces stollii* strains (i) BP054, (j) BP185, and (k) BP462; *Talaromyces amestolkiae* strains (l) BP002, (m) BP257, and (n) BP605. Scale bar: 20 μ m.

Talaromyces species showed circular to oval colonies with short and rough mycelium characterized by a green coloration in the oldest zone and with a white ring on the edges. The isolates identified as *T. amestolkiae* could be distinguished by a red pigmentation visible at the bottom of the Petri dishes. This was in addition to the secretion of a red exudate in the center of the colonies (Figure 10l1–n1). Microscopic observations of *Fusarium* species isolates showed straight (Figure 10a2–c2) and curved fusiform macroconidia (Figure 10d2,e2), while microconidia were ovoidal (Figure 10d2,e2) or ellipsoidal (Figure 10a2–c2,f2–h2). *Ta-*

Talaromyces amestolkiae and *Talaromyces stollii* exhibited aggregated conidia with an ellipsoidal shape (Figure 10i2–n2).

Subsequently to sporulation on PDA, controlled inoculations were performed by injecting a normalized conidia solution into a blossom cup of fruitlets. After 7 days of incubation, fruitlets from low, median, and upper sections of pineapple fruit were extracted and cut into two equal parts. Samples inoculated with sterile water as well as the non-inoculated fruitlets (Mock) did not show any visible FCR symptom. All 5 tested species (13 isolates) led to the development of a vitreous aspect or black spot (Figure 11). Following *F. oxysporum* inoculations, black spot development was observed in both pineapple fruit sections.

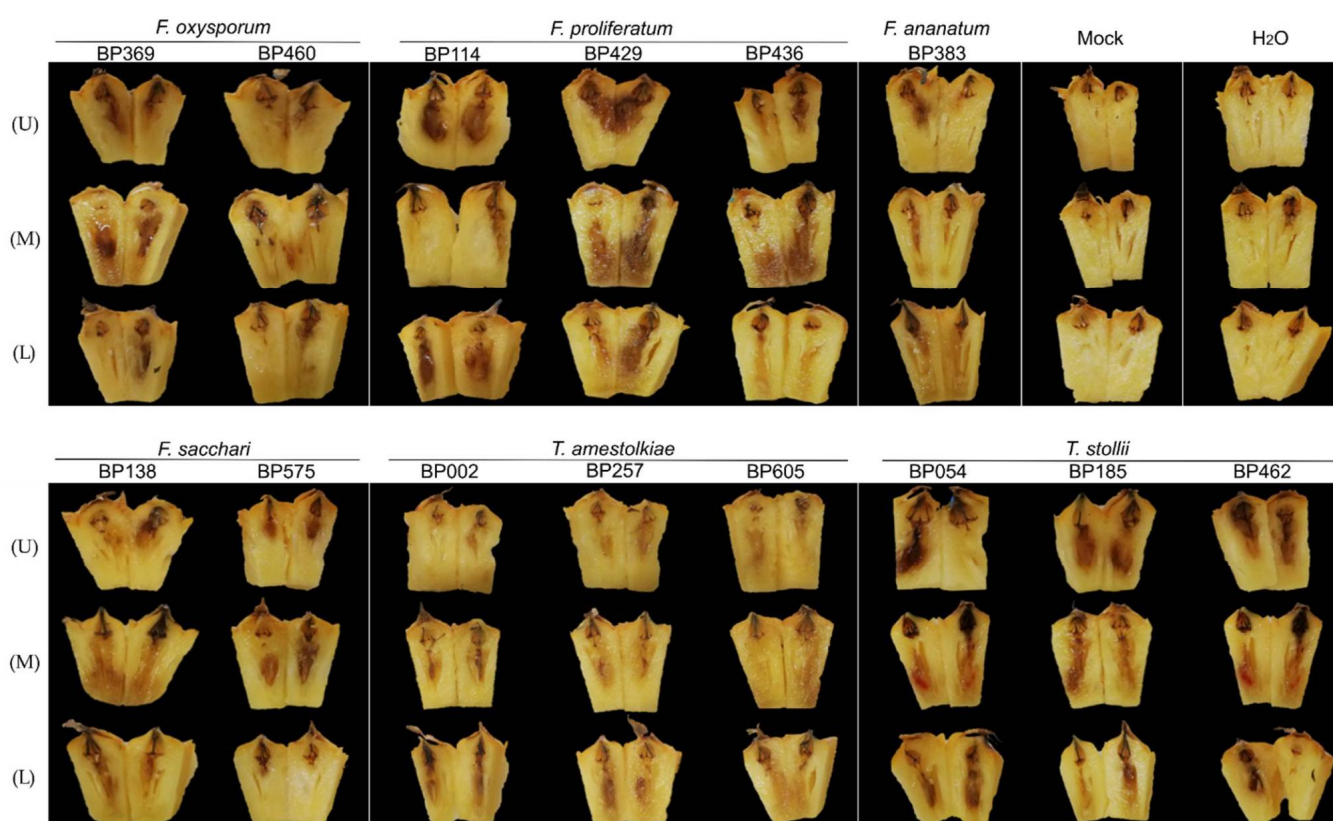


Figure 11. FCR symptoms observed 7 days post-inoculation (dpi) following controlled inoculations of five putative pathogenic fungal species in the blossom cup of fruitlets from the low (L), median (M), and upper (U) parts of ‘Queen Victoria’ pineapple fruit. *F. ananatum* strain BP383 was used as positive control; H₂O: Sterile water-inoculated; mock: No inoculation.

Similar observations were recorded for *F. sacchari*. Interestingly, *F. proliferatum* and *T. stollii* caused strong dark spot development in the fruitlet flesh. Through *T. stollii*-inoculated fruitlets, symptoms came out with a red pigmentation that was visible in samples from the median section only. Inoculations with 3 different isolates of *T. amestolkiae* led to slight necrosis associated with a vitreous appearance. Comparable symptoms were also reported after *F. ananatum* conidia injection, considered in this experiment as a positive control [10].

To fulfill Koch’s postulates, the potential causal agents were recovered by a molecular approach (PCR-DGGE) that allowed for a culture-independent characterization of microbial communities associated with a semi-quantitative determination of band patterns. To this end, *Fusarium*- and *Talaromyces*-inoculated fruitlets were distinguished, but the identification of species could not be achieved. First, a reference gel was performed on pure fungal strains using migration conditions that were similar to that of the inoculated samples (Figure S3). According to the UPGMA construction method, fungal patterns were

structured depending on the genus of the tested strain (Figure 12). Two groups were then identified. One group contained *Fusarium*-inoculated samples and presented the ‘*Fusarium* marker’ according to the reference profiles. The second group was supported by *Talaromyces*-inoculated fruitlets with matching markers and control samples.

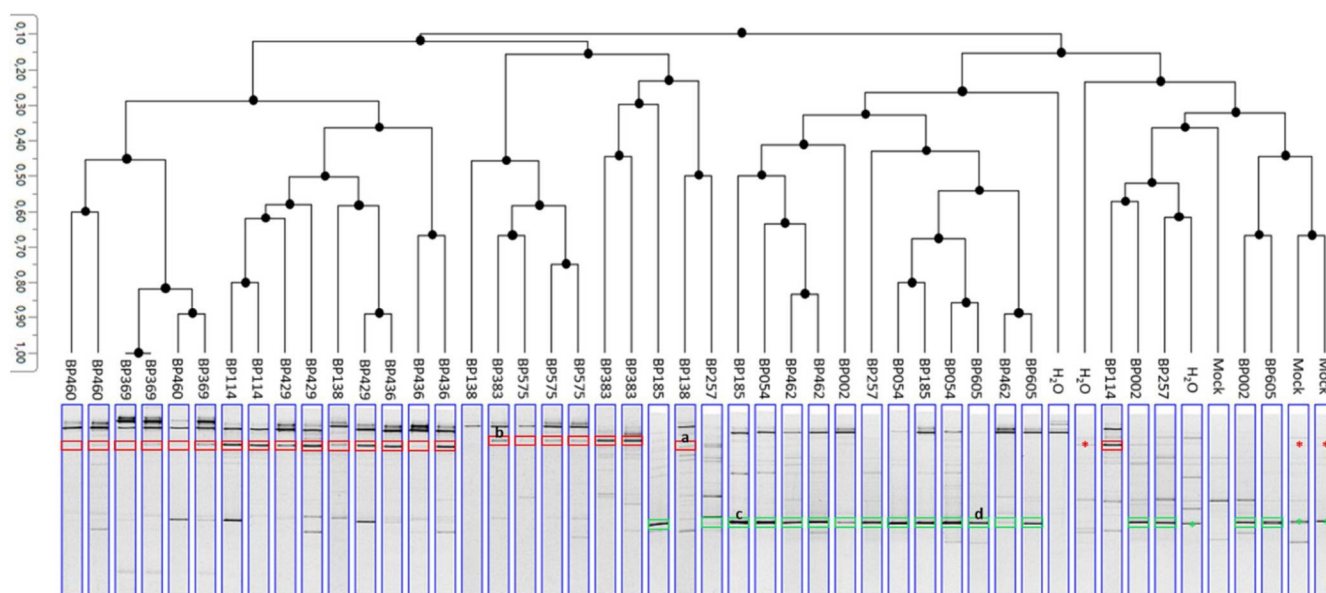


Figure 12. Dendrogram of PCR-DGGE profiles constructed by the UPGMA method. Red rectangles show *Fusarium* markers in *Fusarium*-inoculated fruitlets. Green rectangles show *Talaromyces* markers of *Talaromyces*-inoculated fruitlets. Red arrows indicate naturally present *Fusarium* sp. in control fruitlets. Green arrows indicate naturally present *Talaromyces* sp. in control fruitlets. BP138 and BP575: *Fusarium sacchari*; BP383: *Fusarium ananatum*; BP369 and BP460: *Fusarium oxysporum*; BP114, BP429, and BP436: *Fusarium proliferatum*; BP054, BP185, and BP462: *Talaromyces stollii*; BP002, BP257, and BP605: *Talaromyces amestolkiae*. H₂O: sterile water-inoculated samples; mock: No inoculation. Letters label DNA bands, which were sequenced.

The DNA bands recovered from the gel were sequenced, and the identification results confirmed that the targeted bands corresponded to the *Fusarium* sp. and *Talaromyces* sp. that were used for inoculations (Table S3). Another difficulty relied on the presence of an important natural core mycobiome partly involving *Fusarium* and *Talaromyces* species. The fungal diversity of fruitlets could be appreciated in negative control samples that naturally presented fungal banding patterns corresponding to *Fusarium* (red asterisks) and *Talaromyces* (green asterisks) migration profiles. Fungi-inoculated samples, however, showed an intense band that was not detectable in symptom-free fruitlets that corresponded to the inoculated fungi.

4. Discussion

By analyzing pineapple cultivatable fungal flora, the composition of the mycobiome associated with FCR could be characterized. The data generated in the present study showed that an interaction between biotic and abiotic factors may impact disease spreading over a production area. Moreover, the determination of species contribution to FCR disease led to the discovery of new causal agents.

Our results demonstrate that structuration of the fungal flora of fruitlets was mainly composed of two species complexes that were recently characterized as *Fusarium fujikuroi* and *Talaromyces purpureogenus* [49,50]. This type of association was previously described in FCR through the isolation of *F. verticillioides* and *T. funiculosus* recovered from FCR symptoms [8,51], even though *T. funiculosus* had never been defined as prevalent in Reunion Island before. Previous sampling over eight production areas in the island showed a predominance of *F. ananatum* (72%), *F. oxysporum* (6.67%), *F. proliferatum* (0.67%), and *T.*

stollii (20.67%) [52]. Conjointly, conidia with a fusiform shape were observed in a blossom cup of symptomless pineapples related to the 'Queen Victoria' cultivar, while ellipsoidal conidia were observed in the 'MD-2' defined as the FCR-resistant cultivar of reference [53]. However, the identification of species such as *Disporotrichum dimorphosporum*, *Galactomyces candidus*, and *Clavispora lusitaniae* in freshly cut pineapple (cv. 'Queen Victoria') revealed a higher level of fungal diversity in the fruit flesh [54].

By focusing on a specific plot environment, 49 fungal species were identified through 344 isolates. The infection level was reported over the three plot sections defined for the two experimental plots. Results showed that rows proximal to the mango orchard presented an elevated number of FCR symptoms, and this number was significantly lower in the rows proximal to the jackfruit trees. This suggested the existence of a disease gradient ranging from the mango orchard (high FCR incidence zone) to the jackfruit trees (low FCR incidence zone). Several soil-borne *Fusarium* species can cross long distances through water, especially irrigation water, under the form of chlamydospores. As an example, *F. oxysporum* causing *Fusarium* wilt of banana spread through the soil conductance, leading to pathogen invasion [55]. Nevertheless, the composition of the fungal flora of soil samples from both junctions was not correlated with the disease gradient observed in fruits over row sections. In fact, FCR pathogens such as *Fusarium* species were surprisingly mainly identified over the jackfruit tree interface, and members of the *Talaromyces* genus were poorly represented across soil samples. Mango orchard junctions only demonstrated the presence of *Talaromyces purpureogenus* (one of the four components of the *T. purpureogenus* species complex) as a potential new contributor to FCR. Interestingly, *F. proliferatum* and *F. sacchari* were previously identified as pathogens causing mango malformation disease and leading to an anarchic development of inflorescence [56,57]. Even though this pathology had never been reported in the various mango cultivars of this orchard, it is proposed that mango trees could represent a prevalent habitat for some FCR pathogens. In fact, across a landscape, fungi can switch from an endophyte lifestyle on a specific host to a pathogenic behavior in another host [58,59]. By considering the interaction between crop proximity (adjacent cultures were only 2 m away) and meteorological data, it was suggested that wind could help the dispersion of conidia from a mango tree canopy to pineapple rows, and it was hypothesized that FCR was an airborne disease. This profile also emphasized the significance of row orientation in relation to the main wind direction. The FCR gradient was slightly more pronounced in Plot 2, and the mean level of infestation per fruit was significantly higher. This suggested that the inoculum widespread from the mango tree canopy to the pineapple plot was carried by the wind further than for the plot having rows perpendicular to the major wind direction. In fact, the N-W/S-E-directed rows (Plot 1) showed a strong FCR gradient with a significant difference between plot sections. Thus, plants proximal to the mango orchard appeared as a 'buffer zone' for the shared pathogens and limited the wind dispersion of conidia over the plot. These observations are consistent with Parnell et al., who demonstrated that the spatial configuration of host in a landscape is the keystone determinant of plant-disease epidemics [60].

Among FCR pathogens, *F. ananatum* has been described the most. *F. ananatum* isolates from pineapples of Costa Rica and Ecuador showed a capacity to produce fumonisins B1 (FB₁), fumonisins B2 (FB₂), fumonisins B3 (FB₃), and beauvericin (BEA). However, the production levels observed under in vitro conditions were only in low concentrations, thus constituting a limited risk for food contamination [61]. Following FCR sampling in Reunion Island, *F. proliferatum* and *F. oxysporum* have been described as mycotoxin producers of FB₁, FB₂, and BEA, while no toxigenic profile was detected for *T. stollii*. Significant amounts of FB₁ and BEA have also been detected in naturally infected fruitlets in comparison to healthy ones [52]. It was thus suggested that these species could also be involved in FCR pathogenesis with a significant impact on food safety.

The characterization of the mycobiome related to both healthy and infected fruitlets resulted in the determination of significant pairwise co-occurrence between *Fusarium* and *Talaromyces* species. Although *F. ananatum* was described as the first causal agent of FCR in

Reunion Island [11,52], our data showed a prevalence of *F. proliferatum* (50 strains), which seemed to define a hub over fungal interactions in pineapple. The prevalence of this species was also demonstrated following the sampling of diseased leaves and pineapple fruits in Malaysia [62]. Interestingly, this study identified *F. proliferatum* as the main causal agent of leaf spot disease and pineapple fruit rot, characterized by the development of brown necrosis in the fruit flesh and skin.

Across related abundance profiles and distribution patterns, *F. proliferatum*, *F. sacchari*, *F. oxysporum*, *T. stollii*, and *T. amestolkiae* were investigated for their pathogenicity. Following the injection of conidia in a blossom cup, all of these species showed a capacity to induce symptoms related to FCR. Several symptoms observed after seven days of incubation were only slightly pronounced, particularly for *F. oxysporum* and *T. amestolkiae*. This could be explained by the time variation for fungal development and symptom appearance, which may fluctuate according to the studied species. Surprisingly, the evolution of fungal flora of minimally processed pineapples also revealed the implication of *T. amestolkiae* in fruit spoilage over cold storage conditions [54]. Taken together, these elements illustrate that specific structural arrangement of microbial communities may lead to pathogenesis and/or to fruit quality depreciation [63]. We, therefore, propose the FCR pathosystem to be considered as multi-partite and hypothesize that other species related to *Fusarium fujikuroi* and *Talaromyces purpureogenus* species complexes could be involved. Thus, the presence of multiple pathogens in a production area may increase the epidemiological risks. This partially explained the difficulties faced over the past decades to elucidate the infectious process leading to pineapple FCR susceptibility.

Conventional verification of Koch's postulates had to be adapted for pineapple fruits due to the constraints of dealing with non-microbial-free tissues for in vivo inoculation. As exposed with DGGE migration profiles, the initial (natural) microbial loads in fruitlets contain a high level of fungal diversity as numerous DNA bands that could be detected in symptomless fruitlets. As a comparison, 5–9 bands could be visualized in DGGE lanes of negative controls (H₂O and Mock), while 1–5 species per fruitlets were isolated by a classical microbial procedure. The recovery of the pathogens from inoculated fruitlets was achieved following the establishment of reference migration profiles for each tested species. Over the 13 strains, strong DNA signals corresponded to a highly abundant inoculated species. This showed that the tested strains had been able to extend in the blossom cup of all biological replicates, except for one fruit inoculated with the *F. sacchari* (strain BP138). The inoculation of the tested species also modified the basal fungal communities. Numerous bands with various intensity were detected and contributed to the discrimination between *Fusarium*- and *Talaromyces*-inoculated mycobiomes. Interestingly, the fungal flora profiles of negative control samples were related to *Talaromyces*-inoculated samples rather than to *Fusarium*-inoculated fruitlets. This suggested a pathogen-specific evolution of the fruitlet mycobiome that is much more noticeable following inoculations with *Fusarium* species. The over-representation of these species may thus involve potentially synergistic or antagonistic microbial interactions. As an example, *F. verticillioides*, as a part of the endophytic fungal flora of maize, was described for its capacity to lower corn smut disease severity by limiting the biomass development of the pathogen *Ustilago maydis* [64]. In pineapple, the determination of positive pairwise co-occurrences led to the assumption that mycobiomes of healthy and naturally infected fruitlets were significantly correlated and that species pair combinations thus did not diverge from fruitlet phenotypes. Moreover, phylogenetic trees evidenced strong relationships among strains recovered from both HF and IF, proving that pathogenesis did not correlate with a fungal genetic polymorphism. Nevertheless, the interactions inside fungal communities are complex and may imply numerous species with various communication strategies, as observed in numerous studies [65–68]. It is important to consider that the present approach for the detected fungal species did not enable a determination of either the form (micro/macroconidia and mycelium) or their respective concentration in the fruitlets. The colonization of host tissues could be achieved by prevalent species forming an extensive mycelial network and

could potentially modulate the development of specialized structures of the remaining fungal species [69]. The establishment of cooperative or competitive fungal interactions partly relies on chemical recognition [66,68]. Interestingly, *T. stollii* and *T. amestolkiae* were defined as red-pigment-producing fungi that correspond to azaphilone extrolites [70]. This corroborates the observations performed following the in vitro culture of *T. amestolkiae* and the in vivo development of *T. stollii* [50]. These secondary metabolites are related to antiviral, antimicrobial, antifungal, and other biological activities [71]. This illustrates the necessity to determine how chemical communication between fungi may contribute to FCR establishment. In addition, the present study focused on fungal flora without considering the role of yeast and bacteria, which may also influence the microbial dialog during pathogenesis. In tomato, Wei et al. showed that endophytic microbial communities sharing an ecological niche with the *Ralstonia solanacearum* pathogen exhibited a low disease incidence [72]. As frequently exemplified, *F. oxysporum* has been described for its capacities to secrete and accumulate fusaric acid in order to inhibit the production by *Pseudomonas fluorescens* of 2,4-diacetylphloroglucinol acting as an antibiotic [73]. Biological association between microbial species represented a source for the determination of biocontrol agents. In pineapple, the antagonistic activity of *Trichoderma asperellum* against *F. guttiforme* was already described for the control of fusariosis [74].

In addition, both relative abundance and spatial distribution of fungal species are also fluctuating in accordance with abiotic factors as precipitation, relative humidity, temperature, and wind speed [75]. In the present study, pineapples were cultivated during austral winter, which is considered the prevalent season for FCR incidence in Reunion Island [18]. The SIMPIÑA model was developed to predict the quality of 'Queen Victoria' following total soluble sugar estimation, agricultural practices, and production area [76]. Our data highlighted that the understanding of plant disease outbreaks requires the evaluation of both biotic and abiotic environments. *Fusarium* species belonging to the FFSC, such as *F. sacchari*, *F. verticillioides*, and *F. proliferatum*, were reported as causal agents of several tropical crops, including sugarcane and mango (as previously demonstrated), which constitute preponderant cultures in Reunion Island [77–79]. This supports the potential cross-contribution between shared pathogens and abiotic factors on fungal epidemics in tropical and subtropical regions. The evaluation of influence parameters and their interactions may lead to the definition of risk factors for FCR occurrence. These variables could then be incremented into the VICTORIA database to adapt disease management strategies to various plot contexts [80,81].

5. Conclusions

Our study provided a new understanding of the FCR pathosystem by demonstrating the implications of *F. proliferatum*, *F. oxysporum*, *F. sacchari*, *T. stollii*, and *T. amestolkiae* on pathogenesis. We demonstrated that the mycobiome composition of fruitlets is influenced by adjacent crops that share common pathogens and may cause elevated levels of FCR incidence. It appears essential to consider the plot environment, especially row orientation, in relation to the major wind direction. Finally, similar fungal populations were described in healthy and infected fruitlets, suggesting that pineapple susceptibility may be inherent to chemical communication between pathogens that modulate strategies of host tissue colonization. Determining in vitro and in vivo pathogens interacting-behaviors would be necessary to understand the virulence factors of FCR-pathogens and subsequently investigate the molecular crosstalk of host-multi-pathogens interaction.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2309-608X/7/3/175/s1>, Figure S1: Phylogenetic tree of *Fusarium* species computed by Maximum Likelihood (ML) analysis (JC+I+G as the best fit model) and based on the ITS sequences dataset. Only ML bootstrap branches that support values greater than 70% are shown. Strains isolated from infected and healthy fruitlets are indicated in red and black, respectively. The tree is rooted with *F. sporotrichoides* accession MT218410.1, Figure S2: Phylogenetic tree of *Talaromyces* species computed by Maximum Likelihood (ML) analysis (K80+G as the best fit model) and based on the ITS sequences dataset. Only

ML bootstrap branches that support values greater than 70% are shown. Strains isolated from infected and healthy fruitlets are indicated in red and black, respectively. The tree is rooted with *T. cecidicola* accession MT182957.1, Figure S3: Reference DGGE profiles over ITS-1 region of DNA extracted from pure strains evaluated in Koch's postulates. (Mix) indicates the combined migration of *F. ananatum* strain BP383, *F. sacchari* strain BP575, *F. oxysporum* strain BP460, *F. proliferatum* strain BP429, *T. stollii* strain BP054, and *T. amestolkiae* strain BP002, Table S1: Identification of fungal species isolated from healthy and naturally infected pineapple fruitlets, Table S2: Identification of fungal species isolated from soil coring points of cultures proximal to pineapple plots. Table S3: Identification of fungal species from DNA extracted from DGGE gels corresponding to *Fusarium*- and *Talaromyces*-inoculated pineapple fruitlets.

Author Contributions: Conceptualization, M.V., J.-C.M., and S.S.-G.; methodology, M.V. and C.L.-J.; formal analysis, M.V. and F.C.; investigation, M.V. and C.S.; data curation, M.V.; writing—original draft preparation, M.V.; writing—review and editing, M.V., J.-C.M., C.L.-J., C.S., S.S.-G., F.C., and M.C.; visualization, M.V. and F.C.; supervision, J.-C.M. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for this research, including M.V.'s PhD grant, was provided by the European Regional Development Fund (FEDER Réunion, PO-COSAQ).

Acknowledgments: We acknowledge Pierre Lefeuvre (CIRAD, UMR PVBMT, Reunion Island) for his useful advice and help in phylogenetics. We also kindly thank Bernard Abufera (CIRAD, UPR GECO, Reunion Island) and Georget Tullus (CIRAD, UPR GECO, Reunion Island) for pineapple field maintenance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. *Major Tropical Fruits—Preliminary Market Results 2019*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2020; p. 24.
2. Bartholomew, D.P.; Hawkins, R.A.; Lopez, J.A. Hawaii Pineapple: The Rise and Fall of an Industry. *HortScience* **2012**, *47*, 1390–1398. [[CrossRef](#)]
3. Nassr, M.S.; Abu-Naser, S.S. Knowledge Based System for Diagnosing Pineapple Diseases. *Int. J. Acad. Pedagog. Res.* **2018**, *2*, 12–17.
4. Gu, H.; Zhan, R.L.; Zhan, L.B.; Gong, D.Q.; Jia, Z.W. First Report of *Fusarium Ananatum* Causing Pineapple Fruitlet Core Rot in China. *Plant Dis.* **2015**, *99*, 1653. [[CrossRef](#)]
5. Rohrbach, K.G.; Pfeiffer, J.B. Susceptibility of Pineapple Cultivars to Fruit Diseases Incited by *Penicillium Funiculosum* and *Fusarium Moniliforme*. *Phytopathology* **1976**, *66*, 1386–1390. [[CrossRef](#)]
6. Rohrbach, K.G.; Johnson, M.W. Pests, Diseases and Weeds. In *The Pineapple: Botany, Production and Uses*; Department of Tropical Plant and Soil Science, CTAHR, University of Hawaii at Manoa: Honolulu, HI, USA, 2003; pp. 203–251. [[CrossRef](#)]
7. Seifert, K.A.; Aoki, T.; Baayen, R.P.; Brayford, D.; Burgess, L.W.; Chulze, S.; Gams, W.; Geiser, D.; de Gruyter, J.; Leslie, J.F.; et al. The Name *Fusarium Moniliforme* Should No Longer Be Used. *Mycol. Res.* **2003**, *107*, 643–644. [[CrossRef](#)]
8. Lim, T.-K. Role of *Penicillium Funiculosum* Strains in the Development of Pineapple Fruit Diseases. *Phytopathology* **1980**, *70*, 663. [[CrossRef](#)]
9. Petty, G.J.; Tustin, H.A.; Dicks, H.M. Control of black spot disease / fruitlet core rot in queen pineapple with integrated mealybug, pineapple fruit mite and fungus control programmes. *Acta Hort.* **2006**, 143–149. [[CrossRef](#)]
10. Jacobs, A.; Van Wyk, P.S.; Marasas, W.F.O.; Wingfield, B.D.; Wingfield, M.J.; Coutinho, T.A. *Fusarium Ananatum* Sp. Nov. in the *Gibberella Fujikuroi* Species Complex from Pineapples with Fruit Rot in South Africa. *Fungal Biol.* **2010**, *114*, 515–527. [[CrossRef](#)] [[PubMed](#)]
11. Barral, B.; Chillet, M.; Minier, J.; Léchaudel, M.; Schorr-Galindo, S. Evaluating the Response to *Fusarium Ananatum* Inoculation and Antifungal Activity of Phenolic Acids in Pineapple. *Fungal Biol.* **2017**, *121*, 1045–1053. [[CrossRef](#)] [[PubMed](#)]
12. Mourichon, X. Pineapple Fruitlet Core Rot (Black Spot) and Leathery Pocket: Review and Prospects. *Acta Hort.* **1997**, 501–508. [[CrossRef](#)]
13. Maltz, M.R.; Treseder, K.K.; McGuire, K.L. Links between Plant and Fungal Diversity in Habitat Fragments of Coastal Shrubland. *PLoS ONE* **2017**, *12*, e0184991. [[CrossRef](#)] [[PubMed](#)]
14. Hugoni, M.; Luis, P.; Guyonnet, J.; el Zahar Haichar, F. Plant Host Habitat and Root Exudates Shape Fungal Diversity. *Mycorrhiza* **2018**, *28*, 451–463. [[CrossRef](#)] [[PubMed](#)]
15. Hawksworth, D.L.; Lücking, R. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. In *The Fungal Kingdom*; Heitman, J., Howlett, B.J., Crous, P.W., Stukenbrock, E.H., James, T.Y., Gow, N.A.R., Eds.; ASM Press: Washington, DC, USA, 2017; pp. 79–95. ISBN 978-1-68367-082-7.

16. Dos Santos Souza, C.R.; de Oliveira Barbosa, A.C.; Fortes Ferreira, C.; Vidigal Duarte Souza, F.; de Souza Rocha, L.; de Souza, E.H.; de Oliveira, S.A.S. Diversity of Microorganisms Associated to Ananas Spp. from Natural Environment, Cultivated and Ex Situ Conservation Areas. *Sci. Hortic.* **2019**, *243*, 544–551. [[CrossRef](#)]
17. Rohrbach, K.G.; Taniguchi, G. Effects of Temperature, Moisture, and Stage of Inflorescence Development on Infection of Pineapple by *Penicillium Funiculosum* and *Fusarium Moniliforme* Var. *Subglutinans*. *Phytopathology* **1984**, *74*, 995–1000. [[CrossRef](#)]
18. Fournier, P.; Benneveau, A.; Hardy, C.; Chillet, M.; Léchaudel, M. A Predictive Model Based on a Pluviothermic Index for Leathery Pocket and Fruitlet Core Rot of Pineapple Cv. ‘Queen’. *Eur. J. Plant Pathol.* **2015**, *142*, 449–460. [[CrossRef](#)]
19. Gamliel-Atinsky, E.; Szejnberg, A.; Maymon, M.; Shtienberg, D.; Freeman, S. Inoculum Availability and Conidial Dispersal Patterns of *Fusarium Mangiferae*, the Causal Agent of Mango Malformation Disease. *Phytopathology* **2009**, *99*, 160–166. [[CrossRef](#)] [[PubMed](#)]
20. Rieux, A.; Soubeyrand, S.; Bonnot, F.; Klein, E.K.; Ngando, J.E.; Mehl, A.; Ravigne, V.; Carlier, J.; de Lapeyre de Bellaire, L. Long-Distance Wind-Dispersal of Spores in a Fungal Plant Pathogen: Estimation of Anisotropic Dispersal Kernels from an Extensive Field Experiment. *PLoS ONE* **2014**, *9*, e103225. [[CrossRef](#)] [[PubMed](#)]
21. Aylor, D.E. SPREAD OF PLANT DISEASE ON A CONTINENTAL SCALE: ROLE OF AERIAL DISPERSAL OF PATHOGENS. *Ecology* **2003**, *84*, 1989–1997. [[CrossRef](#)]
22. Rieux, A.; Lenormand, T.; Carlier, J.; de Lapeyre de Bellaire, L.; Ravigné, V. Using Neutral Cline Decay to Estimate Contemporary Dispersal: A Generic Tool and Its Application to a Major Crop Pathogen. *Ecol. Lett.* **2013**, *16*, 721–730. [[CrossRef](#)] [[PubMed](#)]
23. Fredrikson, L.; Skinkis, P.A.; Peachey, E. Cover Crop and Floor Management Affect Weed Coverage and Density in an Establishing Oregon Vineyard. *HortTechnology* **2011**, *21*, 208–216. [[CrossRef](#)]
24. Charest, J.; Dewdney, M.; Paulitz, T.; Philion, V.; Carisse, O. Spatial Distribution of *Venturia Inaequalis* Airborne Ascospores in Orchards. *Phytopathology* **2002**, *92*, 769–779. [[CrossRef](#)] [[PubMed](#)]
25. Osborne, L.E.; Stein, J.M. Epidemiology of *Fusarium* Head Blight on Small-Grain Cereals. *Int. J. Food Microbiol.* **2007**, *119*, 103–108. [[CrossRef](#)] [[PubMed](#)]
26. Dita, M.; Barquero, M.; Heck, D.; Mizubuti, E.S.G.; Staver, C.P. *Fusarium* Wilt of Banana: Current Knowledge on Epidemiology and Research Needs Toward Sustainable Disease Management. *Front. Plant Sci.* **2018**, *9*, 1468. [[CrossRef](#)]
27. Van der Does, H.C.; Constantin, M.E.; Houterman, P.M.; Takken, F.L.W.; Cornelissen, B.J.C.; Haring, M.A.; van den Burg, H.A.; Rep, M. *Fusarium Oxysporum* Colonizes the Stem of Resistant Tomato Plants, the Extent Varying with the R-Gene Present. *Eur. J. Plant Pathol.* **2019**, *154*, 55–65. [[CrossRef](#)]
28. Fournier, P. La Culture de l’ananas Victoria à La Réunion Pour l’exportation. In *Recueil de Bonnes Pratiques*; CIRAD: Reunion Island, France, 2011.
29. Smart Information System (SmartIS)-METEOR-CIRAD-UR AÏDA. Available online: <https://smartis.re/METEOR> (accessed on 25 November 2020).
30. Météo France. Available online: https://donneespubliques.meteofrance.fr/?fond=produit&id_produit=129&id_rubrique=52 (accessed on 30 September 2020).
31. O’Donnell, K.; Cigelnik, E.; Nirenberg, H.I. Molecular Systematics and Phylogeography of the *Gibberella Fujikuroi* Species Complex. *Mycologia* **1998**, *90*, 465–493. [[CrossRef](#)]
32. O’Donnell, K.; Kistler, H.C.; Cigelnik, E.; Ploetz, R.C. Multiple Evolutionary Origins of the Fungus Causing Panama Disease of Banana: Concordant Evidence from Nuclear and Mitochondrial Gene Genealogies. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2044–2049. [[CrossRef](#)] [[PubMed](#)]
33. Liu, X.; Xing, M.; Kong, C.; Fang, Z.; Yang, L.; Zhang, Y.; Wang, Y.; Ling, J.; Yang, Y.; Lv, H. Genetic Diversity, Virulence, Race Profiling, and Comparative Genomic Analysis of the *Fusarium Oxysporum* f. Sp. *Conglutinans* Strains Infecting Cabbages in China. *Front. Microbiol.* **2019**, *10*, 1373. [[CrossRef](#)] [[PubMed](#)]
34. Glass, N.L.; Donaldson, G.C. Development of Primer Sets Designed for Use with the PCR to Amplify Conserved Genes from Filamentous Ascomycetes. *Appl. Environ. Microbiol.* **1995**, *61*, 1323–1330. [[CrossRef](#)] [[PubMed](#)]
35. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification And Direct Sequencing Of Fungal Ribosomal Rna Genes For Phylogenetics. In *PCR Protocols*; Academic Press: Cambridge, MA, USA, 1990; pp. 315–322. ISBN 978-0-12-372180-8.
36. Gardes, M.; Bruns, T.D. ITS Primers with Enhanced Specificity for Basidiomycetes—Application to the Identification of Mycorrhizae and Rusts. *Mol. Ecol.* **1993**, *2*, 113–118. [[CrossRef](#)]
37. Sheffield, V.C.; Cox, D.R.; Lerman, L.S.; Myers, R.M. Attachment of a 40-Base-Pair G + C-Rich Sequence (GC-Clamp) to Genomic DNA Fragments by the Polymerase Chain Reaction Results in Improved Detection of Single-Base Changes. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 232–236. [[CrossRef](#)] [[PubMed](#)]
38. Hamdouche, Y.; Meile, J.C.; Nganou, D.N.; Durand, N.; Teyssier, C.; Montet, D. Discrimination of Post-Harvest Coffee Processing Methods by Microbial Ecology Analyses. *Food Control* **2016**, *65*, 112–120. [[CrossRef](#)]
39. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [[CrossRef](#)] [[PubMed](#)]
40. Edgar, R.C. MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797. [[CrossRef](#)] [[PubMed](#)]
41. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. JModelTest 2: More Models, New Heuristics and Parallel Computing. *Nat. Methods* **2012**, *9*, 772. [[CrossRef](#)]

42. Guindon, S.; Dufayard, J.-F.; Lefort, V.; Anisimova, M.; Hordijk, W.; Gascuel, O. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Syst. Biol.* **2010**, *59*, 307–321. [[CrossRef](#)] [[PubMed](#)]
43. Paradis, E.; Schliep, K. Ape 5.0: An Environment for Modern Phylogenetics and Evolutionary Analyses in R. *Bioinformatics* **2019**, *35*, 526–528. [[CrossRef](#)] [[PubMed](#)]
44. Lê, S.; Josse, J.; Husson, F. FactoMineR: An R Package for Multivariate Analysis. *J. Stat. Softw.* **2008**, *25*. [[CrossRef](#)]
45. Kassambara, A.; Mundt, F. Factoextra. 2020. Available online: <https://cran.r-project.org/package=factoextra> (accessed on 13 January 2021).
46. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; et al. Vegan: Community Ecology Package. 2020. Available online: <http://outputs.worldagroforestry.org/cgi-bin/koha/opac-detail.pl?biblionumber=39504#> (accessed on 13 January 2021).
47. Veech, J.A. A Probabilistic Model for Analysing Species Co-Occurrence: Probabilistic Model. *Glob. Ecol. Biogeogr.* **2013**, *22*, 252–260. [[CrossRef](#)]
48. Griffith, D.M.; Veech, J.A.; Marsh, C.J. Cooccur: Probabilistic Species Co-Occurrence Analysis in R. *J. Stat. Softw.* **2016**, *69*. [[CrossRef](#)]
49. Barral, B.; Chillet, M.; Doizy, A.; Grassi, M.; Ragot, L.; Léchaudel, M.; Durand, N.; Rose, L.J.; Viljoen, A.; Schorr-Galindo, S. Diversity and Toxicogenicity of Fungi That Cause Pineapple Fruitlet Core Rot. *Toxins* **2020**, *12*, 339. [[CrossRef](#)] [[PubMed](#)]
50. Wigmann, É.F.; Behr, J.; Vogel, R.F.; Niessen, L. MALDI-TOF MS Fingerprinting for Identification and Differentiation of Species within the Fusarium Fujikuroi Species Complex. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 5323–5337. [[CrossRef](#)] [[PubMed](#)]
51. Yilmaz, N.; Houbraken, J.; Hoekstra, E.S.; Frisvad, J.C.; Visagie, C.M.; Samson, R.A. Delimitation and Characterisation of Talaromyces Purpurogenus and Related Species. *Persoonia Mol. Phylogeny Evol. Fungi* **2012**, *29*, 39–54. [[CrossRef](#)] [[PubMed](#)]
52. Oxenham, B. Etiology of Fruitlet Core Rot of Pineapple in Queensland. *Qld. J. Agric. Sci.* **1962**, *19*, 27–31.
53. Barral, B.; Chillet, M.; Léchaudel, M.; Lartaud, M.; Verdeil, J.-L.; Conéjéro, G.; Schorr-Galindo, S. An Imaging Approach to Identify Mechanisms of Resistance to Pineapple Fruitlet Core Rot. *Front. Plant Sci.* **2019**, *10*, 1065. [[CrossRef](#)] [[PubMed](#)]
54. Leneveu-Jenvrin, C.; Quentin, B.; Assemat, S.; Hoarau, M.; Meile, J.-C.; Remize, F. Changes of Quality of Minimally-Processed Pineapple (*Ananas Comosus*, Var. 'Queen Victoria') during Cold Storage: Fungi in the Leading Role. *Microorganisms* **2020**, *8*, 185. [[CrossRef](#)] [[PubMed](#)]
55. Ou, Y.; Penton, C.R.; Geisen, S.; Shen, Z.; Sun, Y.; Lv, N.; Wang, B.; Ruan, Y.; Xiong, W.; Li, R.; et al. Deciphering Underlying Drivers of Disease Suppressiveness Against Pathogenic Fusarium Oxysporum. *Front. Microbiol.* **2019**, *10*, 2535. [[CrossRef](#)]
56. Lima, C.S.; Pfenning, L.H.; Costa, S.S.; Campos, M.A.; Leslie, J.F. A New *Fusarium* Lineage within the *Gibberella Fujikuroi* Species Complex Is the Main Causal Agent of Mango Malformation Disease in Brazil. *Plant Pathol.* **2009**, *58*, 33–42. [[CrossRef](#)]
57. Zhan, R.-L.; Yang, S.-J.; Ho, H.-H.; Liu, F.; Zhao, Y.-L.; Chang, J.-M.; He, Y.-B. Mango Malformation Disease in South China Caused by *Fusarium Proliferatum*: Mango Abnormal Growth. *J. Phytopathol.* **2010**, *158*, 721–725. [[CrossRef](#)]
58. García-Guzmán, G.; Heil, M. Life Histories of Hosts and Pathogens Predict Patterns in Tropical Fungal Plant Diseases. *New Phytol.* **2014**, *201*, 1106–1120. [[CrossRef](#)] [[PubMed](#)]
59. Chen, L.; Zhou, J.; Zeng, T.; Miao, Y.; Mei, L.; Yao, G.; Fang, K.; Dong, X.; Sha, T.; Yang, M.; et al. Quantifying the Sharing of Foliar Fungal Pathogens by the Invasive Plant *Ageratina Adenophora* and Its Neighbours. *New Phytol.* **2020**, *227*, 1493–1504. [[CrossRef](#)] [[PubMed](#)]
60. Parnell, S.; Gottwald, T.R.; Gilligan, C.A.; Cunniffe, N.J.; van den Bosch, F. The Effect of Landscape Pattern on the Optimal Eradication Zone of an Invading Epidemic. *Phytopathology* **2010**, *100*, 638–644. [[CrossRef](#)] [[PubMed](#)]
61. Stępień, Ł.; Koczyk, G.; Waśkiewicz, A. Diversity of Fusarium Species and Mycotoxins Contaminating Pineapple. *J. Appl. Genet.* **2013**, *54*, 367–380. [[CrossRef](#)]
62. Ibrahim, N.F.; Mohd, M.H.; Mohamed Nor, N.M.I.; Zakaria, L. Characterization of *Fusarium* Spp. Associated with Pineapple Fruit Rot and Leaf Spot in Peninsular Malaysia. *J. Phytopathol.* **2017**, *165*, 718–726. [[CrossRef](#)]
63. Leneveu-Jenvrin, C.; Quentin, B.; Assemat, S.; Remize, F. Maintaining Physicochemical, Microbiological, and Sensory Quality of Pineapple Juice (*Ananas Comosus*, Var. 'Queen Victoria') through Mild Heat Treatment. *Processes* **2020**, *8*, 1186. [[CrossRef](#)]
64. Rodríguez Estrada, A.E.; Hegeman, A.; Corby Kistler, H.; May, G. In Vitro Interactions between *Fusarium Verticillioides* and *Ustilago Maydis* through Real-Time PCR and Metabolic Profiling. *Fungal Genet. Biol.* **2011**, *48*, 874–885. [[CrossRef](#)] [[PubMed](#)]
65. Hynes, J.; Müller, C.T.; Jones, T.H.; Boddy, L. Changes in Volatile Production During the Course of Fungal Mycelial Interactions Between *Hypholoma Fasciculare* and *Resinicium Bicolor*. *J. Chem. Ecol.* **2006**, *33*, 43–57. [[CrossRef](#)] [[PubMed](#)]
66. Combès, A.; Ndoye, I.; Bance, C.; Bruzaud, J.; Djediat, C.; Dupont, J.; Nay, B.; Prado, S. Chemical Communication between the Endophytic Fungus *Paraconiothyrium Variabile* and the Phytopathogen *Fusarium Oxysporum*. *PLoS ONE* **2012**, *7*, e47313. [[CrossRef](#)]
67. Marmann, A.; Aly, A.; Lin, W.; Wang, B.; Proksch, P. Co-Cultivation—A Powerful Emerging Tool for Enhancing the Chemical Diversity of Microorganisms. *Mar. Drugs* **2014**, *12*, 1043–1065. [[CrossRef](#)] [[PubMed](#)]
68. Barriuso, J.; Hogan, D.A.; Keshavarz, T.; Martínez, M.J. Role of Quorum Sensing and Chemical Communication in Fungal Biotechnology and Pathogenesis. *FEMS Microbiol. Rev.* **2018**, *42*, 627–638. [[CrossRef](#)] [[PubMed](#)]
69. Lu, Z.; Tombolini, R.; Woo, S.; Zeilinger, S.; Lorito, M.; Jansson, J.K. In Vivo Study of Trichoderma-Pathogen-Plant Interactions, Using Constitutive and Inducible Green Fluorescent Protein Reporter Systems. *Appl. Environ. Microbiol.* **2004**, *70*, 3073–3081. [[CrossRef](#)] [[PubMed](#)]

70. Lagashetti, A.C.; Dufossé, L.; Singh, S.K.; Singh, P.N. Fungal Pigments and Their Prospects in Different Industries. *Microorganisms* **2019**, *7*, 604. [[CrossRef](#)] [[PubMed](#)]
71. Osmanova, N.; Schultze, W.; Ayoub, N. Azaphilones: A Class of Fungal Metabolites with Diverse Biological Activities. *Phytochem. Rev.* **2010**, *9*, 315–342. [[CrossRef](#)]
72. Wei, Z.; Yang, T.; Friman, V.-P.; Xu, Y.; Shen, Q.; Jousset, A. Trophic Network Architecture of Root-Associated Bacterial Communities Determines Pathogen Invasion and Plant Health. *Nat. Commun.* **2015**, *6*, 8413. [[CrossRef](#)] [[PubMed](#)]
73. Schouten, A.; van den Berg, G.; Edel-Hermann, V.; Steinberg, C.; Gautheron, N.; Alabouvette, C.; de Vos, C.H.(Ric); Lemanceau, P.; Raaijmakers, J.M. Defense Responses of *Fusarium Oxysporum* to 2,4-Diacetylphloroglucinol, a Broad-Spectrum Antibiotic Produced by *Pseudomonas Fluorescens*. *Mol. Plant-Microbe Interact.* **2004**, *17*, 1201–1211. [[CrossRef](#)] [[PubMed](#)]
74. Santos, C.; Ventura, J.A.; Costa, H.; Fernandes, P.M.B.; Lima, N. MALDI-TOF MS to Identify the Pineapple Pathogen *Fusarium Guttiforme* and Its Antagonist *Trichoderma Asperellum* on Decayed Pineapple. *Trop. Plant Pathol.* **2015**, *40*, 227–232. [[CrossRef](#)]
75. Crandall, S.G.; Gilbert, G.S. Meteorological Factors Associated with Abundance of Airborne Fungal Spores over Natural Vegetation. *Atmos. Environ.* **2017**, *162*, 87–99. [[CrossRef](#)]
76. Dorey, E.; Fournier, P.; Léchaudel, M.; Tixier, P. Modeling Sugar Content of Pineapple under Agro-Climatic Conditions on Reunion Island. *Eur. J. Agron.* **2016**, *73*, 64–72. [[CrossRef](#)]
77. Zheng, Q.; Ploetz, R. Genetic Diversity in the Mango Malformation Pathogen and Development of a PCR Assay: *PCR Assay for Mango Malformation Pathogen*. *Plant Pathol.* **2002**, *51*, 208–216. [[CrossRef](#)]
78. Lin, Z.; Xu, S.; Que, Y.; Wang, J.; Comstock, J.C.; Wei, J.; McCord, P.H.; Chen, B.; Chen, R.; Zhang, M. Species-Specific Detection and Identification of *Fusarium* Species Complex, the Causal Agent of Sugarcane Pokkah Boeng in China. *PLoS ONE* **2014**, *9*, e104195. [[CrossRef](#)] [[PubMed](#)]
79. Filières Végétales—DAAF de La Réunion. Available online: <https://daaf.reunion.agriculture.gouv.fr/Filieres-Vegetales> (accessed on 11 December 2020).
80. Smart Information System (SmartIS)-VICTORIA-CIRAD-UR AÏDA. Available online: <https://smartis.re/VICTORIA> (accessed on 23 October 2020).
81. Dorey, E.; Fournier, P.; Lechaudel, M.; Tixier, P. Validity of the Pineapple Crop Model SIMPIÑA across the Climatic Gradient in Réunion Island. *Eur. J. Agron.* **2015**, *62*, 1–12. [[CrossRef](#)]

Supplementary data – Article 1

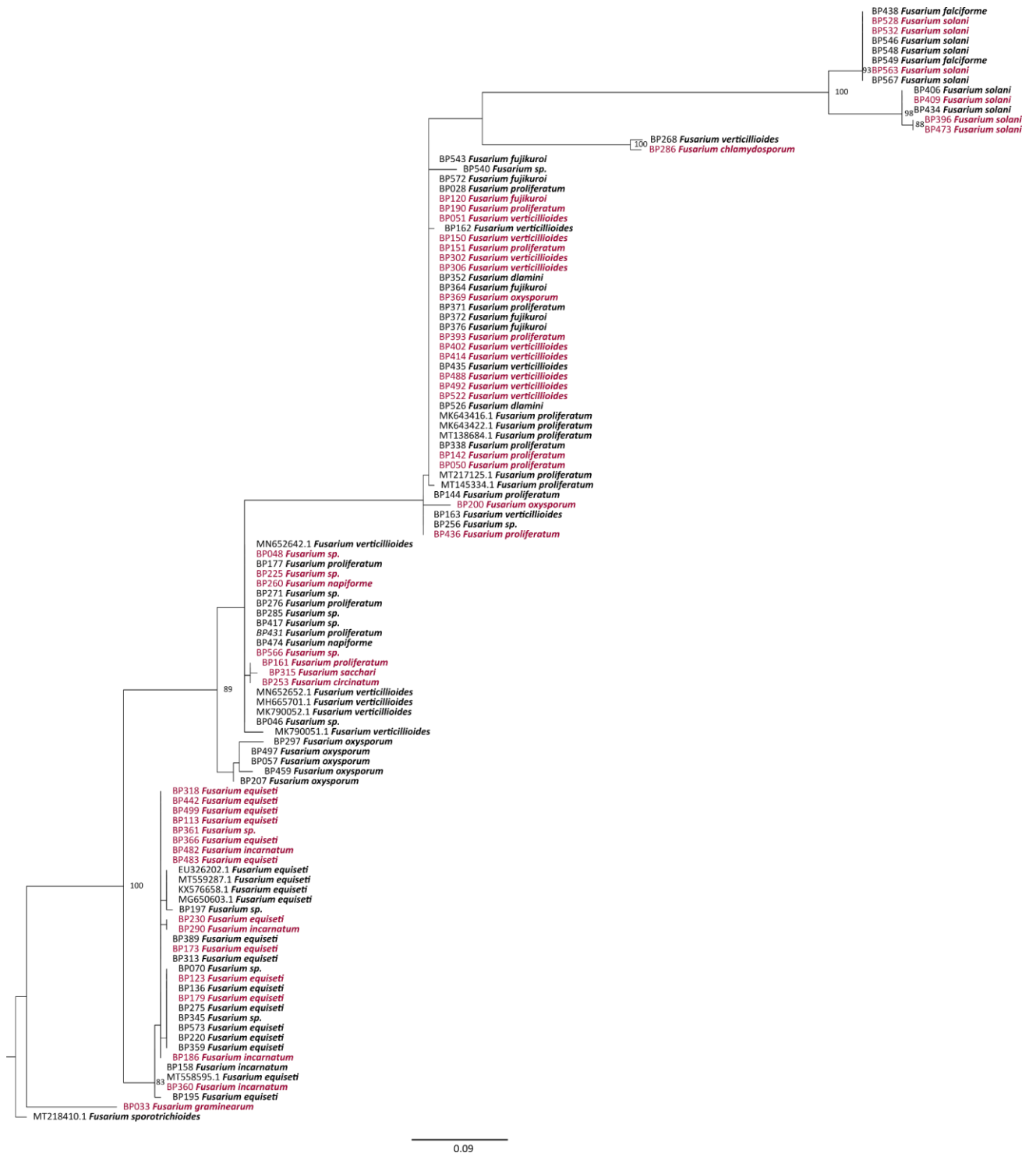


Figure S1. Phylogenetic tree of *Fusarium* species computed by Maximum Likelihood (ML) analysis (JC+I+G as the best fit model) and based on the ITS sequences dataset. Only ML bootstrap branches that support values greater than 70% are shown. Strains isolated from infected and healthy fruitlets are indicated in red and black respectively. The tree is rooted with *F. sporotrichoides* accession MT218410.1.

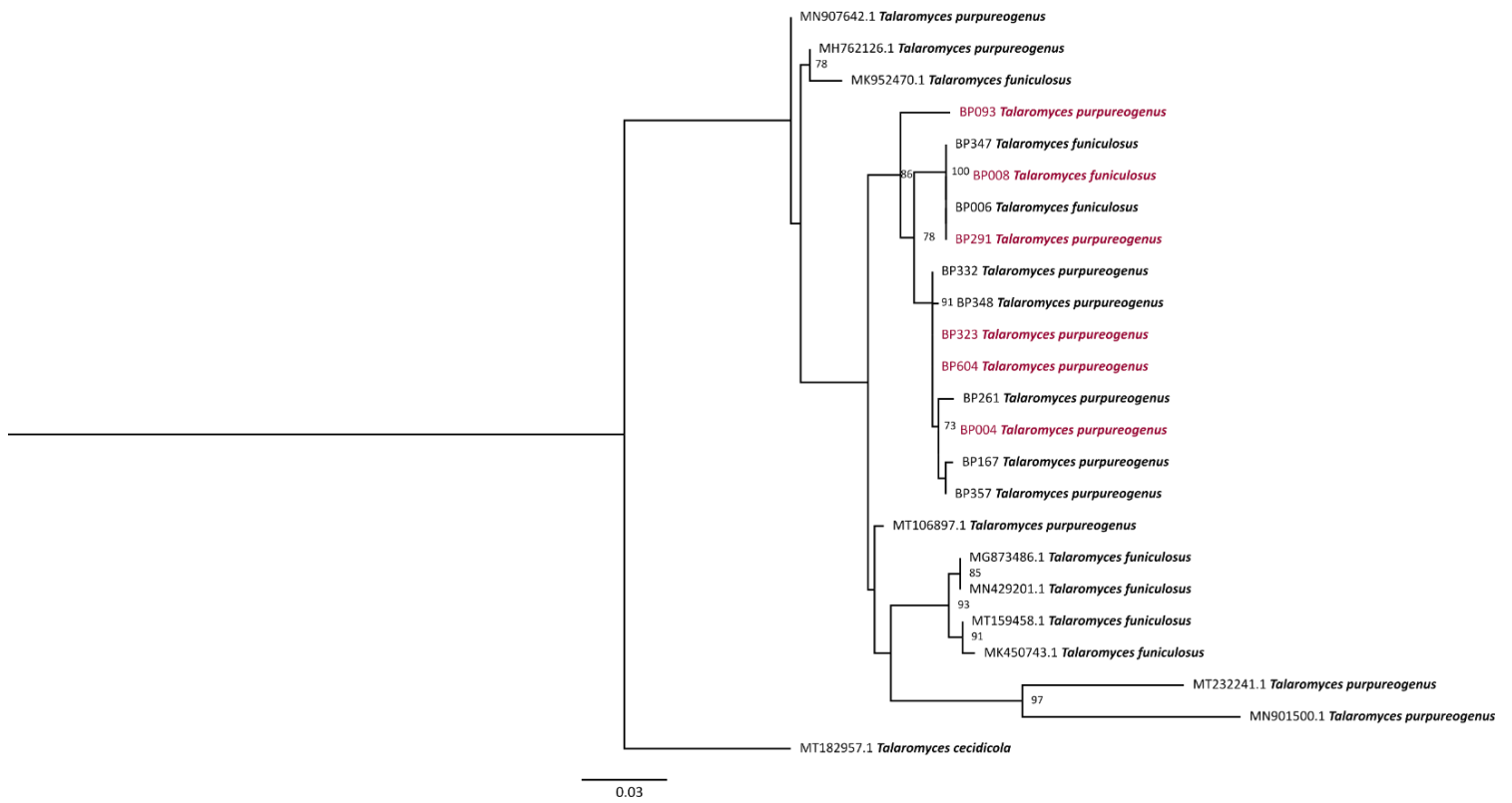


Figure S2. Phylogenetic tree of *Talaromyces* species computed by Maximum Likelihood (ML) analysis (K80+G as the best fit model) and based on the ITS sequences dataset. Only ML bootstrap branches that support values greater than 70% are shown. Strains isolated from infected and healthy fruitlets are indicated in red and black respectively. The tree is rooted with *T. cecidicola* accession MT182957.1.

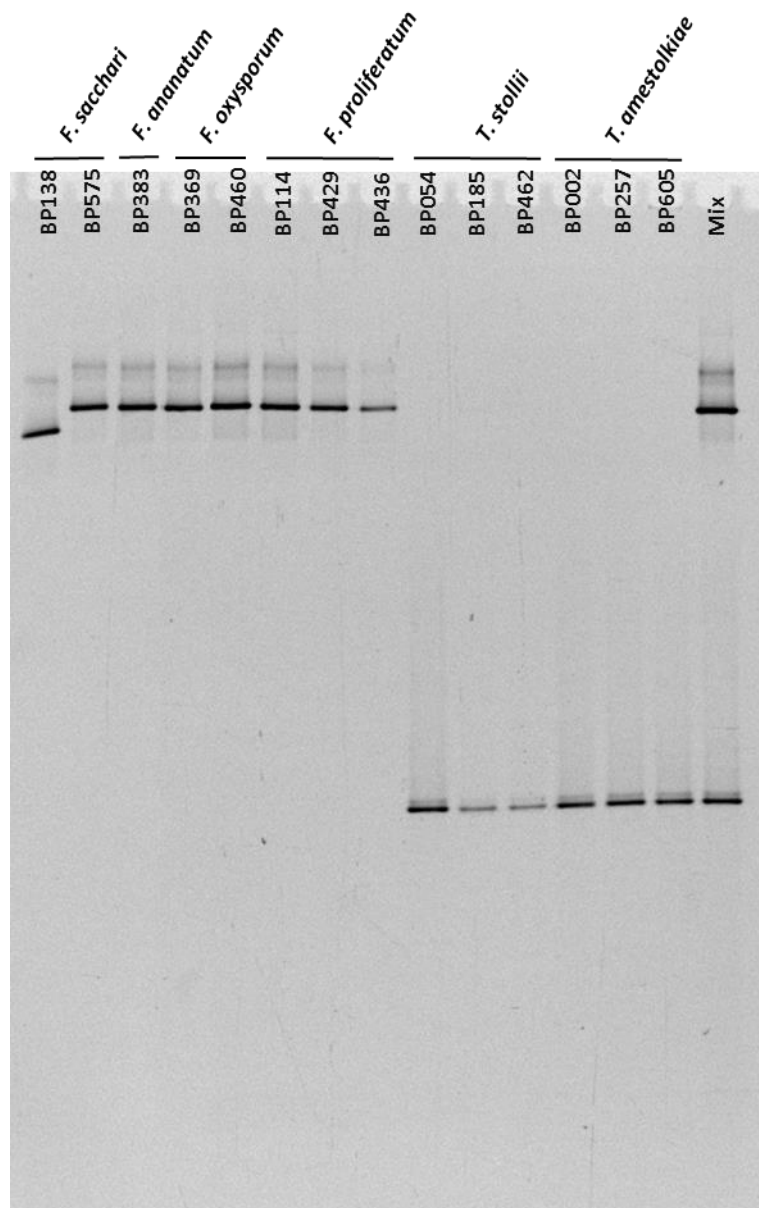


Figure S3. Reference DGGE profiles over ITS-1 region of DNA extracted from pure strains evaluated in Koch's postulates. (Mix) indicates the combined migration of *F. ananatum* strain BP383, *F. sacchari* strain BP575, *F. oxysporum* strain BP460, *F. proliferatum* strain BP429, *T. stollii* strain BP054, and *T. amestolkiae* strain BP002.

Table S1. Identification of fungal species isolated from healthy and naturally infected pineapple fruitlets.

| Isolate ID | Identification | Sampled tissue | Fragment size (bp) | Identity (%) | E-value | Locus | GenBank accession number |
|------------|---------------------------------------|-------------------|--------------------|--------------|-----------------------|-----------|--------------------------|
| BP001 | <i>Talaromyces stollii</i> | healthy fruitlet | 398 | 100 | 0 | β-tubulin | JX315634.1 |
| BP002 | <i>Talaromyces amestolkiae</i> | infected fruitlet | 400 | 99.25 | 0 | β-tubulin | MH287207.1 |
| BP003 | <i>Talaromyces amestolkiae</i> | infected fruitlet | 414 | 100 | 0 | β-tubulin | KJ413360.1 |
| BP004 | <i>Talaromyces purpureogenus</i> | infected fruitlet | 567 | 99.47 | 0 | ITS | MF475916.1 |
| BP006 | <i>Talaromyces funiculosus</i> | healthy fruitlet | 562 | 99.64 | 0 | ITS | AB893941.1 |
| BP008 | <i>Talaromyces funiculosus</i> | infected fruitlet | 548 | 99.64 | 0 | ITS | AB893941.1 |
| BP012 | <i>Talaromyces stollii</i> | infected fruitlet | 320 | 100 | 4.00E ⁻¹⁶⁵ | β-tubulin | JX965359.1 |
| BP014 | <i>Talaromyces stollii</i> | infected fruitlet | 406 | 100 | 0 | β-tubulin | JX315634.1 |
| BP016 | <i>Talaromyces stollii</i> | healthy fruitlet | 406 | 100 | 0 | β-tubulin | JX315634.1 |
| BP018 | <i>Talaromyces stollii</i> | healthy fruitlet | 406 | 100 | 0 | β-tubulin | JX315634.1 |
| BP019 | <i>Talaromyces stollii</i> | infected fruitlet | 406 | 100 | 0 | β-tubulin | JX315634.1 |
| BP020 | <i>Talaromyces stollii</i> | infected fruitlet | 406 | 100 | 0 | β-tubulin | JX315634.1 |
| BP022 | <i>Talaromyces stollii</i> | infected fruitlet | 406 | 100 | 0 | β-tubulin | JX315634.1 |
| BP025 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 414 | 100 | 0 | β-tubulin | MH287207.1 |
| BP027 | <i>Trichoderma trixiae</i> | infected fruitlet | 134 | 100 | 5.00E ⁻⁶² | ITS | MN889512.1 |
| BP028 | <i>Fusarium proliferatum</i> | healthy fruitlet | 532 | 99.81 | 0 | ITS | MN170571.1 |
| BP029 | <i>Trichoderma paraviridescens</i> | infected fruitlet | 587 | 100 | 0 | ITS | MK418756.1 |
| BP030 | <i>Aspergillus niger</i> | infected fruitlet | 579 | 100 | 0 | ITS | JF439461.1 |
| BP031 | <i>Pestalotiopsis vismiae</i> | infected fruitlet | 587 | 99.66 | 0 | ITS | KP747694.1 |
| BP033 | <i>Fusarium graminearum</i> | infected fruitlet | 514 | 100 | 0 | ITS | MK460853.1 |
| BP034 | <i>Talaromyces stollii</i> | healthy fruitlet | 405 | 100 | 0 | β-tubulin | JX315634.1 |
| BP035 | <i>Fusarium cortaderiae</i> | infected fruitlet | 290 | 99.66 | 4.00E ⁻¹⁴⁸ | β-tubulin | AH012626.2 |
| BP037 | <i>Trichoderma sp.</i> | infected fruitlet | 596 | 99.83 | 0 | ITS | KX449479.1 |
| BP038 | <i>Rhizopus stolonifer</i> | infected fruitlet | 788 | 100 | 0 | ITS | MF461025.1 |
| BP039 | <i>Fusarium chlamydosporum</i> | healthy fruitlet | 272 | 100 | 1.00E ⁻¹³⁹ | β-tubulin | KJ125830.1 |
| BP041 | <i>Epicoccum sorghinum</i> | infected fruitlet | 298 | 99.33 | 2.00E ⁻¹⁵⁰ | β-tubulin | MF987525.1 |
| BP042 | <i>Trichoderma harzianum</i> | infected fruitlet | 565 | 100 | 0 | ITS | JN116710.1 |
| BP043 | <i>Aspergillus niger</i> | healthy fruitlet | 450 | 99.56 | 0 | ITS | MN788116.1 |
| BP044 | <i>Fusarium proliferatum</i> | healthy fruitlet | 665 | 100 | 0.0 | EF1α | KF993985.1 |
| BP046 | <i>Fusarium sp.</i> | healthy fruitlet | 528 | 100 | 0 | ITS | HQ630965.1 |
| BP048 | <i>Fusarium sp.</i> | infected fruitlet | 539 | 100 | 0 | ITS | HQ630965.1 |
| BP050 | <i>Fusarium proliferatum</i> | infected fruitlet | 544 | 99.26 | 0 | ITS | MN170571.1 |
| BP051 | <i>Fusarium verticillioides</i> | infected fruitlet | 531 | 100 | 0 | ITS | KR183784.1 |
| BP054 | <i>Talaromyces stollii</i> | infected fruitlet | 406 | 99.51 | 0 | β-tubulin | JX315634.1 |
| BP057 | <i>Fusarium oxysporum</i> | healthy fruitlet | 524 | 100 | 0 | ITS | MH752745.1 |
| BP059 | <i>Aspergillus awamori</i> | healthy fruitlet | 505 | 100 | 0 | β-tubulin | KY416558.1 |
| BP061 | <i>Fusarium ananatum</i> | healthy fruitlet | 632 | 100 | 0 | EF1α | MT010996.1 |
| BP062 | <i>Talaromyces funiculosus</i> | infected fruitlet | 414 | 99.52 | 0 | β-tubulin | MK208457.1 |
| BP063 | <i>Epicoccum sorghinum</i> | healthy fruitlet | 312 | 99.36 | 0 | β-tubulin | MF987525.1 |
| BP065 | <i>Fusarium ananatum</i> | healthy fruitlet | 663 | 100 | 0 | EF1α | MT010996.1 |
| BP070 | <i>Fusarium sp.</i> | healthy fruitlet | 523 | 99.81 | 0 | ITS | MG664720.1 |
| BP071 | <i>Trichoderma paraviridescens</i> | healthy fruitlet | 586 | 100 | 0 | ITS | MF782827.1 |
| BP072 | <i>Fusarium proliferatum</i> | healthy fruitlet | 668 | 100 | 0 | EF1α | KU872092.1 |
| BP076 | <i>Fusarium proliferatum</i> | healthy fruitlet | 669 | 99.85 | 0 | EF1α | KF993985.1 |
| BP077 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 422 | 100 | 0 | β-tubulin | MH287207.1 |
| BP078 | <i>Pestalotiopsis trachicarpicola</i> | healthy fruitlet | 440 | 100 | 0 | β-tubulin | MN295594.1 |
| BP081 | <i>Talaromyces stollii</i> | healthy fruitlet | 398 | 100 | 0 | β-tubulin | JX315634.1 |
| BP082 | <i>Talaromyces amestolkiae</i> | infected fruitlet | 401 | 100 | 0 | β-tubulin | MH287207.1 |
| BP084 | <i>Aspergillus niger</i> | healthy fruitlet | 508 | 100 | 0 | β-tubulin | HQ632734.1 |

Table S1. Continued.

| Isolate ID | Identification | Sampled tissue | Fragment size (bp) | Identity (%) | E-value | Locus | GenBank accession number |
|------------|---------------------------------------|-------------------|--------------------|--------------|-----------------------|-----------|--------------------------|
| BP086 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 414 | 100 | 0 | β-tubulin | KJ413360.1 |
| BP087 | <i>Talaromyces stollii</i> | infected fruitlet | 406 | 99.75 | 0 | β-tubulin | JX315634.1 |
| BP088 | <i>Talaromyces stollii</i> | infected fruitlet | 398 | 100 | 0 | β-tubulin | JX315634.1 |
| BP091 | <i>Trichoderma asperellum</i> | infected fruitlet | 43 | 100 | 1.00E ⁻¹³ | ITS | KX538815.1 |
| BP093 | <i>Talaromyces purpureogenus</i> | infected fruitlet | 523 | 100 | 0 | ITS | MH185977.1 |
| BP096 | <i>Pestalotiopsis trachicarpicola</i> | infected fruitlet | 440 | 100 | 0 | β-tubulin | MN295594.1 |
| BP097 | <i>Talaromyces amestolkiae</i> | infected fruitlet | 422 | 100 | 0 | β-tubulin | KJ413360.1 |
| BP100 | <i>Aspergillus niger</i> | infected fruitlet | 573 | 100 | 0 | ITS | KY357318.1 |
| BP101 | <i>Fusarium ananatum</i> | infected fruitlet | 666 | 100 | 0 | EF1α | MT010996.1 |
| BP102 | <i>Talaromyces amestolkiae</i> | infected fruitlet | 408 | 100 | 0 | β-tubulin | KJ413360.1 |
| BP104 | <i>Talaromyces amestolkiae</i> | infected fruitlet | 408 | 100 | 0 | β-tubulin | LT559068.1 |
| BP106 | <i>Clonostachys weningii</i> | infected fruitlet | 496 | 100 | 0 | ITS | NR_119651.1 |
| BP107 | <i>Aspergillus flavus</i> | infected fruitlet | 424 | 100 | 0 | ITS | MN955851.1 |
| BP109 | <i>Fusarium proliferatum</i> | healthy fruitlet | 659 | 100 | 0 | EF1α | KF993985.1 |
| BP113 | <i>Fusarium equiseti</i> | infected fruitlet | 514 | 100 | 0 | ITS | MK680159.1 |
| BP114 | <i>Fusarium proliferatum</i> | infected fruitlet | 658 | 100 | 0 | EF1α | KF993985.1 |
| BP118 | <i>Fusarium proliferatum</i> | healthy fruitlet | 655 | 99.85 | 0 | EF1α | MN386746.1 |
| BP120 | <i>Fusarium fujikuroi</i> | infected fruitlet | 527 | 100 | 0 | ITS | MF281281.2 |
| BP121 | <i>Fusarium ananatum</i> | infected fruitlet | 646 | 100 | 0 | EF1α | MT010996.1 |
| BP123 | <i>Fusarium equiseti</i> | infected fruitlet | 511 | 100 | 0 | ITS | KF624787.1 |
| BP127 | <i>Fusarium proliferatum</i> | infected fruitlet | 646 | 100 | 0 | EF1α | MN386746.1 |
| BP129 | <i>Trichoderma sp.</i> | infected fruitlet | 598 | 99.83 | 0 | ITS | MK870964.1 |
| BP130 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 414 | 100 | 0 | β-tubulin | KJ413360.1 |
| BP136 | <i>Fusarium equiseti</i> | healthy fruitlet | 516 | 100 | 0 | ITS | KF624787.1 |
| BP138 | <i>Fusarium sacchari</i> | infected fruitlet | 663 | 100 | 0 | EF1α | MN193868.1 |
| BP139 | <i>Clonostachys sp.</i> | healthy fruitlet | 503 | 100 | 0 | ITS | MH681594.1 |
| BP141 | <i>Bionectria ochroleuca</i> | healthy fruitlet | 550 | 100 | 0 | ITS | EU552110.1 |
| BP144 | <i>Fusarium proliferatum</i> | healthy fruitlet | 528 | 100 | 0 | ITS | MH712288.1 |
| BP147 | <i>Fusarium proliferatum</i> | infected fruitlet | 644 | 99.84 | 0 | EF1α | MN175178.1 |
| BP148 | <i>Fusarium proliferatum</i> | healthy fruitlet | 654 | 99.85 | 0 | EF1α | KU872092.1 |
| BP150 | <i>Fusarium verticillioides</i> | infected fruitlet | 537 | 99.63 | 0 | ITS | MT594370.1 |
| BP151 | <i>Fusarium proliferatum</i> | infected fruitlet | 523 | 100 | 0 | ITS | MK336501.1 |
| BP154 | <i>Fusarium incarnatum</i> | infected fruitlet | 292 | 99.66 | 3.00E ⁻¹⁴⁹ | β-tubulin | MK439850.1 |
| BP155 | <i>Fusarium proliferatum</i> | healthy fruitlet | 644 | 99.84 | 0 | EF1α | KF993985.1 |
| BP156 | <i>Fusarium proliferatum</i> | healthy fruitlet | 663 | 100 | 0 | EF1α | KF993985.1 |
| BP158 | <i>Fusarium incarnatum</i> | healthy fruitlet | 525 | 99.81 | 0 | ITS | MN882829.1 |
| BP159 | <i>Fusarium proliferatum</i> | healthy fruitlet | 663 | 100 | 0 | EF1α | FJ538242.1 |
| BP161 | <i>Fusarium proliferatum</i> | infected fruitlet | 531 | 99.62 | 0 | ITS | MH591759.1 |
| BP162 | <i>Fusarium verticillioides</i> | healthy fruitlet | 541 | 99.82 | 0 | ITS | MT594370.1 |
| BP163 | <i>Fusarium verticillioides</i> | healthy fruitlet | 527 | 100 | 0 | ITS | MT126609.1 |
| BP165 | <i>Fusarium proliferatum</i> | healthy fruitlet | 292 | 99.66 | 1.00E ⁻¹⁴⁸ | β-tubulin | MH398186.1 |
| BP167 | <i>Talaromyces purpureogenus</i> | healthy fruitlet | 513 | 99.61 | 0 | ITS | MF476006.1 |
| BP169 | <i>Glomus clarum</i> | healthy fruitlet | 102 | 100 | 0 | ITS | AY035654.1 |
| BP170 | <i>Diaporthe masirevicii</i> | infected fruitlet | 487 | 99.79 | 0 | β-tubulin | MF668289.1 |
| BP173 | <i>Fusarium equiseti</i> | infected fruitlet | 512 | 99.8 | 0 | ITS | MG650603.1 |
| BP177 | <i>Fusarium proliferatum</i> | healthy fruitlet | 530 | 100 | 0 | ITS | MH591759.1 |
| BP179 | <i>Fusarium equiseti</i> | infected fruitlet | 523 | 100 | 0 | ITS | KF624787.1 |
| BP180 | <i>Fusarium proliferatum</i> | infected fruitlet | 657 | 100 | 0 | EF1α | MK442093.1 |
| BP185 | <i>Talaromyces stollii</i> | infected fruitlet | 398 | 100 | 0 | β-tubulin | JX315634.1 |
| BP186 | <i>Fusarium incarnatum</i> | infected fruitlet | 467 | 100 | 0 | ITS | MK336548.1 |

Table S1. Continued.

| Isolate ID | Identification | Sampled tissue | Fragment size (bp) | Identity (%) | E-value | Locus | GenBank accession number |
|------------|---------------------------------------|-------------------|--------------------|--------------|-----------------------|-----------|--------------------------|
| BP187 | <i>Fusarium ananatum</i> | healthy fruitlet | 306 | 98.39 | 1.00E ⁻¹⁴⁴ | β-tubulin | LC386036.1 |
| BP188 | <i>Fusarium ananatum</i> | infected fruitlet | 644 | 100 | 0 | EF1α | MT010996.1 |
| BP190 | <i>Fusarium proliferatum</i> | infected fruitlet | 539 | 99.81 | 0 | ITS | MH055399.1 |
| BP191 | <i>Fusarium equiseti</i> | healthy fruitlet | 294 | 100 | 5.00E ⁻¹⁵² | β-tubulin | MK278900.1 |
| BP194 | <i>Talaromyces stollii</i> | infected fruitlet | 398 | 100 | 0 | β-tubulin | JX315634.1 |
| BP195 | <i>Fusarium equiseti</i> | healthy fruitlet | 512 | 99.80 | 0 | ITS | MN589630.1 |
| BP197 | <i>Fusarium sp.</i> | healthy fruitlet | 517 | 100 | 0 | ITS | MG664720.1 |
| BP199 | <i>Aspergillus fumigatus</i> | infected fruitlet | 504 | 100 | 0 | β-tubulin | MH844690.1 |
| BP200 | <i>Fusarium oxysporum</i> | infected fruitlet | 88 | 98.86 | 6.00E ⁻³⁶ | ITS | MK729614.1 |
| BP202 | <i>Aspergillus niger</i> | healthy fruitlet | 543 | 100 | 0 | ITS | MK336494.1 |
| BP204 | <i>Fusarium oxysporum</i> | infected fruitlet | 658 | 100 | 0 | EF1α | CP053267.1 |
| BP207 | <i>Fusarium oxysporum</i> | infected fruitlet | 527 | 100 | 0 | ITS | KU847855.1 |
| BP208 | <i>Fusarium ficicrescens</i> | healthy fruitlet | 286 | 99.3 | 7.00E ⁻¹⁴³ | β-tubulin | KP662895.1 |
| BP210 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 408 | 100 | 0 | β-tubulin | LT559068.1 |
| BP211 | <i>Talaromyces stollii</i> | infected fruitlet | 398 | 100 | 0 | β-tubulin | JX315634.1 |
| BP212 | <i>Pestalotiopsis trachicarpicola</i> | infected fruitlet | 434 | 100 | 0 | β-tubulin | MN295594.1 |
| BP213 | <i>Talaromyces stollii</i> | infected fruitlet | 398 | 100 | 0 | β-tubulin | JX315634.1 |
| BP215 | <i>Talaromyces stollii</i> | healthy fruitlet | 398 | 100 | 0 | β-tubulin | JX315634.1 |
| BP216 | <i>Pestalotiopsis trachicarpicola</i> | healthy fruitlet | 441 | 100 | 0 | β-tubulin | MN295594.1 |
| BP218 | <i>Fusarium proliferatum</i> | healthy fruitlet | 663 | 99.85 | 0 | EF1α | KF993985.1 |
| BP219 | <i>Fusarium oxysporum</i> | healthy fruitlet | 660 | 100 | 0 | EF1α | MG000154.1 |
| BP220 | <i>Fusarium equiseti</i> | healthy fruitlet | 511 | 100 | 0 | ITS | MN589630.1 |
| BP222 | <i>Fusarium proliferatum</i> | infected fruitlet | 661 | 100 | 0 | EF1α | KF993985.1 |
| BP224 | <i>Fusarium chlamydosporum</i> | healthy fruitlet | 272 | 100 | 2.00E ⁻¹³⁸ | β-tubulin | KJ125830.1 |
| BP225 | <i>Fusarium sp.</i> | infected fruitlet | 528 | 100 | 0 | ITS | HQ630965.1 |
| BP230 | <i>Fusarium equiseti</i> | infected fruitlet | 487 | 99.79 | 0 | ITS | MN589630.1 |
| BP236 | <i>Fusarium ananatum</i> | infected fruitlet | 664 | 100 | 0 | EF1α | MT010996.1 |
| BP238 | <i>Fusarium ananatum</i> | infected fruitlet | 641 | 100 | 0 | EF1α | MT010996.1 |
| BP240 | <i>Talaromyces stollii</i> | infected fruitlet | 392 | 100 | 0 | β-tubulin | JX315634.1 |
| BP241 | <i>Fusarium ananatum</i> | healthy fruitlet | 652 | 99.85 | 0 | EF1α | MT010996.1 |
| BP243 | <i>Fusarium ananatum</i> | healthy fruitlet | 656 | 100 | 0 | EF1α | MT010996.1 |
| BP247 | <i>Fusarium ananatum</i> | healthy fruitlet | 653 | 100 | 0 | EF1α | MT010996.1 |
| BP251 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 408 | 100 | 0 | β-tubulin | MH287207.1 |
| BP253 | <i>Fusarium circinatum</i> | infected fruitlet | 515 | 100 | 0 | ITS | MK334369.1 |
| BP254 | <i>Aspergillus novoparasiticus</i> | infected fruitlet | 562 | 100 | 0 | ITS | MH279415.1 |
| BP256 | <i>Fusarium sp.</i> | healthy fruitlet | 517 | 100 | 0 | ITS | MK355727.1 |
| BP257 | <i>Talaromyces amestolkiae</i> | infected fruitlet | 407 | 100 | 0 | β-tubulin | KJ413360.1 |
| BP259 | <i>Penicillium sp.</i> | healthy fruitlet | 563 | 100 | 0 | ITS | EU330619.1 |
| BP260 | <i>Fusarium napiforme</i> | infected fruitlet | 515 | 100 | 0 | ITS | MH862670.1 |
| BP261 | <i>Talaromyces purpureogenus</i> | healthy fruitlet | 502 | 99.8 | 0 | ITS | MF476006.1 |
| BP263 | <i>Diaporthe sp.</i> | healthy fruitlet | 461 | 98.05 | 0 | β-tubulin | MH220834.1 |
| BP265 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 401 | 100 | 0 | β-tubulin | KJ413360.1 |
| BP268 | <i>Fusarium verticillioides</i> | healthy fruitlet | 543 | 100 | 0 | ITS | MH863977.1 |
| BP269 | <i>Aspergillus awamori</i> | healthy fruitlet | 506 | 100 | 0 | β-tubulin | KY416558.1 |
| BP271 | <i>Fusarium sp.</i> | healthy fruitlet | 506 | 100 | 0 | ITS | MH777054.1 |
| BP273 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 421 | 100 | 0 | β-tubulin | KJ413360.1 |
| BP275 | <i>Fusarium equiseti</i> | healthy fruitlet | 511 | 100 | 0 | ITS | MN589627.1 |
| BP276 | <i>Fusarium proliferatum</i> | healthy fruitlet | 529 | 100 | 0 | ITS | MH591759.1 |
| BP278 | <i>Fusarium oxysporum</i> | healthy fruitlet | 645 | 100 | 0 | EF1α | CP053267.1 |
| BP280 | <i>Fusarium incarnatum</i> | healthy fruitlet | 251 | 100 | 8.00E ⁻¹²⁷ | β-tubulin | MK439849.1 |

Table S1. Continued.

| Isolate ID | Identification | Sampled tissue | Fragment size (bp) | Identity (%) | E-value | Locus | GenBank accession number |
|------------|----------------------------------|-------------------|--------------------|--------------|-----------|------------------|--------------------------|
| BP285 | <i>Fusarium</i> sp. | healthy fruitlet | 514 | 100 | 0 | ITS | MT530119.1 |
| BP286 | <i>Fusarium chlamydosporum</i> | infected fruitlet | 533 | 99.81 | 0 | ITS | MG250448.1 |
| BP288 | <i>Diaporthe kongii</i> | infected fruitlet | 544 | 99.26 | 0 | ITS | KR024740.1 |
| BP290 | <i>Fusarium incarnatum</i> | infected fruitlet | 496 | 99.8 | 0 | ITS | MN882829.1 |
| BP291 | <i>Talaromyces purpureogenus</i> | infected fruitlet | 547 | 99.63 | 0 | ITS | MK749843.1 |
| BP293 | <i>Fusarium proliferatum</i> | healthy fruitlet | 676 | 100 | 0 | EF1 α | KF993985.1 |
| BP296 | <i>Fusarium equiseti</i> | healthy fruitlet | 294 | 99.66 | 5.00E-149 | β -tubulin | MK278900.1 |
| BP297 | <i>Fusarium oxysporum</i> | infected fruitlet | 364 | 100 | 0 | ITS | EU273520.1 |
| BP302 | <i>Fusarium verticillioides</i> | infected fruitlet | 529 | 100 | 0 | ITS | MT594370.1 |
| BP305 | <i>Fusarium ananatum</i> | healthy fruitlet | 659 | 100 | 0 | EF1 α | MT010996.1 |
| BP306 | <i>Fusarium verticillioides</i> | infected fruitlet | 541 | 99.82 | 0 | ITS | MT594370.1 |
| BP308 | <i>Fusarium ananatum</i> | infected fruitlet | 671 | 100 | 0 | EF1 α | MT010996.1 |
| BP313 | <i>Fusarium equiseti</i> | healthy fruitlet | 506 | 99.8 | 0 | ITS | MN589629.1 |
| BP315 | <i>Fusarium sacchari</i> | infected fruitlet | 438 | 100 | 0 | ITS | KC464631.1 |
| BP316 | <i>Lasiodiplodia mahajangana</i> | healthy fruitlet | 398 | 100 | 0 | ITS | MH057188.1 |
| BP318 | <i>Fusarium equiseti</i> | infected fruitlet | 514 | 100 | 0 | ITS | MH578585.1 |
| BP319 | <i>Fusarium proliferatum</i> | healthy fruitlet | 658 | 100 | 0 | EF1 α | KU872092.1 |
| BP320 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 414 | 100 | 0 | β -tubulin | KJ413360.1 |
| BP323 | <i>Talaromyces purpureogenus</i> | infected fruitlet | 566 | 100 | 0 | ITS | MF475966.1 |
| BP324 | <i>Fusarium proliferatum</i> | healthy fruitlet | 659 | 100 | 0 | EF1 α | MK442093.1 |
| BP326 | <i>Fusarium ananatum</i> | infected fruitlet | 671 | 100 | 0 | EF1 α | MT010996.1 |
| BP331 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 414 | 100 | 0 | β -tubulin | KJ413360.1 |
| BP332 | <i>Talaromyces purpureogenus</i> | healthy fruitlet | 556 | 99.82 | 0 | ITS | KJ528885.1 |
| BP334 | <i>Talaromyces stollii</i> | infected fruitlet | 398 | 100 | 0 | β -tubulin | JX315634.1 |
| BP335 | <i>Curvularia lunata</i> | infected fruitlet | 533 | 100 | 0 | ITS | MN971669.1 |
| BP338 | <i>Fusarium proliferatum</i> | healthy fruitlet | 538 | 100 | 0 | ITS | MT466521.1 |
| BP340 | <i>Talaromyces stollii</i> | healthy fruitlet | 398 | 100 | 0 | β -tubulin | JX315634.1 |
| BP341 | <i>Penicillium</i> sp. | infected fruitlet | 576 | 99.48 | 0 | ITS | KF931337.1 |
| BP342 | <i>Fusarium ananatum</i> | healthy fruitlet | 661 | 100 | 0 | EF1 α | MT010996.1 |
| BP343 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 422 | 99.29 | 0 | β -tubulin | KJ413360.1 |
| BP344 | <i>Talaromyces stollii</i> | infected fruitlet | 398 | 100 | 0 | β -tubulin | JX315634.1 |
| BP345 | <i>Fusarium</i> sp. | healthy fruitlet | 511 | 100 | 0 | ITS | KM979504.1 |
| BP346 | <i>Talaromyces stollii</i> | healthy fruitlet | 396 | 99.75 | 0 | β -tubulin | JX315634.1 |
| BP347 | <i>Talaromyces funiculosus</i> | healthy fruitlet | 501 | 99.6 | 0 | ITS | AB893941.1 |
| BP348 | <i>Talaromyces purpureogenus</i> | healthy fruitlet | 556 | 99.82 | 0 | ITS | KJ528885.1 |
| BP349 | <i>Penicillium</i> sp. | healthy fruitlet | 557 | 99.82 | 0 | ITS | EU330619.1 |
| BP351 | <i>Trichoderma erinaceum</i> | infected fruitlet | 228 | 100 | 2.00E-115 | ITS | MK109820.1 |
| BP352 | <i>Fusarium dlamini</i> | infected fruitlet | 548 | 100 | 0 | ITS | MN173109.1 |
| BP353 | <i>Fusarium ananatum</i> | infected fruitlet | 656 | 100 | 0 | EF1 α | MT010996.1 |
| BP356 | <i>Fusarium ananatum</i> | healthy fruitlet | 656 | 100 | 0 | EF1 α | MT010996.1 |
| BP357 | <i>Talaromyces purpureogenus</i> | healthy fruitlet | 513 | 99.81 | 0 | ITS | MF476006.1 |
| BP359 | <i>Fusarium equiseti</i> | healthy fruitlet | 487 | 100 | 0 | ITS | MN589627.1 |
| BP360 | <i>Fusarium incarnatum</i> | infected fruitlet | 471 | 100 | 0 | ITS | MN882828.1 |
| BP361 | <i>Fusarium</i> sp. | infected fruitlet | 527 | 99.81 | 0 | ITS | EU330623.1 |
| BP364 | <i>Fusarium fujikuroi</i> | healthy fruitlet | 533 | 100 | 0 | ITS | MF281281.2 |
| BP366 | <i>Fusarium equiseti</i> | infected fruitlet | 514 | 100 | 0 | ITS | MK680159.1 |
| BP367 | <i>Fusarium ananatum</i> | infected fruitlet | 669 | 100 | 0 | EF1 α | MT010996.1 |
| BP368 | <i>Fusarium oxysporum</i> | infected fruitlet | 663 | 99.7 | 0 | EF1 α | MN386739.1 |
| BP369 | <i>Fusarium oxysporum</i> | infected fruitlet | 539 | 100 | 0 | ITS | KC119203.1 |
| BP370 | <i>Aspergillus flavus</i> | infected fruitlet | 336 | 100 | 0 | ITS | MF152938.1 |

Table S1. Continued.

| Isolate ID | Identification | Sampled tissue | Fragment size (bp) | Identity (%) | E-value | Locus | GenBank accession number |
|------------|---------------------------------|-------------------|--------------------|--------------|-----------------------|------------------|--------------------------|
| BP371 | <i>Fusarium proliferatum</i> | healthy fruitlet | 538 | 100 | 0 | ITS | MH055399.1 |
| BP372 | <i>Fusarium fujikuroi</i> | healthy fruitlet | 539 | 100 | 0 | ITS | MF281281.2 |
| BP373 | <i>Fusarium proliferatum</i> | infected fruitlet | 671 | 100 | 0 | EF1 α | MK442093.1 |
| BP376 | <i>Fusarium fujikuroi</i> | healthy fruitlet | 531 | 100 | 0 | ITS | MF281281.2 |
| BP379 | <i>Fusarium ananatum</i> | healthy fruitlet | 658 | 100 | 0 | EF1 α | MT010996.1 |
| BP383 | <i>Fusarium ananatum</i> | infected fruitlet | 656 | 100 | 0 | EF1 α | MT010996.1 |
| BP384 | <i>Fusarium ananatum</i> | infected fruitlet | 655 | 100 | 0 | EF1 α | MT010996.1 |
| BP388 | <i>Fusarium ananatum</i> | healthy fruitlet | 671 | 100 | 0 | EF1 α | MT010996.1 |
| BP389 | <i>Fusarium equiseti</i> | infected fruitlet | 507 | 100 | 0 | ITS | MH879584.1 |
| BP391 | <i>Fusarium proliferatum</i> | infected fruitlet | 660 | 100 | 0 | EF1 α | MN539103.1 |
| BP393 | <i>Fusarium proliferatum</i> | infected fruitlet | 531 | 99.81 | 0 | ITS | MH055399.1 |
| BP395 | <i>Fusarium solani</i> | infected fruitlet | 686 | 100 | 0 | EF1 α | MN200289.1 |
| BP396 | <i>Fusarium solani</i> | infected fruitlet | 549 | 100 | 0 | ITS | MK968891.1 |
| BP400 | <i>Fusarium ananatum</i> | healthy fruitlet | 669 | 100 | 0 | EF1 α | MT010996.1 |
| BP402 | <i>Fusarium verticillioides</i> | infected fruitlet | 526 | 100 | 0 | ITS | MT594370.1 |
| BP405 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 414 | 100 | 0 | β -tubulin | MH287203.1 |
| BP406 | <i>Fusarium solani</i> | healthy fruitlet | 548 | 100 | 0 | ITS | JX173101.1 |
| BP407 | <i>Fusarium ananatum</i> | infected fruitlet | 670 | 100 | 0 | EF1 α | MT010996.1 |
| BP409 | <i>Fusarium solani</i> | infected fruitlet | 526 | 100 | 0 | ITS | KF030977.1 |
| BP410 | <i>Fusarium ananatum</i> | healthy fruitlet | 673 | 100 | 0 | EF1 α | MT010996.1 |
| BP413 | <i>Fusarium proliferatum</i> | healthy fruitlet | 669 | 99.85 | 0 | EF1 α | KF993985.1 |
| BP414 | <i>Fusarium verticillioides</i> | infected fruitlet | 524 | 100 | 0 | ITS | MT594370.1 |
| BP416 | <i>Talaromyces amestolkiae</i> | infected fruitlet | 406 | 100 | 0 | β -tubulin | KJ413360.1 |
| BP417 | <i>Fusarium sp.</i> | healthy fruitlet | 513 | 100 | 0 | ITS | MH777054.1 |
| BP418 | <i>Aspergillus niger</i> | infected fruitlet | 569 | 100 | 0 | ITS | KY357318.1 |
| BP420 | <i>Fusarium verticillioides</i> | healthy fruitlet | 662 | 100 | 0 | EF1 α | MN861780.1 |
| BP421 | <i>Fusarium verticillioides</i> | infected fruitlet | 661 | 100 | 0 | EF1 α | MT095057.1 |
| BP422 | <i>Fusarium ananatum</i> | healthy fruitlet | 636 | 100 | 0 | EF1 α | MT010996.1 |
| BP423 | <i>Fusarium ananatum</i> | infected fruitlet | 656 | 100 | 0 | EF1 α | MT010996.1 |
| BP426 | <i>Fusarium incarnatum</i> | infected fruitlet | 290 | 99.66 | 4.00E ⁻¹⁴⁸ | β -tubulin | MK752398.1 |
| BP428 | <i>Fusarium proliferatum</i> | infected fruitlet | 661 | 100 | 0 | EF1 α | MN539103.1 |
| BP429 | <i>Fusarium proliferatum</i> | infected fruitlet | 676 | 100 | 0 | EF1 α | FJ538242.1 |
| BP430 | <i>Fusarium ananatum</i> | infected fruitlet | 659 | 100 | 0 | EF1 α | MT010996.1 |
| BP431 | <i>Fusarium proliferatum</i> | healthy fruitlet | 512 | 100 | 0 | ITS | MT563410.1 |
| BP432 | <i>Cosmospora butyri</i> | healthy fruitlet | 530 | 100 | 0 | ITS | KU204560.1 |
| BP433 | <i>Fusarium proliferatum</i> | healthy fruitlet | 283 | 100 | 7.00E ⁻¹⁴⁶ | β -tubulin | MH398186.1 |
| BP434 | <i>Fusarium solani</i> | healthy fruitlet | 532 | 100 | 0 | ITS | MK968891.1 |
| BP435 | <i>Fusarium verticillioides</i> | healthy fruitlet | 541 | 100 | 0 | ITS | MT594370.1 |
| BP436 | <i>Fusarium proliferatum</i> | infected fruitlet | 530 | 100 | 0 | ITS | MH712288.1 |
| BP438 | <i>Fusarium falciforme</i> | healthy fruitlet | 547 | 100 | 0 | ITS | MT251175.1 |
| BP441 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 531 | 99.26 | 0 | ITS | AB278180.1 |
| BP442 | <i>Fusarium equiseti</i> | infected fruitlet | 498 | 100 | 0 | ITS | MH578585.1 |
| BP443 | <i>Fusarium ananatum</i> | infected fruitlet | 659 | 100 | 0 | EF1 α | MT010996.1 |
| BP444 | <i>Fusarium sp.</i> | healthy fruitlet | 651 | 98.79 | 0 | EF1 α | JF740861.1 |
| BP445 | <i>Fusarium oxysporum</i> | healthy fruitlet | 676 | 100 | 0 | EF1 α | MN417196.1 |
| BP447 | <i>Fusarium ananatum</i> | healthy fruitlet | 656 | 100 | 0 | EF1 α | MT010996.1 |
| BP449 | <i>Fusarium oxysporum</i> | healthy fruitlet | 675 | 99.7 | 0 | EF1 α | MN417202.1 |
| BP450 | <i>Fusarium incarnatum</i> | healthy fruitlet | 306 | 99.35 | 1.00E ⁻¹⁵⁴ | β -tubulin | MK752398.1 |
| BP454 | <i>Fusarium oxysporum</i> | healthy fruitlet | 666 | 100 | 0 | EF1 α | CP053267.1 |
| BP458 | <i>Fusarium oxysporum</i> | healthy fruitlet | 671 | 100 | 0 | EF1 α | MN507111.1 |

Table S1. Continued.

| Isolate ID | Identification | Sampled tissue | Fragment size (bp) | Identity (%) | E-value | Locus | GenBank accession number |
|------------|----------------------------------|-------------------|--------------------|--------------|-----------------------|------------------|--------------------------|
| BP459 | <i>Fusarium oxysporum</i> | infected fruitlet | 454 | 99.34 | 0 | ITS | MH777057.1 |
| BP460 | <i>Fusarium oxysporum</i> | infected fruitlet | 666 | 100 | 0 | EF1 α | MN507111.1 |
| BP462 | <i>Talaromyces stollii</i> | infected fruitlet | 398 | 100 | 0 | β -tubulin | JX315634.1 |
| BP463 | <i>Fusarium ananatum</i> | infected fruitlet | 664 | 100 | 0 | EF1 α | MT010996.1 |
| BP465 | <i>Fusarium ananatum</i> | infected fruitlet | 661 | 100 | 0 | EF1 α | MT010996.1 |
| BP468 | <i>Fusarium ananatum</i> | healthy fruitlet | 483 | 99.44 | 0 | EF1 α | MT010996.1 |
| BP469 | <i>Fusarium verticillioides</i> | healthy fruitlet | 662 | 100 | 0 | EF1 α | MN861780.1 |
| BP473 | <i>Fusarium solani</i> | infected fruitlet | 508 | 100 | 0 | ITS | MK968891.1 |
| BP474 | <i>Fusarium napiforme</i> | healthy fruitlet | 517 | 100 | 0 | ITS | MH685904.1 |
| BP475 | <i>Fusarium verticillioides</i> | healthy fruitlet | 659 | 100 | 0 | EF1 α | MT095057.1 |
| BP476 | <i>Fusarium oxysporum</i> | healthy fruitlet | 290 | 100 | 9.00E ⁻¹⁵⁰ | β -tubulin | MF806523.1 |
| BP477 | <i>Fusarium proliferatum</i> | infected fruitlet | 660 | 100 | 0 | EF1 α | MN386746.1 |
| BP478 | <i>Fusarium proliferatum</i> | infected fruitlet | 661 | 100 | 0 | EF1 α | MN386746.1 |
| BP482 | <i>Fusarium incarnatum</i> | infected fruitlet | 526 | 100 | 0 | ITS | MH712152.1 |
| BP483 | <i>Fusarium equiseti</i> | infected fruitlet | 524 | 100 | 0 | ITS | MT558602.1 |
| BP484 | <i>Fusarium sp.</i> | infected fruitlet | 146 | 99.32 | 7.00E ⁻⁶⁸ | ITS | MH777054.1 |
| BP485 | <i>Fusarium proliferatum</i> | healthy fruitlet | 659 | 100 | 0 | EF1 α | MN539103.1 |
| BP486 | <i>Fusarium proliferatum</i> | infected fruitlet | 660 | 100 | 0 | EF1 α | MK442093.1 |
| BP487 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 519 | 99.43 | 0 | ITS | AB278180.1 |
| BP488 | <i>Fusarium verticillioides</i> | infected fruitlet | 541 | 100 | 0 | ITS | MT594370.1 |
| BP490 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 535 | 99.44 | 0 | ITS | AB278180.1 |
| BP492 | <i>Fusarium verticillioides</i> | infected fruitlet | 528 | 100 | 0 | ITS | MT594370.1 |
| BP493 | <i>Phialemoniopsis curvata</i> | healthy fruitlet | 508 | 99.41 | 0 | ITS | AB278180.1 |
| BP494 | <i>Fusarium ananatum</i> | healthy fruitlet | 661 | 100 | 0 | EF1 α | MT010996.1 |
| BP495 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 511 | 99.42 | 0 | ITS | AB278180.1 |
| BP496 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 518 | 99.42 | 0 | ITS | AB278180.1 |
| BP497 | <i>Fusarium oxysporum</i> | healthy fruitlet | 510 | 100 | 0 | ITS | EU152473.1 |
| BP499 | <i>Fusarium equiseti</i> | infected fruitlet | 527 | 99.81 | 0 | ITS | MH879584.1 |
| BP500 | <i>Fusarium ananatum</i> | infected fruitlet | 659 | 100 | 0 | EF1 α | MT010996.1 |
| BP501 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 508 | 99.41 | 0 | ITS | AB278180.1 |
| BP503 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 506 | 99.41 | 0 | ITS | AB278180.1 |
| BP504 | <i>Phialemoniopsis curvata</i> | healthy fruitlet | 507 | 99.21 | 0 | ITS | AB278180.1 |
| BP507 | <i>Lasiodiplodia mahajangana</i> | healthy fruitlet | 401 | 100 | 0 | ITS | MH057188.1 |
| BP511 | <i>Fusarium verticillioides</i> | healthy fruitlet | 660 | 100 | 0 | EF1 α | MN861780.1 |
| BP512 | <i>Fusarium verticillioides</i> | healthy fruitlet | 660 | 100 | 0 | EF1 α | MN861772.1 |
| BP513 | <i>Fusarium proliferatum</i> | healthy fruitlet | 671 | 100 | 0 | EF1 α | KF993985.1 |
| BP515 | <i>Aspergillus oryzae</i> | infected fruitlet | 562 | 100 | 0 | ITS | MN648727.1 |
| BP516 | <i>Aspergillus flavus</i> | infected fruitlet | 515 | 100 | 0 | ITS | MN955851.1 |
| BP518 | <i>Fusarium verticillioides</i> | healthy fruitlet | 640 | 100 | 0 | EF1 α | MN861768.1 |
| BP519 | <i>Phialemoniopsis curvata</i> | healthy fruitlet | 505 | 99.41 | 0 | ITS | AB278180.1 |
| BP520 | <i>Clonostachys rosea</i> | healthy fruitlet | 406 | 97.78 | 0 | ITS | MH047188.1 |
| BP521 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 519 | 99.43 | 0 | ITS | AB278180.1 |
| BP522 | <i>Fusarium verticillioides</i> | infected fruitlet | 529 | 100 | 0 | ITS | MT594370.1 |
| BP523 | <i>Fusarium proliferatum</i> | healthy fruitlet | 656 | 99.7 | 0 | EF1 α | KU872092.1 |
| BP526 | <i>Fusarium dlamini</i> | healthy fruitlet | 538 | 100 | 0 | ITS | MN173109.1 |
| BP527 | <i>Fusarium proliferatum</i> | infected fruitlet | 660 | 100 | 0 | EF1 α | KF993985.1 |
| BP528 | <i>Fusarium solani</i> | infected fruitlet | 541 | 100 | 0 | ITS | MT594367.1 |
| BP529 | <i>Fusarium verticillioides</i> | infected fruitlet | 636 | 100 | 0 | EF1 α | MN861780.1 |
| BP530 | <i>Fusarium ananatum</i> | healthy fruitlet | 656 | 100 | 0 | EF1 α | MT010996.1 |
| BP532 | <i>Fusarium solani</i> | infected fruitlet | 541 | 100 | 0 | ITS | MT594367.1 |

Table S1. Continued.

| Isolate ID | Identification | Sampled tissue | Fragment size (bp) | Identity (%) | E-value | Locus | GenBank accession number |
|------------|----------------------------------|-------------------|--------------------|--------------|-----------|------------------|--------------------------|
| BP533 | <i>Fusarium verticillioides</i> | infected fruitlet | 656 | 100 | 0 | EF1 α | MH496632.1 |
| BP534 | <i>Fusarium proliferatum</i> | infected fruitlet | 660 | 100 | 0 | EF1 α | MK442093.1 |
| BP536 | <i>Fusarium verticillioides</i> | healthy fruitlet | 658 | 100 | 0 | EF1 α | MT095057.1 |
| BP537 | <i>Phialemoniopsis curvata</i> | healthy fruitlet | 389 | 99.49 | 0 | ITS | AB278180.1 |
| BP538 | <i>Lasiodiplodia citricola</i> | healthy fruitlet | 416 | 100 | 0 | β -tubulin | KU530119.1 |
| BP539 | <i>Lasiodiplodia theobromae</i> | healthy fruitlet | 415 | 100 | 0 | β -tubulin | KR260829.1 |
| BP540 | <i>Fusarium sp.</i> | healthy fruitlet | 514 | 100 | 0 | ITS | FJ008991.1 |
| BP543 | <i>Fusarium fujikuroi</i> | healthy fruitlet | 378 | 100 | 0 | ITS | MN963682.1 |
| BP544 | <i>Fusarium proliferatum</i> | infected fruitlet | 659 | 100 | 0 | EF1 α | MT095058.1 |
| BP546 | <i>Fusarium solani</i> | healthy fruitlet | 535 | 100 | 0 | ITS | JN006817.1 |
| BP548 | <i>Fusarium solani</i> | healthy fruitlet | 548 | 100 | 0 | ITS | KX621960.1 |
| BP549 | <i>Fusarium falciforme</i> | healthy fruitlet | 538 | 100 | 0 | ITS | MT251175.1 |
| BP550 | <i>Aspergillus flavus</i> | infected fruitlet | 576 | 100 | 0 | ITS | MF120213.1 |
| BP551 | <i>Aspergillus niger</i> | infected fruitlet | 565 | 100 | 0 | ITS | MN788116.1 |
| BP555 | <i>Fusarium sp.</i> | healthy fruitlet | 610 | 99.02 | 0 | EF1 α | JF740861.1 |
| BP556 | <i>Fusarium proliferatum</i> | infected fruitlet | 661 | 100 | 0 | EF1 α | MN386746.1 |
| BP559 | <i>Fusarium sp.</i> | healthy fruitlet | 645 | 98.77 | 0 | EF1 α | JF740861.1 |
| BP563 | <i>Fusarium solani</i> | infected fruitlet | 550 | 100 | 0 | ITS | KX621960.1 |
| BP564 | <i>Fusarium proliferatum</i> | healthy fruitlet | 661 | 100 | 0 | EF1 α | MN386746.1 |
| BP565 | <i>Fusarium ananatum</i> | healthy fruitlet | 658 | 100 | 0 | EF1 α | MT010996.1 |
| BP566 | <i>Fusarium sp.</i> | infected fruitlet | 516 | 100 | 0 | ITS | MT530119.1 |
| BP567 | <i>Fusarium solani</i> | healthy fruitlet | 543 | 100 | 0 | ITS | MT594367.1 |
| BP570 | <i>Fusarium proliferatum</i> | healthy fruitlet | 663 | 100 | 0 | EF1 α | KF993986.1 |
| BP572 | <i>Fusarium fujikuroi</i> | healthy fruitlet | 452 | 100 | 0 | ITS | MN963682.1 |
| BP573 | <i>Fusarium equiseti</i> | healthy fruitlet | 495 | 100 | 0 | ITS | MN589630.1 |
| BP574 | <i>Fusarium solani</i> | infected fruitlet | 638 | 99.69 | 0 | EF1 α | KY486693.1 |
| BP575 | <i>Fusarium sacchari</i> | infected fruitlet | 660 | 100 | 0 | EF1 α | MN193868.1 |
| BP576 | <i>Phialemoniopsis curvata</i> | healthy fruitlet | 511 | 99.42 | 0 | ITS | AB278180.1 |
| BP578 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 519 | 99.44 | 0 | ITS | AB278180.1 |
| BP579 | <i>Fusarium chlamydosporum</i> | healthy fruitlet | 270 | 100 | 1.00E-138 | β -tubulin | KJ125830.1 |
| BP580 | <i>Aspergillus flavus</i> | infected fruitlet | 548 | 100 | 0 | ITS | MN955851.1 |
| BP582 | <i>Davidiella sp.</i> | healthy fruitlet | 577 | 100 | 0 | ITS | KX621979.1 |
| BP583 | <i>Aspergillus flavus</i> | healthy fruitlet | 558 | 100 | 0 | ITS | MN006669.1 |
| BP584 | <i>Aspergillus welwitschiae</i> | healthy fruitlet | 560 | 100 | 0 | ITS | MH374611.1 |
| BP585 | <i>Aspergillus flavus</i> | healthy fruitlet | 242 | 100 | 0 | ITS | MK850352.1 |
| BP586 | <i>Aspergillus flavus</i> | infected fruitlet | 552 | 99.82 | 0 | ITS | MN238861.1 |
| BP589 | <i>Talaromyces stollii</i> | infected fruitlet | 405 | 100 | 0 | β -tubulin | JX315634.1 |
| BP590 | <i>Fusarium sp.</i> | healthy fruitlet | 651 | 98.79 | 0 | EF1 α | JF740861.1 |
| BP594 | <i>Penicillium sp.</i> | healthy fruitlet | 496 | 100 | 0 | ITS | KU556148.1 |
| BP595 | <i>Fusarium sp.</i> | healthy fruitlet | 627 | 98.58 | 0 | EF1 α | JF740861.1 |
| BP596 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 518 | 99.43 | 0 | ITS | AB278180.1 |
| BP597 | <i>Phialemoniopsis curvata</i> | healthy fruitlet | 520 | 99.44 | 0 | ITS | AB278180.1 |
| BP598 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 520 | 99.44 | 0 | ITS | AB278180.1 |
| BP599 | <i>Fusarium fujikuroi</i> | healthy fruitlet | 524 | 100 | 0 | ITS | CP023090.1 |
| BP601 | <i>Phialemoniopsis curvata</i> | infected fruitlet | 506 | 99.21 | 0 | ITS | AB278180.1 |
| BP603 | <i>Talaromyces amestolkiae</i> | healthy fruitlet | 403 | 100 | 0 | β -tubulin | MH287203.1 |
| BP604 | <i>Talaromyces purpureogenus</i> | infected fruitlet | 548 | 100 | 0 | ITS | KJ528885.1 |
| BP605 | <i>Talaromyces amestolkiae</i> | infected fruitlet | 402 | 100 | 0 | β -tubulin | KJ413360.1 |
| BP611 | <i>Fusarium oxysporum</i> | infected fruitlet | 665 | 100 | 0 | EF1 α | CP053267.1 |
| BP613 | <i>Fusarium verticillioides</i> | healthy fruitlet | 659 | 100 | 0 | EF1 α | MN861780.1 |

Table S2. Identification of fungal species isolated from soil coring points of cultures proximal to pineapple plots.

| Isolate ID | Identification | Adjacent crop | Fragment size (bp) | Identity (%) | E-value | GenBank accession number |
|------------|-------------------------------------|-----------------|--------------------|--------------|-----------------------|--------------------------|
| SBP050 | <i>Acrostalagmus sp.</i> | jackfruit trees | 411 | 100 | 0 | MH482850.1 |
| SBP027 | <i>Aspergillus flavipes</i> | mango orchard | 569 | 99.82 | 0 | KF624764.1 |
| SBP035 | <i>Aspergillus flavipes</i> | mango orchard | 550 | 100 | 0 | KF624764.1 |
| SBP068 | <i>Aspergillus flavipes</i> | mango orchard | 576 | 100 | 0 | KF624764.1 |
| SBP002 | <i>Aspergillus sclerotiorum</i> | mango orchard | 588 | 100 | 0 | KC478519.1 |
| SBP010 | <i>Aspergillus sp.</i> | mango orchard | 410 | 100 | 0 | MK312294.1 |
| SBP005 | <i>Aspergillus subramanianii</i> | mango orchard | 483 | 100 | 0 | MK952313.1 |
| SBP044 | <i>Aspergillus tardicrescens</i> | jackfruit trees | 449 | 99.78 | 0 | KY087686.1 |
| SBP020 | <i>Aureobasidium namibiae</i> | mango orchard | 196 | 100 | 2.00E ⁻⁹⁶ | MK794387.1 |
| SBP071 | <i>Bartalinia pondoensis</i> | mango orchard | 470 | 100 | 0 | NR153599.1 |
| SBP060 | <i>Bartalinia pondoensis</i> | jackfruit trees | 564 | 100 | 0 | NR153599.1 |
| SBP040 | <i>Cladosporium cladosporioides</i> | jackfruit trees | 479 | 98.96 | 0 | MF475948.1 |
| SBP012 | <i>Cladosporium sp.</i> | mango orchard | 418 | 100 | 0 | MK336600.1 |
| SBP029 | <i>Cladosporium sp.</i> | mango orchard | 542 | 100 | 0 | MK336600.1 |
| SBP062 | <i>Cladosporium sp.</i> | jackfruit trees | 542 | 100 | 0 | MN275867.1 |
| SBP043 | <i>Cladosporium tenuissimum</i> | jackfruit trees | 547 | 99.63 | 0 | KX621982.1 |
| SBP083 | <i>Cystofilobasidiales sp.</i> | jackfruit trees | 36 | 97.22 | 1.00E ⁻⁰⁶ | MF615083.1 |
| SBP053 | <i>Diaporthe helianthi</i> | jackfruit trees | 556 | 100 | 0 | KM979926.1 |
| SBP065 | <i>Diaporthe helianthi</i> | jackfruit trees | 563 | 100 | 0 | KM979926.1 |
| SBP006 | <i>Edenia gomezpompae</i> | mango orchard | 526 | 99.81 | 0 | NR156217.1 |
| SBP022 | <i>Fennellia nivea</i> | mango orchard | 577 | 100 | 0 | FJ155814.1 |
| SBP069 | <i>Fennellia nivea</i> | mango orchard | 563 | 100 | 0 | FJ155814.1 |
| SBP017 | <i>Fusarium cf. solani</i> | mango orchard | 434 | 99.77 | 0 | MK336488.1 |
| SBP037 | <i>Fusarium equiseti</i> | jackfruit trees | 492 | 100 | 0 | MG650603.1 |
| SBP076 | <i>Fusarium oxysporum</i> | mango orchard | 412 | 100 | 0 | MH777057.1 |
| SBP051 | <i>Fusarium polyphialidicum</i> | jackfruit trees | 517 | 100 | 0 | HQ607880.1 |
| SBP054 | <i>Fusarium sp.</i> | jackfruit trees | 508 | 100 | 0 | KM099504.1 |
| SBP064 | <i>Fusarium sp.</i> | jackfruit trees | 396 | 100 | 0 | KM099504.1 |
| SBP023 | <i>Heterocephalum aurantiacum</i> | mango orchard | 374 | 99.73 | 0 | MH858803.1 |
| SBP079 | <i>Heterocephalum aurantiacum</i> | mango orchard | 419 | 100 | 0 | MH858803.1 |
| SBP021 | <i>Lecanicillium saksenae</i> | mango orchard | 565 | 100 | 0 | AB360363.1 |
| SBP034 | <i>Lecanicillium saksenae</i> | mango orchard | 537 | 100 | 0 | AB360363.1 |
| SBP073 | <i>Lecanicillium saksenae</i> | mango orchard | 543 | 100 | 0 | KY320616.1 |
| SBP028 | <i>Leptosphaeria spgazzinii</i> | mango orchard | 524 | 99.81 | 0 | MG664743.1 |
| SBP038 | <i>Mortierella horticola</i> | jackfruit trees | 350 | 99.71 | 6.00E ⁻¹⁸⁰ | KC018245.1 |
| SBP007 | <i>Mortierella sp.</i> | mango orchard | 515 | 95.34 | 0 | KU612370.1 |
| SBP004 | <i>Penicillium citrinum</i> | mango orchard | 410 | 100 | 0 | KX363454.1 |
| SBP009 | <i>Penicillium guanacastense</i> | mango orchard | 523 | 99.24 | 0 | MH374548.1 |
| SBP052 | <i>Penicillium janthinellum</i> | jackfruit trees | 444 | 100 | 0 | MG845261.1 |
| SBP067 | <i>Penicillium multicolor</i> | mango orchard | 521 | 100 | 0 | MH864874.1 |
| SBP003 | <i>Penicillium ochrochloron</i> | mango orchard | 555 | 99.82 | 0 | MG661735.1 |
| SBP019 | <i>Penicillium sclerotigenum</i> | mango orchard | 561 | 100 | 0 | MK334370.1 |
| SBP011 | <i>Penicillium sp.</i> | mango orchard | 564 | 100 | 0 | KT336528.1 |

Table S2. Continued.

| Isolate ID | Identification | Adjacent crop | Fragment size (bp) | Identity (%) | E-value | GenBank accession number |
|------------|------------------------------------|-----------------|--------------------|--------------|-----------------------|--------------------------|
| SBP018 | <i>Penicillium sp.</i> | mango orchard | 556 | 100 | 0 | HQ608086.1 |
| SBP026 | <i>Penicillium spinulosum</i> | mango orchard | 565 | 99.65 | 0 | MF476047.1 |
| SBP039 | <i>Penicillium sumatraense</i> | jackfruit trees | 529 | 100 | 0 | MH864547.1 |
| SBP041 | <i>Penicillium sumatraense</i> | jackfruit trees | 558 | 100 | 0 | MH864547.1 |
| SBP045 | <i>Penicillium sumatraense</i> | jackfruit trees | 529 | 100 | 0 | MH864547.1 |
| SBP072 | <i>Peniophora sp.</i> | mango orchard | 826 | 100 | 0 | HQ608067.1 |
| SBP056 | <i>Pestalotiopsis sp.</i> | jackfruit trees | 267 | 100 | 0 | LC040892.1 |
| SBP049 | <i>Phoma sp.</i> | jackfruit trees | 413 | 100 | 0 | JN207265.1 |
| SBP059 | <i>Plectosphaerella cucumerina</i> | jackfruit trees | 516 | 100 | 0 | AB469880.1 |
| SBP063 | <i>Pleosporales sp.</i> | jackfruit trees | 517 | 100 | 0 | MK247832.1 |
| SBP008 | <i>Purpureocillium lilacinum</i> | mango orchard | 582 | 99.66 | 0 | LN809016.1 |
| SBP058 | <i>Pyrenochaeta sp.</i> | jackfruit trees | 503 | 99.6 | 0 | EU750693.1 |
| SBP025 | <i>Robillarda sessilis</i> | mango orchard | 553 | 99.46 | 0 | KR873255.1 |
| SBP030 | <i>Robillarda sessilis</i> | mango orchard | 568 | 99.47 | 0 | KR873255.1 |
| SBP057 | <i>Roussoella siamensis</i> | jackfruit trees | 478 | 100 | 0 | NR155716.1 |
| SBP075 | <i>Talaromyces purpurogenus</i> | mango orchard | 536 | 100 | 0 | AB872822.1 |
| SBP055 | <i>Trichoderma guizhouense</i> | jackfruit trees | 293 | 100 | 4.00E ⁻¹⁵⁰ | MN170570.1 |
| SBP001 | <i>Trichoderma harzianum</i> | mango orchard | 440 | 100 | 0 | MN326481.1 |
| SBP066 | <i>Xepicula leucotricha</i> | jackfruit trees | 569 | 100 | 0 | MH858391.1 |

Table S3. Identification of fungal species from DNA extracted from DGGE gels corresponding to *Fusarium*- and *Talaromyces*-inoculated pineapple fruitlets.

| Band | Inoculated species | Identification | Identity (%) | E-value | GenBank accession number |
|------|--------------------------------|--------------------------------|--------------|------------------------|--------------------------|
| a | <i>Fusarium sacchari</i> | <i>Fusarium sp.</i> | 100 | 2 × 10 ⁻³³ | FJ008992.1 |
| b | <i>Fusarium ananatum</i> | <i>Fusarium sp.</i> | 99.51 | 4 × 10 ⁻¹⁰⁰ | FJ210605.1 |
| c | <i>Talaromyces stollii</i> | <i>Talaromyces stollii</i> | 99.58 | 9 × 10 ⁻¹¹⁷ | AB910938.1 |
| d | <i>Talaromyces amestolkiae</i> | <i>Talaromyces amestolkiae</i> | 100 | 1 × 10 ⁻¹⁰⁴ | MT441607.1 |

CHAPITRE II

Influence des interactions inter-pathogènes sur la croissance et la toxinogénèse

**Article 2 - Interactions between fungal pathogens of
pineapple Fruitlet Core Rot modulate their
mycotoxigenic potential**

Suite à la caractérisation des mycobiomes de fruits sains et symptomatiques, il a été suggéré que la production de métabolites secondaires d'origine fongique puisse conditionner la structuration du cortège pathogène *in planta*. Plus particulièrement, le rôle des mycotoxines a été étudié suite à la mise en évidence des potentiels toxigènes des espèces pathogènes de la tache noire (Barral *et al.*, 2020). En effet, les toxines fongiques constituent une problématique majeure tant pour leur implication dans les pathosystèmes de par leur rôle de facteurs de virulence, que pour le risque de santé publique associé à la contamination des fruits frais et transformés.

Ce second chapitre est présenté au format de l'article intitulé « **Interactions between fungal pathogens of pineapple Fruitlet Core Rot modulate their mycotoxigenic potential** » soumis dans la revue *Toxins*. Cet axe de recherche a permis de déterminer la nature des interactions mises en œuvre lors de confrontations menées *in vitro* entre les quatre principaux pathogènes de la tache noire : *F. proliferatum*, *F. ananatum*, *F. oxysporum* et *T. stollii*. Les résultats obtenus ont montré que *T. stollii* possède une importante activité antagoniste sur la croissance et la capacité de colonisation des espèces *F. proliferatum* et *F. oxysporum*. Par ailleurs, ces interactions inter-pathogènes ont mis en évidence une modulation des potentiels de production en fumonisines B₁, B₂ et en beauvericine des espèces pathogènes de *Fusarium* au cours des confrontations *Fusarium* spp. – *T. stollii* mais également *Fusarium* spp. – *Fusarium* spp.. De plus, l'apparition d'une coloration rouge a été observée au niveau de la zone de contact au cours du suivi dynamique des interactions *Fusarium* spp. – *T. stollii* sans que la (ou les) molécule(s) impliquée(s) puisse(ent) être identifiée(s) ou quantifiée(s). Ces éléments suggèrent qu'*in situ*, une co-occurrence de ces espèces induit d'importants remaniements du mycobiome des fruits pouvant conditionner l'établissement de la tache noire chez l'ananas 'Queen Victoria'.

Article

Interactions between fungal pathogens of pineapple Fruitlet Core Rot modulate their mycotoxigenic potential

Manon Vignassa ^{1,3,*}, Christian Soria ^{1,3}, Noël Durand ^{2,3}, Charlie Poss ^{2,3}, Jean-Christophe Meile ^{1,3}, Marc Chillet ^{1,3} and Sabine Schorr-Galindo ³

¹ CIRAD, UMR Qualisud, F-97410 Saint-Pierre, Réunion, France; christian.soria@cirad.fr (C.S.); meile@cirad.fr (J.-C.M.); marc.chillet@cirad.fr (M.C.)

² CIRAD, UMR Qualisud, F-34398 Montpellier, France; noel.durand@cirad.fr (N.D.); charlie.poss@cirad.fr (C.P.)

³ Qualisud, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, IRD, Université de La Réunion, Montpellier, France; sabine.galindo@umontpellier.fr (S.S.-G.)

* Correspondence: manon.vignassa@cirad.fr; Tel.: +262-2-62-49-28-62

Abstract: The Fruitlet Core Rot (FCR) of pineapple is a fungal disease characterized by a multi-pathogen pathosystem. Recently, *Fusarium proliferatum*, *Fusarium oxysporum* and *Talaromyces stollii* joined the set of FCR pathogens until then exclusively supported by *Fusarium ananatum*. The particularity of FCR relies on the presence of healthy and diseased fruitlets within a same fruit. The mycobiome associated to these two types of tissues suggested that disease occurrence may be triggered or linked to a specific chemical communication among pathogens. Interactions between the four recently identified pathogens were determined by dual-culture bioassays. Both fungal growth and mycotoxin production patterns were monitored according to a 10-day kinetic. Results evidenced that *Talaromyces stollii* was the main growth inhibitor of *Fusarium* species. A collapse of fumonisins and beauvericin contents was observed when FCR-pathogens were crossed challenged. Antagonism between *Fusarium* species and *Talaromyces stollii* was supported by the diffusion of a red pigmentation and droplets of red exudates at the mycelium surface. This study revealed that secondary metabolites could play a role in shaping the fungal network of pineapple fruitlet and contribute to virulence promoting FCR establishment.

Keywords: *Ananas comosus*, beauvericin, dual-culture, Fruitlet Core Rot, fumonisins, *Fusarium*, in vitro, LC/MS-MS, *Talaromyces*

Key Contribution: *Fusarium ananatum*, *Fusarium proliferatum*, *Fusarium oxysporum* and *Talaromyces stollii* constitute the fungal pathogenic cortege inducing pineapple Fruitlet Core Rot. *Talaromyces stollii* was demonstrated as a strong antagonist of *Fusarium* spp.. Confrontations between pathogens modulate their production of fumonisin B₁, fumonisin B₂ and beauvericin.

1. Introduction

The Fruitlet Core Rot of pineapple (FCR) is one of the major issue in the worldwide market exportation causing severe fruit quality depreciation [1]. The particularity of the FCR pathosystem lies in that both healthy and diseased fruitlets are present within a same pineapple fruit. For several years, FCR was studied with *Fusarium ananatum* as the main causal agent with the leading role even though other species as *Fusarium proliferatum*, *Fusarium oxysporum* and *Talaromyces stollii* were identified in both healthy and infected fruitlets [2,3]. FCR pathogen contaminations occur at pre-flowering stages but it is later (during fruit development) that fungi spread through the septal nectaries and achieve the

blossom cup colonization. The pathogens then benefit from the pineapple sugar accumulation and in some case, successfully counterbalance the host defenses leading to the browning of the fruitlet flesh defined as ‘black spot’ [4]. Recently, *Fusarium proliferatum*, *Fusarium oxysporum* and *Talaromyces stollii* were characterized for inducing similar symptoms to those caused by infection of *F. ananatum* thus, confirming their implication in the FCR pathosystem [5]. The determination of the mycobiomes of healthy and diseased fruitlets also revealed that the four FCR pathogens concomitantly constituted the most abundant fungal species, suggesting that pathogenesis does not only rely on fruitlet contamination. Additionally, the in vitro study of FCR-pathogens also demonstrated significant change in the fungal community composition that is promoted following inoculation with the *Fusarium*-FCR pathogens [5]. This hypothesizes a transition from an endophytic to a pathogenic behavior related to the increase of fungal biomass in the host tissues.

In plant diseases, *Fusarium proliferatum* and *Fusarium oxysporum* species are widely studied for their ability to produce mycotoxins, which are harmful substances for human health [6–9]. Mycotoxins are fungal secondary metabolites known for their potential or demonstrated mutagen and carcinogen effects causing severe toxicity on animal cells. The biohazard of these compounds led to the establishment of limit thresholds in food and feed products by the European Food Safety Authority (EFSA) [10]. Considering FCR incidence over various production areas, isolates of *Fusarium* species obtained from FCR-diseased pineapple fruits were elucidated to be producers of Fumonisin B₁ (FB₁), Fumonisin B₂ (FB₂), Fumonisin B₃ (FB₃), Moniliformin (MON) and Beauvericin (BEA) in in vitro culture conditions [11]. Thereafter, FB₁ and BEA were also quantified in higher concentrations in naturally diseased fruitlets than in healthy ones suggesting that these toxins could constitute key factors for fungal virulence [3]. In pineapple, pre- and post-harvest conditions are defined as influent parameters for both FCR pathogenesis and introduction of mycotoxins in the food chains [5,12–14]. Abiotic parameters such as pH, sugar content, temperature and a_w (water activity) related to hygrometry modulate the growth and toxigenicity of fungal strains [15]. In fruits production and transformation schemes, the main food safety issue relies on the production of patulin, ochratoxin A, aflatoxins and *Alternaria* toxins by fungal species [16,17]. As an example, *Penicillium expansum* causes the blue mold disease in apples and pears and is an important producer of patulin leading to the contamination of fruits and their co-products (juices, mash and fresh cut) [18–20].

In plant-pathogen dialogue, mycotoxins are suspected to be involved in the modulation of host defenses then constituting influent parameters for signal pathways manipulation [21]. For example, deletion of *Fusarium verticillioides* *SET1* resulted in both growth and fumonisins accumulation failing thus limiting the colonization process of the pathogen into maize stalks and kernel [8]. *F. verticillioides* *FUG1* (*Fungal Unknown Gene 1*) deletion mutant presented weaker microconidia production with no macroconidia formation. Following inoculation on kernel, *FUG1* mutant also revealed a defective profile of FB₁ biosynthesis while FB₂ concentration was quantified in higher levels than in wild-type [22]. These studies demonstrated that toxins could support the fungal virulence and influence the bypass of the plant defenses [23].

Plant microbiomes are being explored and appeared as promising tools to get insight into the structure and function of microbial communities [24]. Previous studies on microbial interaction reveals the implication of fungal secondary metabolites in the defense against other microorganisms, in order to ensure for an ecological niche [25–27]. Specifically, mycotoxins have been suggested as major compounds during interspecific competition related to the colonization of a habitat [28]. These interactions could be supported by exploitation competition, which is characterized by an extensive use of resources by one of the competing species thus limiting the nutrient availability for competitors [29,30]. Alternatively, interference competition occurred when a competitor inhibits the growth

and the spread of the concomitant species by secretion of antibiotic metabolites in a given space [31,32]. Particularly, various microbial species are described to interact or antagonize *Fusarium* species identified from plant microbiomes. This leads to the modulation of the *Fusarium* toxins accumulation in host cellular compartments and results in a decrease of fungal virulence. As an example, bacterial species such as *Pseudomonas fluorescens* has the ability to degrade and detoxify the secreted mycotoxins leading to the drop of deoxynivalenol (DON) content accumulated by *Fusarium culmorum* [33]. Interestingly, fungi can also display similar behaviors. In the context of the *Fusarium* head blight (FHB), the confrontations between *Fusarium culmorum* and *Alternaria tenuissima* on wheat kernels showed that toxins produced by *A. tenuissima* were reduced to less than 5% while the biosynthesis of DON and zearalenone (ZEA) by *F. culmorum* were 11- and 36-fold higher respectively [34]. The competitive in vitro culture of *Fusarium graminearum* and *Aspergillus ochraceus* showed more elevated accumulation of DON and ZEA during the early stages of interaction than for unchallenged culture of *F. graminearum* [35]. Considering their broad host range, *Fusarium* species can also interact with each other within a microbial community. Ridout et al. showed that in in vitro conditions, *Fusarium poae* reduced by more than 95% the fumonisin production of *Fusarium proliferatum* [36]. Similarly, *Fusarium temperatum* decreased to 32% the fumonisins content accumulated by *F. verticillioides*. Furthermore, beauvericin is considered as a fungal virulence factor for plant host and has been described as a growth inhibitor for the endophyte *Paraconiothyrium variabile* [37]. However, a recent study demonstrated that, during interaction, *P. variabile* can metabolize beauvericin produced in high concentrations by the pathogen *F. oxysporum* in order to protect its ecological niche [38]. These inter-species interactions emphasize the importance of considering fungi secondary metabolites production in the structuration of microbial networks to accurately determine the role of toxins in the pathogenesis [39].

In the present study, we propose to provide relevant information on the behavior of FCR-pathogens under in vitro dual-culture conditions. First, the evolution of changes in fungal spreading was monitored and the corresponding growth inhibition profiles were defined. Second, we characterized the influence of FCR-pathogens dual-culture on their patterns of mycotoxin accumulation according to a 10-day kinetic approach. Altogether, the data provided new insight into the microbial communication that may directly influence the occurrence of FCR disease and the mycotoxin content in pineapple.

2. Results

2.1. Dynamic evolution of fungal colonies

In Reunion Island, *Fusarium ananatum*, *Fusarium proliferatum*, *Fusarium oxysporum* and *Talaromyces stollii* were identified in pineapple FCR-diseased tissues and were defined as prevalent species of the fruit associated mycobiome. Recently, their ability to promote black spot symptoms was confirmed resulting in the characterization of three novel FCR-pathogens [5]. Searching for chemical communication implemented in the structuration of the fungal network, four isolates related to these species were selected for dual-culture bioassays.

First, a phenotypic monitoring was performed on both single- and dual-cultures across five time points in order to detect changes in mycelial growth and coloration. In single-cultures, all studied *Fusarium* species showed a white mycelium after 2 days of incubation. From day 4, these species were characterized by a coloration ranging from salmon pink (*F. ananatum*), to violet (*F. oxysporum*) and beige (*F. proliferatum*) on PDA (Figure 1 A-D). *F. oxysporum* and *F. proliferatum* exhibited an aerial mycelium while *F. ananatum* was characterized by a short and dense mycelium surface. From this time point, no phenotypic variation was noticed until the end of the monitoring. Moreover, *Fusarium* species reached the mycelial full coverage of the wells within six days of incubation (Figure 1 A-C).

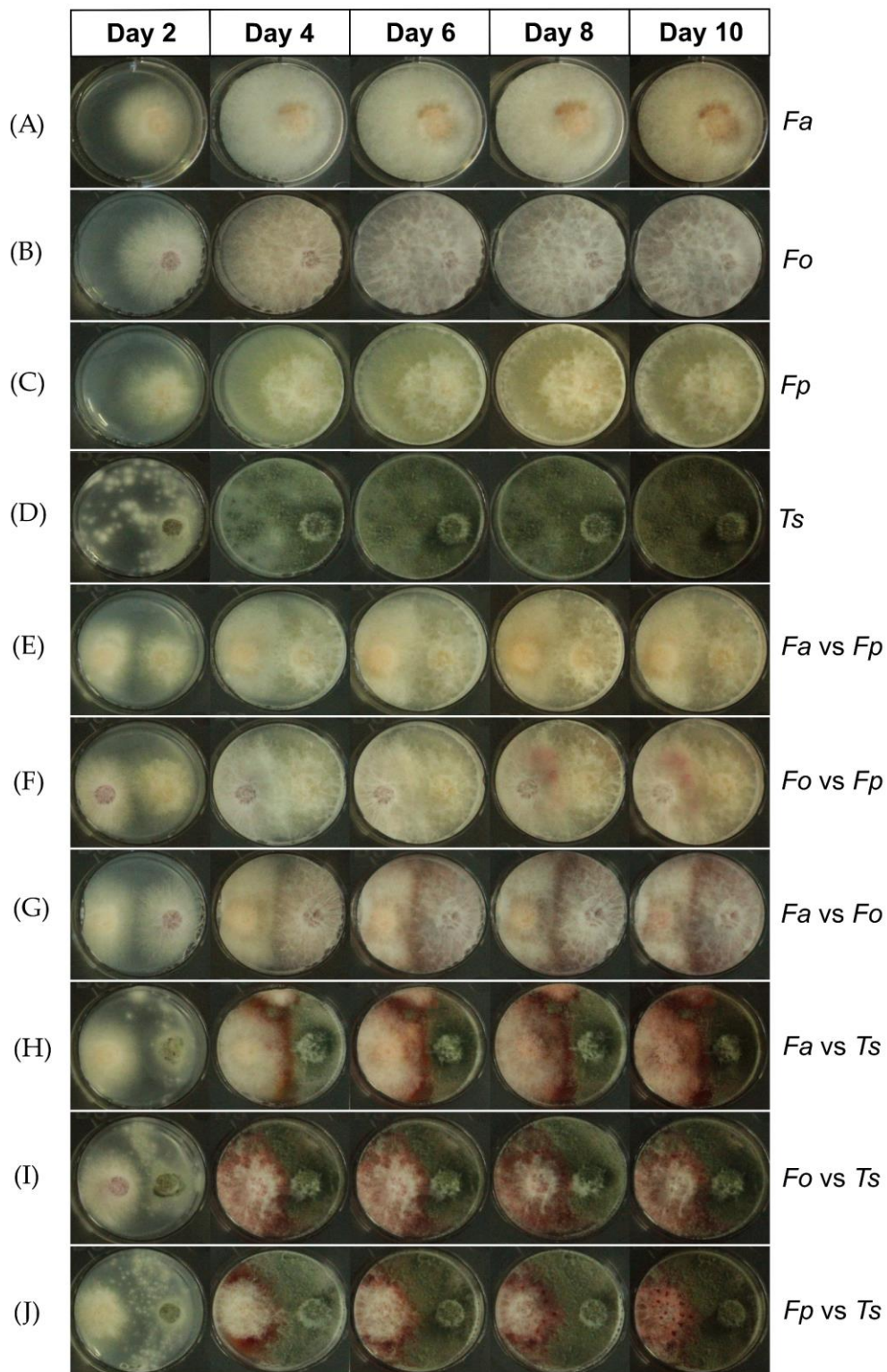


Figure 1. FCR-pathogens colony aspects on PDA after incubation in single-culture of (A) *Fusarium ananatum* (*Fa*), (B) *Fusarium oxysporum* (*Fo*), (C) *Fusarium proliferatum* (*Fp*) and (D) *Talaromyces stollii* (*Ts*) or in condition of dual-culture corresponding to (E) *F. ananatum* versus *F. proliferatum* (*Fa vs Fp*), (F) *F. oxysporum* versus *F. proliferatum* (*Fo vs Fp*), (G) *F. ananatum* versus *F. oxysporum* (*Fa vs Fo*), (H) *F. ananatum* versus *T. stollii* (*Fa vs Ts*), (I) *F. oxysporum* versus *T. stollii* (*Fo vs Ts*), (J) *F. proliferatum* versus *T. stollii* (*Fp vs Ts*) along a 10-day incubation time.

Secondly, *Talaromyces stollii* showed a phenotype that was remarkably different from *Fusarium* species with a spotted white and light green colored mycelium at day 2. *T. stollii* fully colonized the medium surface after 6 days of incubation, similarly to the *Fusarium* species (Figure 1D). The morphology of the colonies was characterized by a short and powdery dark green mycelium. Interestingly, important changes in phenotypes were observed among the co-cultivation of the FCR-pathogens. Following dual-culture of *F. oxysporum* and *F. proliferatum*, mycelial contacts were observed starting from day 4 while hyphae aerial profiles remained unchanged all along the incubation period. From day 8, a red-violet pigmentation appeared at the mycelia contact zone and extend until day 10 (Figure 1F). The pigmentation was exclusively localized at the end of hyphae with no diffusion in the agar. In addition, the co-cultivation of *F. ananatum* and *F. proliferatum* showed no visible change in the surface colonization patterns with colony growth morphologies similar to the control conditions. Contact between mycelia was also reported at day 4 and remain subsequently indistinguishable from the single cultures (Figure 1E). Considering *Fusarium* co-cultures, confrontation between *F. ananatum* and *F. oxysporum* exhibited the most visible change in growth evolution. The mycelia colorations observed at day 2 and day 4 were similar to single-cultures of the corresponding species (Figure 1G). Nevertheless, a strong red-violet pigmentation was noticeable from day 6 to day 10 on the colony surface and even more intense on the reverse side of the wells (Figure S2 G). Mycelia of both species were in contact from day 6 to the last time point.

Trying to define the chemical communication that structured interactions between FCR-pathogens, the most evident change occurred following dual-cultures of *Fusarium* sp. with *T. stollii* (Figure 1 H-J). Interestingly, growth inhibition was observed for all culture conditions implicating these species combinations. A zone line characterized by appearance of a red pigmentation was observed at the mycelia contact area for both *F. oxysporum* and *F. proliferatum* in the presence of *T. stollii* on day 4 and day 6 (Figure 1 I-J). On the subsequent time points, the intense red pigmentation spread all over the growth surface. In these conditions and from day 8, the *F. proliferatum* colony surface accumulated droplets of red exudate (Figure 1J). Dual-culture of *F. ananatum* and *T. stollii* also lead to the appearance of red a pigmentation. However, this constituted the only species combination presenting an inhibition zone from day 4 and until the end of the monitoring (Figure 1H). Finally, the red pigmentation highlighted over the set of *Fusarium-Talaromyces* dual-cultures, was localized and highly intense in the bottom side of the *Fusarium* colonies as shown in Figure S2 (Figure S2 H-J).

2.2. Influence of FCR-pathogens co-cultivation on growth and inhibition patterns

In order to characterize interactions between the FCR-pathogens, mycelial growth rates and their related inhibition ratio were defined. Results showed that in single-culture, the four studied species supported an exponential growth phase from day 2 to day 4 (Figure 2A, C, E, G). At this time point, the medium surface was completely colonized and a slower or stationary growth phase was then observed after 6 days of incubation. In dual-culture, the exponential growth was still running until day 6 for all experimental conditions. The data described antagonistic interactions consequently to dual-culture of *Fusarium* spp. and *T. stollii*. In fact, *T. stollii* was described as the prevalent competitor with average inhibition ratios (av. \pm sd) of $5.1 \pm 5.9\%$, $16.4 \pm 7.2\%$ and $22.4 \pm 3.1\%$ against *F. ananatum*, *F. oxysporum* and *F. proliferatum* respectively (Figure 2B, D, F).

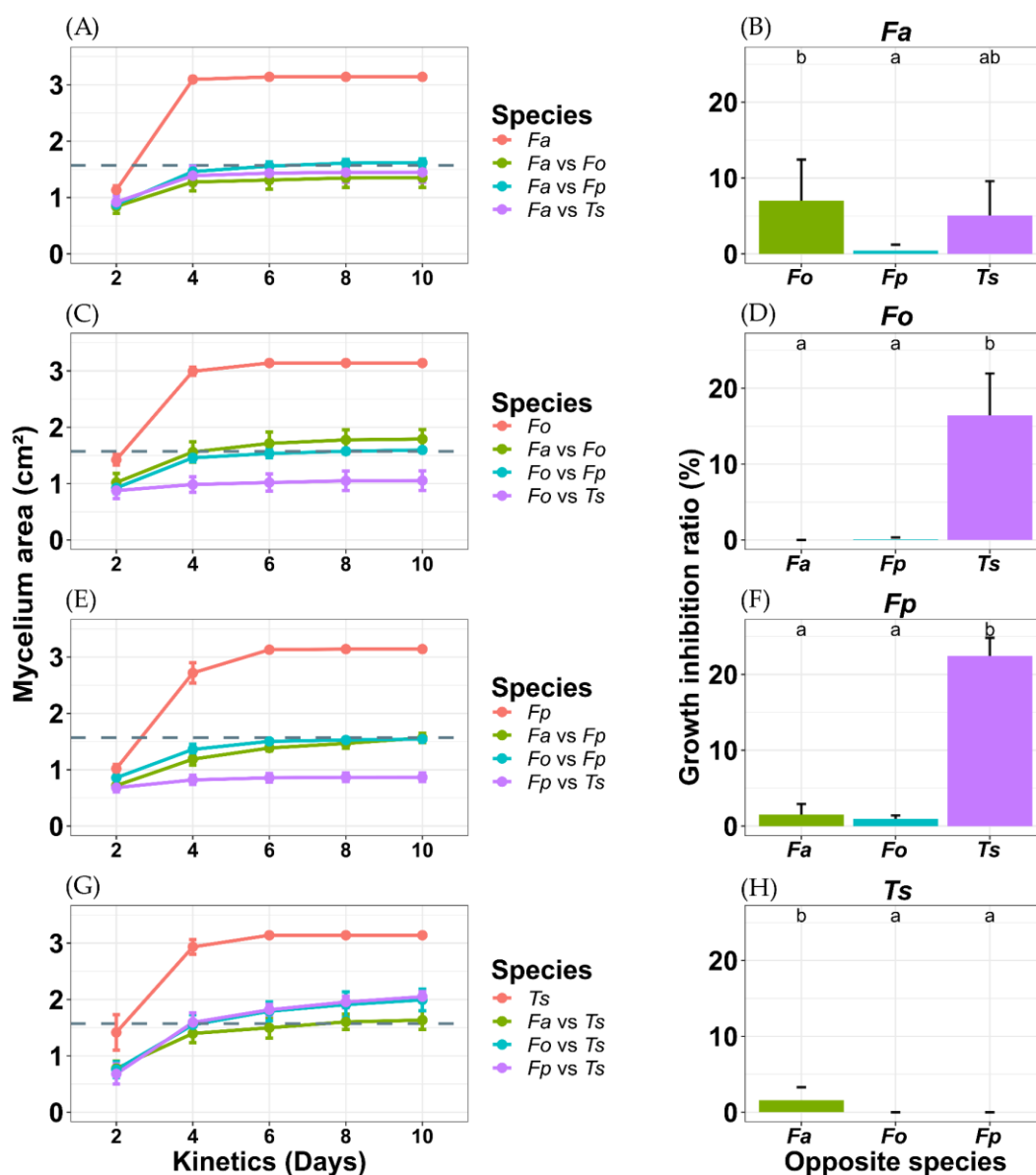


Figure 2. Influence of interactions between FCR-pathogens on colony growth parameters. (A), (C), (E) and (G) show the evolution of growth rates during a 10-day kinetics for dual-cultures and the corresponding single-cultures. The grey dashed lines represent 50% of well area (1,57 cm²). (B), (D), (F) and (H) indicate the corresponding growth inhibition ratio calculated from data at day 10 for each dual-culture condition. (A) and (B); (C) and (D); (E) and (F); (G) and (H) show the growth profiles and inhibition potential of *F. ananatum*, *F. oxysporum*, *F. proliferatum* and *T. stollii* respectively. Vertical bars represent standard errors of means (n = 9). Letters show significantly different inhibition ratios for each species following Tukey's multiple comparison test at $p < 0.05$. *Fa*: *Fusarium ananatum*; *Fo*: *Fusarium oxysporum*; *Fp*: *Fusarium proliferatum* and *Ts*: *Talaromyces stollii*.

T. stollii represented the species with the more elevated potential of inhibition on *F. oxysporum* growth by comparison to *F. ananatum* ($p < 0.001$) and *F. proliferatum* ($p < 0.001$). The growth of *F. proliferatum* was significantly reduced by *T. stollii* compared to dual-cultures with *F. ananatum* ($p < 0.001$) and *F. oxysporum* ($p < 0.001$). The mycelial growth of *F. ananatum* was significantly reduced by *F. oxysporum* in comparison to *F. proliferatum* ($p = 0.04$). Interestingly, no significant difference was evidenced among inhibition potentials of *F. oxysporum* and *T. stollii* on *F. ananatum*. In fact, *F. oxysporum* revealed the highest inhibition ratio of $7.03 \pm 7.02\%$ against *F. ananatum*. This is the only *Fusarium-Fusarium*

combination characterizing a growth antagonism. The remaining *Fusarium-Fusarium* conditions described an equal occupation of the wells corresponding to an area of 1.57 cm² (grey dashed line on figure 2 A, C, E and G). By contrast to others *Fusarium* species, *F. ananatum* presented a slight but significant reduction of *T. stollii* growth with an inhibition by 1.58% of the medium coverage compared to confrontations against *F. oxysporum* ($p = 0.02$) and *F. proliferatum* ($p = 0.02$) (Figure 2H).

2.3. Modulation profiling of mycotoxins production

For each *Fusarium* species toxigenic patterns in control conditions described the exclusive production of FB₁, FB₂ and BEA. In the sample set corresponding to *F. ananatum*, BEA was the only mycotoxin detected and was supported by a maximum average concentration (av. \pm sd) of $985.8 \pm 233.6 \mu\text{g kg}^{-1}$ recorded at day 6 (Figure 3A, D, E). Moreover, the *Fusarium* strains present contrasted toxins production profiles. The highest concentrations of fumonisins were recorded for *F. oxysporum* with $1455.5 \pm 77.1 \mu\text{g kg}^{-1}$ of FB₁ and $476.9 \pm 268.9 \mu\text{g kg}^{-1}$ of FB₂ at day 8 (Figure 3C, D, F). The tested strain of *F. proliferatum* is a consistent producer of FB₁ with a peak of production at $767.4 \pm 4.4 \mu\text{g kg}^{-1}$ after 8 days of incubation. However, its biosynthesis patterns of FB₂ and BEA were slightly pronounced and correspond at their maximum to 78.1 ± 49.3 and $130.6 \pm 0.9 \mu\text{g kg}^{-1}$ respectively (Figure 3B, E, F). No toxin was identified and quantified from *T. stollii* single-cultures even after 10 days of incubation (Figure 3A, B, C).

When compared to the single-culture of *F. ananatum*, the dual-culture of *F. ananatum* and *T. stollii* significantly reduced the synthesis of BEA from 4 to 10 days of incubation. Particularly, the concentration of BEA was 34.7-fold lower ($p < 0.001$) in *F. ananatum* versus *T. stollii* ($28.4 \pm 4.7 \mu\text{g kg}^{-1}$) condition at day 6 when compared to control condition of *F. ananatum* ($985.8 \pm 233.6 \mu\text{g kg}^{-1}$) (Figure 3A). For the confrontation between *F. oxysporum* and *T. stollii*, the most significant drop in toxin amount was observed for BEA at day 6 ($56.6 \pm 66.3 \mu\text{g kg}^{-1}$) with a 13.5-fold reduction when compared to *F. oxysporum* control conditions ($761.9 \pm 58.7 \mu\text{g kg}^{-1}$, $p < 0.001$). The most significant inhibitions of FB₁ and FB₂ were supported by a down accumulation by 1.3- and 3.1-fold and were observed for days 8 ($p = 0.01$) and 10 ($p = 0.04$) respectively (Figure 3C). Across *F. proliferatum* versus *T. stollii*, BEA concentrations were significantly reduced when compared to single-culture of *F. proliferatum*. BEA amounts were thus close to the LOD and LOQ (Figure 3B). Interestingly, the confrontation with *T. stollii* less drastically decreased both FB₁ and FB₂ production of *F. proliferatum*. Notably, the most significant difference occurred at day 6 with an FB₁ concentration of $890.1 \pm 47.6 \mu\text{g kg}^{-1}$ in *F. proliferatum* versus *T. stollii* compared to $740.4 \pm 71.5 \mu\text{g kg}^{-1}$ in *F. proliferatum* single-culture ($p = 0.02$). This is the sole condition that revealed a higher toxins concentration in dual-culture than in control. Day 8 constituted the only kinetic point for which FB₁ was found at lower concentrations in dual-cultures than in the single ones. Then, the FB₁ concentrations were quite homogeneous among single- and dual-cultures at day 10 with an average of $512.9 \pm 98.0 \mu\text{g kg}^{-1}$. Conjointly, interaction between *Fusarium*-FCR pathogens also depicted a drop in mycotoxin content. At day 6, *F. proliferatum* versus *F. ananatum* dual-culture exhibited a reduction by 2.2- ($p < 0.001$) and 3.1-fold ($p = 0.003$) of the accumulation of FB₁ and BEA in comparison to *F. proliferatum* and *F. ananatum* respectively (Figure 3E). Similarly, *F. oxysporum* vs *F. ananatum* conditions promoted the highest reduction of FB₁, FB₂ and BEA contents by 1.4- ($p = 0.01$), 2.8- ($p < 0,001$) and 2.2-fold ($p = 0.02$) respectively in contrast to *F. oxysporum* single-culture at day 6. When compared to *F. ananatum* single-culture, BEA concentration of *F. oxysporum* versus *F. ananatum* was 2.8-fold lower for the same time point ($p = 0.003$). Nevertheless, this profile is inverted at day 8 with $553.7 \pm 95.6 \mu\text{g kg}^{-1}$ accumulated in *F. ananatum* versus *F. oxysporum* condition against $279.1 \pm 50.2 \mu\text{g kg}^{-1}$ synthesized by *F. ananatum* in single-culture (Figure 3D). The interaction between *F. oxysporum* and *F. proliferatum* demonstrated accumulation patterns similar to the main trend observed through the five other modalities of FCR-pathogens dual-cultures.

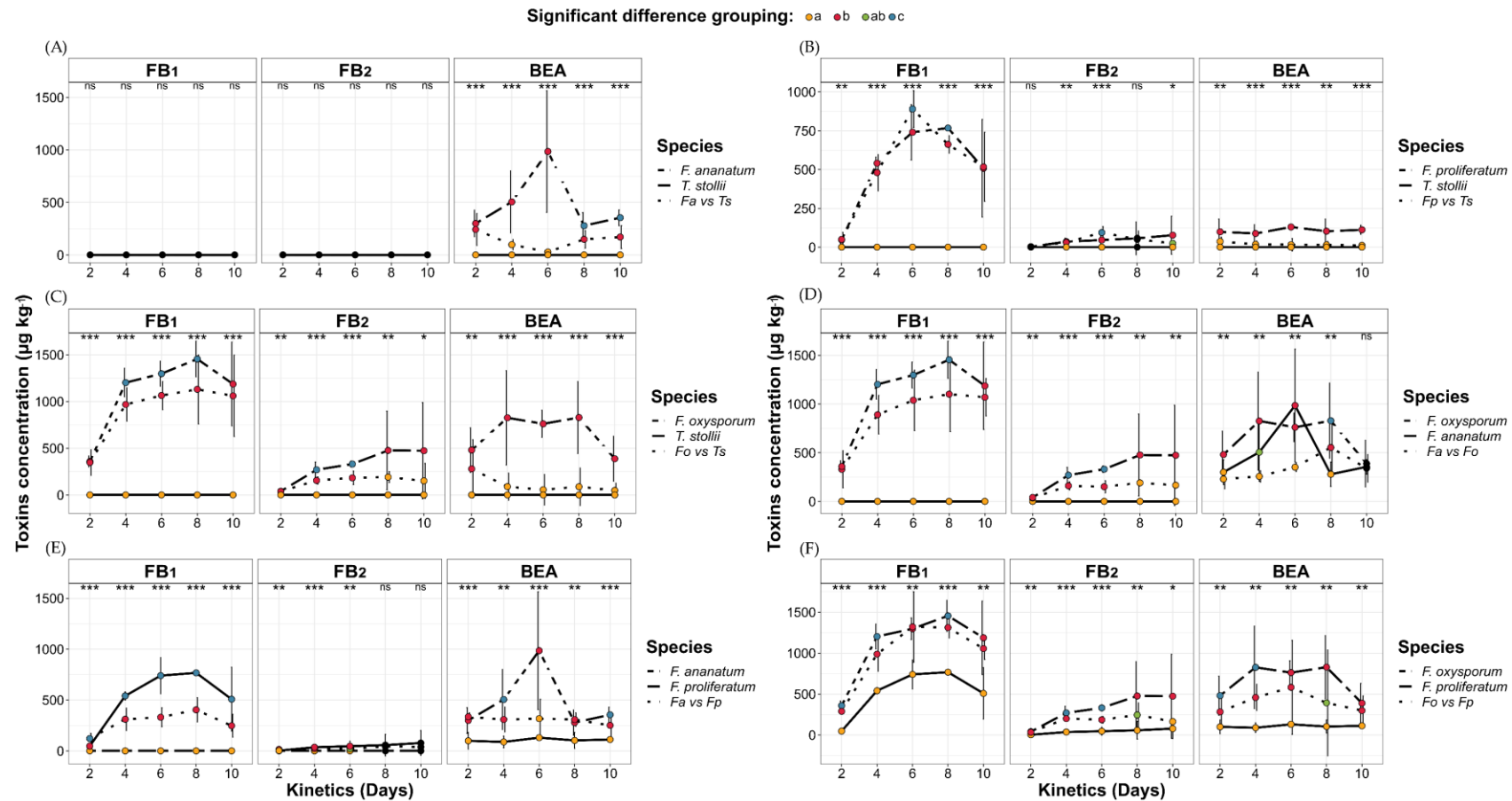


Figure 3. Dynamics of fumonisins B₁ (FB₁), fumonisins B₂ (FB₂) and beauvericin (BEA) production from FCR-fungal pathogens single and dual-cultures on PDA medium. (A) *F. ananatum* versus *T. stollii* (Fa vs Ts), (B) *F. proliferatum* vs *T. stollii* (Fp vs Ts), (C) *F. oxysporum* versus *T. stollii* (Fo vs Ts), (D) *F. oxysporum* versus *F. ananatum* (Fo vs Fa), (E) *F. ananatum* versus *F. proliferatum* (Fa vs Fp) and (F) *F. oxysporum* versus *F. proliferatum* (Fo vs Fp). Vertical bars represent standard error of means (n = 3). Differences between single- and dual-culture were either significant at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) or non-significant (ns) for each toxin and each kinetic point. Dots colors indicates significant differences according to Tukey's multiple comparison test at $p < 0,05$ and correspond to 'a' (yellow dots), 'b' (red dots), 'c' (blue dots) and 'ab' (green dots).

Then, the most significant differences in FB₁ and BEA levels were noticed at day 4 with a respective reduction by 1.21- ($p = 0.01$) and 1.80-fold ($p = 0.02$) compared to *F. oxysporum* control (Figure 3F). The highest significant change in FB₂ was demonstrated at day 10 with a drop in concentration of 2.9-fold ($p = 0.04$) for the dual-culture *F. oxysporum* versus *F. proliferatum* in comparison to *F. oxysporum* single-culture toxigenicity.

3. Discussion

Focusing on the interactions that shape the fungal flora is primordial for the definition of the host-pathogen(s) dialogue especially in a multi-partite pathosystem such as pineapple FCR. Previously, species belonging to the *Fusarium fujikuroi* (FFSC) [40] and *Talaromyces purpureogenus* species complexes [41] were identified as the main risk of FCR occurrence in pineapple. Moreover, considering the relative abundance of species that constituted the mycobiomes of healthy and diseased fruitlets, *T. stollii* corresponded to the third prevalent species, coming after *F. proliferatum* and *F. ananatum*. Its occurrence was 2-fold higher in diseased tissues than in healthy ones [5]. It was thus hypothesized that specific fungal communities' arrangements may determine the balance between healthy or diseased pineapple fruitlets. Considering the known toxinogenicity of these *Fusarium* species, it was important to determine the impact of such interactions on mycotoxin patterns in pineapple in order to estimate potential issue for the consumer health.

The FCR-pathogens dual-culture approach highlighted important changes in the evolution of mycelial growth. First of all, the growth curves and their relative inhibition ratios show that *T. stollii* is the main competitor of the FCR complex with the highest inhibitory effect on growth and colonization of the three *Fusarium* species assayed. In fact, similar antagonistic patterns were observed following challenged cultivation of *F. proliferatum* and *F. oxysporum* with *T. stollii* suggesting an interference competition beneficial to *T. stollii*. By comparison, co-cultivation of *F. ananatum* and *T. stollii* differ with the presence of an inhibition zone as defined previously [42]. Growth patterns showed that *F. ananatum* partially contained the spreading on free space and nutrients access of *T. stollii*. This is the only *Fusarium* species which, although being inhibited, succeeded to challenge *T. stollii*. These results are in accordance with Losada et al. which demonstrated that the secondary metabolism of the competitor is the key determinant of the fungal competitive fitness [43]. Interestingly, the main growth inhibition ratio obtained from *T. stollii* is performed against *F. proliferatum* which was previously described as the most abundant species in relation to the proximal environment of pineapple plots [5]. These modulation patterns facilitate the exploitation of resources from artificial medium by *T. stollii*. This suggest that *T. stollii* biomass may be widely set up into the blossom cup of fruitlets. *Fusarium* species could then be restricted in host tissue in the form of conidia and remained detectable by classical microbiological approach. Moreover, inside FFSC, *F. oxysporum* also demonstrated an antagonistic behavior against *F. ananatum* with a growth inhibition similar to that induced by *T. stollii*. Additionally, these antagonistic interactions all evidenced a pigmentation that could result from a pigment secretion, in some case with a concomitant red exudate. Particularly, when species belonging to FFSC were confronted to *T. stollii*, the appearance of a red pigmentation was noticeable from day 4. Across the single-culture of the four species assayed, no such red pigmentation was observed suggesting that this coloration specifically resulted from the antagonistic interaction between *Fusarium* spp. and *T. stollii*. Fungi are widely described for their ability to produced bioactive compounds that supported the interconnecting microbial network within an ecological niche [44,45]. Inside the diversity of secondary metabolites accumulated by fungi, pigments have been describe for their large scale of activities such as antimicrobial, antifungal, antiviral and others biological activities [46]. Although *T. stollii* was never studied in confrontation procedures, several

data show that *Talaromyces* species such as *T. purpureogenus* and *T. amestolkiae* could produce and diffuse red azaphilones polyketide pigments ('Monascus pigments') [47]. Alternatively, the culture of species belonging to the FFSC could also be associated with the production of pigments on the mycelium or by diffusion in the agar. In these conditions, excretion of red bikaverin defined as a mycotoxin, has been elucidated in *F. fujikuroi* and *F. oxysporum* cultures [48,49]. *Fusarium* species are defined as major producers of secondary red colored metabolites such as bostrycoidin, norjavanicin and 8-O-methylfusarubin in conditions of environmental stresses [50,51]. It is thus hypothesized that in antagonistic condition, *Fusarium*-FCR-pathogens could constitute red pigment secretors in order to manage their competitor. This is in the line with Son et al., that demonstrated the biocontrol efficiency of bikaverin and fusaric acid produced by *F. oxysporum* on the growth and development of *Phytophthora infestans* [52]. Moreover, this is relevant with the presence of a red exudate that was observed at the mycelial surface of *F. proliferatum* after 8 days of co-cultivation with *T. stollii*. In spite of the various mycotoxin standards used in this study, this specific secondary metabolite was not identified. Therefore, we cannot conclude on the role and importance of this compound in the fungal networks and de facto in the occurrence of FCR.

Following the analysis of toxins, fumonisins B₁ and B₂, and beauvericin were the only identifiable and quantifiable compounds in our samples. Contrary to previous studies, and in accordance with Barral et al., the *Fusarium* strains isolated in Reunion Island did not produce fumonisin B₃ or moniliformin [3,53,54]. This demonstrate that pineapple fruit constitute a model of interest in the study of *Fusarium* toxins in fresh fruits. Concerning the dynamic of mycotoxins productions patterns, fumonisins and beauvericin were drastically affected by dual-culture assay except for the interaction between *F. proliferatum* and *T. stollii* which exhibited after 6 days of incubation, a rise in FB₁ and FB₂ content. This highlighted the fact that chemical dialogue is species dependent [55]. Interestingly, most of the significant changes in mycotoxins concentration considering dual-culture and their respective single-culture occurred at, or after, the sixth day of interaction. This is consistent with the growth profiles observed in control conditions where the studied species fully colonized the medium surface within 6 days. Thereby, fungal species development seemed to be controlled by modulation of its metabolism by the competitor. Such regulations were evidenced from *Pseudomonas aeruginosa* phenazine-derived metabolites that regulated the switch from vegetative growth to conidiation of *Aspergillus fumigatus* [56]. Fumonisins have notably been described for acting as a systemic signal with accumulation of FB₁ in leaf maize during roots colonization by *F. verticillioides* [57].

In the present study, we show that *T. stollii* could compete with several species belonging to the FFSC by negatively impacting their growth and toxins production. This is consistent with Camardo Leggieri *et al.* which demonstrated that the in vitro co-cultivation of *Fusarium verticillioides* and *Aspergillus flavus* result in a significant reduction of colony diameter. Moreover, they also evidenced that productions of aflatoxin B₁ and fumonisins (B₁ and B₂) were significantly impacted by the culture condition (single or dual) in interaction with temperature of incubation [58]. Since mycotoxins could be considered as virulence factor for fungal species, such modulation could decrease the pathogenicity of *F. ananatum*, *F. proliferatum* and *F. oxysporum* leading thus to an increase of host resistance. Similarly, the dual-culture of *Fusarium verticillioides* versus *Ustilago maydis* promoted the secretion of fusaric acid by *F. verticillioides* that inhibited the growth of *U. maydis* and significantly decreased symptoms of the Corn Smut disease [59–61]. However, the molecular and biochemical mechanisms that characterized the FCR pathosystem are also supported by the biomass of each protagonist. Then, a low content in *T. stollii* biomass may result in a weak inhibition of *Fusarium* species. This may lead to an important host tissue colonization and a rise in FB₁/FB₂/BEA accumulations by *F. ananatum*, *F. proliferatum*

and/or *F. oxysporum*. Thereafter, toxins acting as virulence factors could promote host susceptibility and disease expression [62]. Such theory is consistent with the levels of FB₁ and BEA quantified in significantly higher concentrations in infected fruitlets than in healthy ones [3]. It is important to notice that based on the results obtained by Barral *et al.* and by the present study, toxins were quantified at lower levels in pineapple flesh than in vitro approaches. Additionally, it seems essential to consider spatial and temporal patterns of species contamination. Fungi spreading is defined by landscape contextualization (*i.e.* configuration of host in its environment, proximal crops) and meteorological factors (*i.e.* wind, temperatures, and hygrometry) resulting in asynchronous species dispersions [63,64]. Also, environmental factors are considered as crucial determinant for both plant resistance and fungal pathogens growth and virulence [65]. Thus, FCR occurrence could be supported by a specific temporal sequence of contamination. This sequence of fungal penetration in the blossom cup probably determined, through the modulation of pathogens interactions, the evolution of prevalent fungal biomass and the future of pineapple resistance. This was previously been proposed in *Gibberella* and *Fusarium* ear rot of maize by demonstrating that infection partly relies on the combination of competitive and facilitative interactions between the pathogens *Fusarium graminearum* and *Fusarium verticillioides* [66]. Moreover, *Fusarium* pathogens are widely studied for their ability to spread over fruits crops via irrigation water or wind [67,68]. This demonstrated that an initial contamination achieved by one of the consider *Fusarium* species could result in elevated amounts of mycotoxins and, once again, to the establishment of pineapple fruit susceptibility. In addition to the economic loss, FCR could thus constituted a hazard for human health in derivative products such as juice or transformed pineapple flesh.

In Reunion Island, *F. ananatum* and *F. proliferatum* were alternatively identified as prevalent species in accordance with the numerous climate and production areas present on the Island [14]. When challenged against *T. stollii*, *F. proliferatum* showed toxins patterns different from the other *Fusarium* species-*T. stollii* combinations. Even if growth rates were the most impacted by *T. stollii* competition (growth inhibition of 22.4%), this constituted the unique *Fusarium* species that presented similar or superior FB₁ and FB₂ levels in at least 4 of the 5 time points. Additionally, *F. ananatum* appeared as the only studied *Fusarium* species competent for limiting growth and spreading of *T. stollii*. Thus, in interaction with *T. stollii*, *F. proliferatum* constituted the only one FCR-pathogens able to preserve its mycotoxigenic potential while *F. ananatum* represent the main growth challenger. It is hypothesized that in vivo, these two *Fusarium* species could contribute to a pairwise management of *T. stollii* but with an impact on sanitary quality due to their mycotoxin production. This would be in concordance with their respective distribution and abundance profiles across the landscape of Reunion Island.

4. Conclusions

Our study provided original understanding on interspecific competitions between *Fusarium proliferatum*, *Fusarium ananatum*, *Fusarium oxysporum* and *Talaromyces stollii*, the four fungal pathogens recently described as contributors to FCR epidemics in pineapple. We evidenced that *Talaromyces stollii* represent the main antagonist of the FCR-pathogenic *Fusarium* species. Inhibitory effects on growth and mycotoxin accumulation reported specific chemical arrangements in the network of pineapple fruitlet mycobiome. It appears that the fine modulation of colonization profiles may rely on specific sequence of contamination which, subsequently, could constitute a key determinant of biomass evolution in host tissues. Determining the influence of temporal and environmental factors on fungal virulence would help to precisely define the scenario of FCR expression. Future prospects may also be necessary to characterized whether the drop in fumonisins and beauvericin contents in species interactions result from a lack of biosynthesis or a detoxification process. This would help to define the toxigenic risks associated with the various pineapple production areas in order to ensure food safety for the consumers.

5. Materials and Methods

5.1. Microbial material

5.1.1. Fungal strains

Four fungal strains previously isolated from pineapple black spots in Reunion Island were selected for their pathogenicity in FCR: *Fusarium ananatum* (clp001), *Fusarium proliferatum* (BP429), *Fusarium oxysporum* (BP369) and *Talaromyces stollii* (BP462). Isolates were stored in the fungi collection at CIRAD Ligne-Paradis, La Réunion, France at -80 °C in 20% glycerol [5,69]. Prior to analysis, pre-cultures of each strain were grown in 9 cm Petri dishes on potato dextrose agar (PDA, VWR International, Fontenay-Sous-Bois, France) for 9 days at 27 °C in the dark.

5.1.2. Dual-culture bioassays

According to a previous optimization procedure, 2 mL of PDA were distributed in each well of 12-well culture plates (ThermoFisher Scientific, Illkirch, France) [70]. Plates used for fungi cultivation were constituted by wells with an internal diameter of 2 cm. The dual-culture assays were strains were challenged as follows: *F. ananatum* (clp001) versus *F. oxysporum* (BP369), *F. ananatum* (clp001) versus *F. proliferatum* (BP429), *F. ananatum* (clp001) versus *T. stollii* (BP462), *F. oxysporum* (BP369) versus *F. proliferatum* (BP429), *F. oxysporum* (BP369) versus *T. stollii* (BP462) and *F. proliferatum* (BP429) versus *T. stollii* (BP462). Wells were inoculated with agar plugs of 2 mm diameter obtained from pre-cultures. For dual-culture condition, agar plugs were deposited at respectively 1 and 3 quarters positions along the well diameter (at 0.5 cm distance from the margin of the well). Controls consisted of well inoculated with a single strain. Both confronted and control conditions were performed in triplicate. Thus, a plate was devoted to one dual-culture condition and composed by 3 wells of single-cultures of both strain *A* and *B*, 3 wells of dual-culture of strain *A* versus strain *B* and 3 non-inoculated wells as 'Mock' (Figure S1). Incubation was performed following 2-, 4-, 6-, 8- and 10-days kinetics at 27 °C in the dark. For each time point, the 3 wells replicates were sampled (mycelium and PDA), pooled and frozen in liquid nitrogen. 3 technical replicates were performed corresponding to a total of 90 plates (1 plate per dual-culture condition and per time point, 6 dual-cultures, 5 time points, and 3 technical replicates).

5.2. Mycotoxin identification and quantification

5.2.1. Extraction procedure

For this assay, the 3 pooled well contents were grinded at 5 m s⁻¹ for 15 s with two ceramic beads using the FastPrep-24 Instrument (MP Biomedicals®, Illkirch, France). From the homogenized matrix, 2.5 g were weighed and 14 mL of acetonitrile/H₂O/acetic acid (79:5:1, v/v/v) were added. Samples were then vortexed and placed in a shaking incubator for 30 min at 200 rpm (Gerhardt Analytical Systems, Königswinter, Germany). After 3 min of centrifugation at 1000 rpm, 1 mL of supernatant was transferred in amber glass vial and dried at 40 °C under nitrogen flow. After evaporation, 1 mL of 99.5/0.5% (v/v) water/acetic acid solution of internal standard mix was added and the solution was filtered using a 0.45 µm PTFE (polytetrafluoroethylene) syringe filter (ClearLine, Issy-les-Moulineaux, France). The filtrate was therefore used for the determination of mycotoxins.

5.2.2. Tandem mass spectrometry assay

5.2.2.1. UHPLC MS/MS analysis

For all samples, mycotoxins were detected and confirmed by Ultra High-Performance Liquid Chromatography (UHPLC, Shimadzu, Tokyo, Japan) coupled with a mass spectrometer (8040, Shimadzu, Tokyo, Japan) and quantified by isotope labelled internal standard (IS) following the protocol described by Capodanno *et al.* and Moreau and Levi [71,72]. The data were analyzed using LabSolutions software (v5.91/2017, Shimadzu, Tokyo, Japan, 2017). The limit of detection (LOD) and limit of quantification (LOQ) were respectively: 0.03/0.1 ng mL⁻¹ for FB₁, 0.01/0.05 ng mL⁻¹ for FB₂ and 0.05/0.15 ng mL⁻¹ for BEA.

5.2.2.2. UHPLC conditions

Chromatographic separation was carried out using a Kinetex 2.6 µm C18 100A 50 × 2.1 mm ID column (Phenomenex, Torrance, CA, USA). The column temperature was maintained at 50 °C and injection was performed with a volume of 50 µL. The mobile phase A consisted of 99.5% water and 0.5% acetic acid (HPLC MS Grade, Sigma-Aldrich, Darmstadt, Germany). Mobile phase B consisted of 99.5% isopropanol (HPLC MS Grade, Biosolve, Dieuze, France) and 0.5% acetic acid (HPLC MS Grade, Sigma-Aldrich, Darmstadt, Germany). A mobile phase gradient program was started at 90% A (0.01 min), 45% A at 1.5 min, 15% A at 3.5 min, 20% A at 4 min, 98% A at 4.01 min and finally 98% A at 11 min. The flow rate was 0.4 mL min⁻¹.

5.2.2.3. MS/MS conditions

The mass spectrometer was operated in electrospray ionisation, positive (ESI+) and negative (ESI-) ionisation mode, and two multiple reaction monitoring (MRM) transitions for each analyte were monitored for Quantification (Q) and Qualification (q). The parameters of the mass spectrometer were: desolvation line: 250 °C; heater block: 400 °C; nebulizing gas: 3 L min⁻¹; Drying gas: 15 L min⁻¹; CID gas pressure: 270 kPa (Argon).

5.2.2.4. Standard solution

The certified isotope labelled standard solutions were purchased from Romer Labs Diagnostic GmbH (Tulln, Austria). The certified standard solutions of aflatoxins (B₁, B₂, G₁ and G₂), fumonisins (B₁ and B₂), trichothecenes (DON, nivalenol, 15ADON, 3ADON, HT-2 toxin, T2 toxin), zearalenone (ZEA), citrinin, ochratoxin A (OTA) and beauvericin (BEA) were purchased from R-Biopharm (Saint-Didier-au-Mont-d'Or, France). A composite standard and Internal standard (IS) working solution of all of the mycotoxins was prepared by dissolving appropriate volumes of each compound in a mobile phase A: 99.5% water and 0.5% acetic acid. Stock solutions were then diluted with the mobile phase A, in order to obtain the appropriate working solutions for the calibration. All solutions were stored at -20 °C in amber glass vials and darkness before use.

5.3. Computational analysis

5.3.1. Calculations of areas and inhibition ratios

According to the sampling kinetics, culture plates were photographed and the colony growth rate was determined by image analysis using the ImageJ software (v.1.53.a, U. S. National Institutes of Health, Bethesda, MD, USA, 2020) considering the mycelium area in each well for both single- and dual-cultures [73].

Patterns of inhibition growth were calculated for each dual-culture condition after 10 days of incubation following the formula:

$$I\% = [(R1 - R2) / R1] \times 100] - 50$$

Where R1 corresponds to the mycelial area of the studied species in control condition (single-culture) and R2 to the mycelial area of the studied species in dual-culture [74].

5.3.2. Statistical analysis

Data were analyzed with the R programming language v4.0.3 (R Core Team, 2020). First, significant differences between inhibition ratios for each studied species in condition of dual-culture was tested with a deviance test and a Tukey's multiple comparison test.

Then, the effect of species culture conditions on toxins accumulation was tested for each mycotoxin identified and each kinetic point with a deviance test and independent generalized linear models. Multiple comparisons between means of mycotoxin concentrations at the different incubation kinetics and for the different culture conditions were tested using the Tukey's test.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Dual-culture procedure performed with fungal species defined as FCR-pathogens of pineapple on 12-well culture plates. 1,2: single-cultures, 3: dual-cultures, 4: Mock., Figure S2. Fungal colony aspects on PDA view from the bottom of plates according to a 10-day kinetics. Conditions are defined as incubation in single-culture of (A) *Fusarium ananatum* (Fa), (B) *Fusarium oxysporum* (Fo), (C) *Fusarium proliferatum* (Fp) and (D) *Talaromyces stollii* (Ts) or confrontation by dual-culture of (E) *F. ananatum* versus *F. proliferatum* (Fa vs Fp), (F) *F. oxysporum* versus *F. proliferatum* (Fo vs Fp), (G) *F. ananatum* versus *F. oxysporum* (Fa vs Fo), (H) *F. ananatum* versus *T. stollii* (Fa vs Ts), (I) *F. oxysporum* versus *T. stollii* (Fo vs Ts), (J) *F. proliferatum* versus *T. stollii* (Fp vs Ts).

Author Contributions: Conceptualization, M.V.; methodology, M.V. and N.D.; investigation, M.V., C.S., N.D. and C.P.; formal analysis, M.V.; data curation, M.V.; writing—original draft preparation, M.V.; writing—review and editing, M.V., J.-C.M., C.S., M.C., and S.S.-G.; visualization, M.V. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for this research, including M.V.'s PhD grant, was provided by the European Regional Development Fund (FEDER Réunion, PO-COSAQ).

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Gu; Zhan, R.L.; Zhan, L.B.; Gong, D.Q.; Jia, Z.W. First Report of *Fusarium Ananatum* Causing Pineapple Fruitlet Core Rot in China. *Plant Disease* **2015**, *99*, 1653–16, doi:10.1094/PDIS-03-15-0279-PDN.
2. Jacobs, A.; Van Wyk, P.S.; Marasas, W.F.O.; Wingfield, B.D.; Wingfield, M.J.; Coutinho, T.A. *Fusarium Ananatum* Sp. Nov. in the Gibberella Fujikuroi Species Complex from Pineapples with Fruit Rot in South Africa. *Fungal Biology* **2010**, *114*, 515–527, doi:10.1016/j.funbio.2010.03.013.
3. Barral, B.; Chillet, M.; Doizy, A.; Grassi, M.; Ragot, L.; Léchaudel, M.; Durand, N.; Rose, L.J.; Viljoen, A.; Schorr-Galindo, S. Diversity and Toxigenicity of Fungi That Cause Pineapple Fruitlet Core Rot. *Toxins* **2020**, *12*, 339, doi:10.3390/toxins12050339.
4. Mourichon, X. Pineapple Fruitlet Core Rot (Black Spot) and Leathery Pocket : Review and Prospects. *Acta Horticulturae* **1997**, 501–508.
5. Vignassa, M.; Meile, J.-C.; Chiroleu, F.; Soria, C.; Leneveu-Jenvrin, C.; Schorr-Galindo, S.; Chillet, M. Pineapple Mycobiome Related to Fruitlet Core Rot Occurrence and the Influence of Fungal Species Dispersion Patterns. *JoF* **2021**, *29*.
6. Li, Z.F.; He, C.L.; Wang, Y.; Li, M.J.; Dai, Y.J.; Wang, T.; Lin, W. Enhancement of Trichothecene Mycotoxins of *Fusarium Oxysporum* by Ferulic Acid Aggravates Oxidative Damage in *Rehmannia Glutinosa* Libosch. *Sci Rep* **2016**, *6*, 33962, doi:10.1038/srep33962.
7. Gálvez, L.; Urbaniak, M.; Waśkiewicz, A.; Stepień, Ł.; Palmero, D. *Fusarium Proliferatum* – Causal Agent of Garlic Bulb Rot in Spain: Genetic Variability and Mycotoxin Production. *Food Microbiology* **2017**, *67*, 41–48, doi:10.1016/j.fm.2017.05.006.
8. Li, T.; Gong, L.; Jiang, G.; Wang, Y.; Kumar Gupta, V.; Qu, H.; Duan, X.; Wang, J.; Jiang, Y. Carbon Sources Influence Fumonisin Production in *Fusarium Proliferatum*. *Proteomics* **2017**, *17*, 16, doi:10.1002/pmic.201700070.
9. Shao, C.; Xiang, D.; Wei, H.; Liu, S.; Yi, G.; Lyu, S.; Guo, L.; Li, C. Predicting Virulence of *Fusarium Oxysporum* f. Sp. *Cubense* Based on the Production of Mycotoxin Using a Linear Regression Model. *Toxins* **2020**, *12*, 254, doi:10.3390/toxins12040254.

10. Mycotoxins Available online: <https://www.efsa.europa.eu/en/topics/topic/mycotoxins>.
11. Stępień, Ł.; Koczyk, G.; Waśkiewicz, A. Diversity of Fusarium Species and Mycotoxins Contaminating Pineapple. *J Appl Genetics* **2013**, *54*, 367–380, doi:10.1007/s13353-013-0146-0.
12. Jackson, L.S.; Al-Taher, F. Factors Affecting Mycotoxin Production in Fruits. In *Mycotoxins in Fruits and Vegetables*; Elsevier, 2008; pp. 75–104 ISBN 978-0-12-374126-4.
13. Kumar, D.; Barad, S.; Sionov, E.; Keller, N.; Prusky, D. Does the Host Contribute to Modulation of Mycotoxin Production by Fruit Pathogens? *Toxins* **2017**, *9*, 280, doi:10.3390/toxins9090280.
14. Fournier, P.; Benneveau, A.; Hardy, C.; Chillet, M.; Léchaudel, M. A Predictive Model Based on a Pluviothermic Index for Leathery Pocket and Fruitlet Core Rot of Pineapple Cv. 'Queen.' *Eur J Plant Pathol* **2015**, *142*, 449–460, doi:10.1007/s10658-015-0625-8.
15. Sanzani, S.M.; Reverberi, M.; Geisen, R. Mycotoxins in Harvested Fruits and Vegetables: Insights in Producing Fungi, Biological Role, Conducive Conditions, and Tools to Manage Postharvest Contamination. *Postharvest Biology and Technology* **2016**, *122*, 95–105, doi:10.1016/j.postharvbio.2016.07.003.
16. Moss, M.O. Fungi, Quality and Safety Issues in Fresh Fruits and Vegetables. *J Appl Microbiol* **2008**, *104*, 1239–1243, doi:10.1111/j.1365-2672.2007.03705.x.
17. Fernández-Cruz, M.L.; Mansilla, M.L.; Tadeo, J.L. Mycotoxins in Fruits and Their Processed Products: Analysis, Occurrence and Health Implications. *Journal of Advanced Research* **2010**, *1*, 113–122, doi:10.1016/j.jare.2010.03.002.
18. Spadaro, D.; Ciavorella, A.; Frati, S.; Garibaldi, A.; Gullino, M.L. Incidence and Level of Patulin Contamination in Pure and Mixed Apple Juices Marketed in Italy. *Food Control* **2007**, *18*, 1098–1102, doi:10.1016/j.foodcont.2006.07.007.
19. Puel, O.; Galtier, P.; Oswald, I. Biosynthesis and Toxicological Effects of Patulin. *Toxins* **2010**, *2*, 613–631, doi:10.3390/toxins2040613.
20. Coton, M.; Bregier, T.; Poirier, E.; Debaets, S.; Arnich, N.; Coton, E.; Dantigny, P. Production and Migration of Patulin in Penicillium Expansum Molded Apples during Cold and Ambient Storage. *International Journal of Food Microbiology* **2020**, *313*, 108377, doi:10.1016/j.ijfoodmicro.2019.108377.
21. Diamond, M.; Reape, T.J.; Rocha, O.; Doyle, S.M.; Kacprzyk, J.; Doohan, F.M.; McCabe, P.F. The Fusarium Mycotoxin Deoxynivalenol Can Inhibit Plant Apoptosis-Like Programmed Cell Death. *PLoS ONE* **2013**, *8*, e69542, doi:10.1371/journal.pone.0069542.
22. Ridenour, J.B.; Bluhm, B.H. The Novel Fungal-Specific Gene FUG1 Has a Role in Pathogenicity and Fumonisin Biosynthesis in Fusarium Verticillioides: FUG1, a Novel Gene in Fusarium Verticillioides. *Molecular Plant Pathology* **2017**, *18*, 513–528, doi:10.1111/mpp.12414.
23. Ma, L.-J.; Geiser, D.M.; Proctor, R.H.; Rooney, A.P.; O'Donnell, K.; Trail, F.; Gardiner, D.M.; Manners, J.M.; Kazan, K. Fusarium Pathogenomics. *Annu. Rev. Microbiol.* **2013**, *67*, 399–416, doi:10.1146/annurev-micro-092412-155650.
24. Banerjee, S.; Schlaeppli, K.; van der Heijden, M.G.A. Keystone Taxa as Drivers of Microbiome Structure and Functioning. *Nat Rev Microbiol* **2018**, *16*, 567–576, doi:10.1038/s41579-018-0024-1.
25. Vinale, F.; Marra, R.; Scala, F.; Ghisalberti, E.L.; Lorito, M.; Sivasithamparam, K. Major Secondary Metabolites Produced by Two Commercial Trichoderma Strains Active against Different Phytopathogens. *Lett Appl Microbiol* **2006**, *43*, 143–148, doi:10.1111/j.1472-765X.2006.01939.x.
26. You, F.; Han, T.; Wu, J.; Huang, B.; Qin, L. Antifungal Secondary Metabolites from Endophytic Verticillium Sp. *Biochemical Systematics and Ecology* **2009**, *37*, 162–165, doi:10.1016/j.bse.2009.03.008.
27. Combès, A.; Ndoye, I.; Bance, C.; Bruzaud, J.; Djediat, C.; Dupont, J.; Nay, B.; Prado, S. Chemical Communication between the Endophytic Fungus Paraconiothyrium Variabile and the Phytopathogen Fusarium Oxysporum. *PLoS ONE* **2012**, *7*, e47313, doi:10.1371/journal.pone.0047313.

28. Knowles, S.L.; Raja, H.A.; Wright, A.J.; Lee, A.M.L.; Caesar, L.K.; Cech, N.B.; Mead, M.E.; Steenwyk, J.L.; Ries, L.N.A.; Goldman, G.H.; et al. Mapping the Fungal Battlefield: Using in Situ Chemistry and Deletion Mutants to Monitor Interspecific Chemical Interactions Between Fungi. *Front. Microbiol.* **2019**, *10*, 285, doi:10.3389/fmicb.2019.00285.
29. Sarrocco, S.; Mauro, A.; Battilani, P. Use of Competitive Filamentous Fungi as an Alternative Approach for Mycotoxin Risk Reduction in Staple Cereals: State of Art and Future Perspectives. *Toxins* **2019**, *11*, 701, doi:10.3390/toxins11120701.
30. Pellan, L.; Durand, N.; Martinez, V.; Fontana, A.; Schorr-Galindo, S.; Strub, C. Commercial Biocontrol Agents Reveal Contrasting Compartments Against Two Mycotoxigenic Fungi in Cereals: *Fusarium Graminearum* and *Fusarium Verticillioides*. *Toxins* **2020**, *12*, 152, doi:10.3390/toxins12030152.
31. Dekker, M. The Fungal Community: Its Organization and Role in the Ecosystem. *Supplied by U.S. Dept. of Agric., National Center for Agricultural Utilization Research, Peoria, IL 7627*, 11.
32. Ghou, M.; Mitri, S. The Ecology and Evolution of Microbial Competition. *Trends in Microbiology* **2016**, *24*, 833–845, doi:10.1016/j.tim.2016.06.011.
33. Khan, M.R.; Doohan, F.M. Bacterium-Mediated Control of *Fusarium* Head Blight Disease of Wheat and Barley and Associated Mycotoxin Contamination of Grain. *Biological Control* **2009**, *48*, 42–47, doi:10.1016/j.biocontrol.2008.08.015.
34. Müller, M.E.H.; Steier, I.; Köppen, R.; Siegel, D.; Proske, M.; Korn, U.; Koch, M. Cocultivation of Phytopathogenic *Fusarium* and *Alternaria* Strains Affects Fungal Growth and Mycotoxin Production. *J Appl Microbiol* **2012**, *113*, 874–887, doi:10.1111/j.1365-2672.2012.05388.x.
35. Milles, J.; Krämer, J.; Prange, A. In Vitro Competitive Interactions of *Fusarium Graminearum* with *Aspergillus Ochraceus* and *Penicillium Verrucosum* with Regard to Mycotoxin Production. *Journal of Food, Agriculture & Environment* **2007**, *6*.
36. Ridout, M.E.; Godfrey, B.; Newcombe, G. Effects of Antagonists on Mycotoxins of Seedborne *Fusarium* Spp. in Sweet Corn. *Toxins* **2019**, *11*, 438, doi:10.3390/toxins11080438.
37. López-Berges, M.S.; Hera, C.; Sulyok, M.; Schäfer, K.; Capilla, J.; Guarro, J.; Di Pietro, A. The Velvet Complex Governs Mycotoxin Production and Virulence of *Fusarium Oxysporum* on Plant and Mammalian Hosts: Velvet Governs Mycotoxins and Virulence in *Fusarium*. *Molecular Microbiology* **2013**, *87*, 49–65, doi:10.1111/mmi.12082.
38. Bärenstrauch, M.; Mann, S.; Jacquemin, C.; Bibi, S.; Sylla, O.-K.; Baudouin, E.; Buisson, D.; Prado, S.; Kunz, C. Molecular Crosstalk between the Endophyte *Paraconiothyrium Variabile* and the Phytopathogen *Fusarium Oxysporum* – Modulation of Lipoyxygenase Activity and Beauvericin Production during the Interaction. *Fungal Genetics and Biology* **2020**, *139*, 103383, doi:10.1016/j.fgb.2020.103383.
39. Marmann, A.; Aly, A.; Lin, W.; Wang, B.; Proksch, P. Co-Cultivation—A Powerful Emerging Tool for Enhancing the Chemical Diversity of Microorganisms. *Marine Drugs* **2014**, *12*, 1043–1065, doi:10.3390/md12021043.
40. Wigmann, É.F. MALDI-TOF MS Fingerprinting for Identification and Differentiation of Species within the *Fusarium Fujikuroi* Species Complex. *Appl Microbiol Biotechnol* **2019**, *15*.
41. Yilmaz, N.; Houbraken, J.; Hoekstra, E.S.; Frisvad, J.C.; Visagie, C.M.; Samson, R.A. Delimitation and Characterisation of *Talaromyces Purpurogenus* and Related Species. *Pers - Int Mycol J* **2012**, *29*, 39–54, doi:10.3767/003158512X659500.
42. Bertrand, S.; Schumpp, O.; Bohni, N.; Bujard, A.; Azzollini, A.; Monod, M.; Gindro, K.; Wolfender, J.-L. Detection of Metabolite Induction in Fungal Co-Cultures on Solid Media by High-Throughput Differential Ultra-High Pressure Liquid Chromatography–Time-of-Flight Mass Spectrometry Fingerprinting. *Journal of Chromatography A* **2013**, *1292*, 219–228, doi:10.1016/j.chroma.2013.01.098.
43. Losada, L.; Ajayi, O.; Frisvad, J.C.; Yu, J.; Nierman, W.C. Effect of Competition on the Production and Activity of Secondary Metabolites in *Aspergillus* Species. *Med Mycol* **2009**, *47*, S88–S96, doi:10.1080/13693780802409542.
44. Fox, E.M.; Howlett, B.J. Secondary Metabolism: Regulation and Role in Fungal Biology. *Current Opinion in Microbiology* **2008**, *11*, 481–487, doi:10.1016/j.mib.2008.10.007.

45. Nirlane da Costa Souza, P.; Luiza Bim Grigoletto, T.; Alberto Beraldo de Moraes, L.; Abreu, L.M.; Henrique Souza Guimarães, L.; Santos, C.; Ribeiro Galvão, L.; Gomes Cardoso, P. Production and Chemical Characterization of Pigments in Filamentous Fungi. *Microbiology* **2016**, *162*, 12–22, doi:10.1099/mic.0.000168.
46. Sánchez-Muñoz, S.; Mariano-Silva, G.; Leite, M.O.; Mura, F.B.; Verma, M.L.; da Silva, S.S.; Chandel, A.K. Production of fungal and bacterial pigments and their applications. In *Biotechnological Production of Bioactive Compounds*; Elsevier, 2020; pp. 327–361 ISBN 978-0-444-64323-0.
47. Yilmaz, N.; Visagie, C.M.; Houbraeken, J.; Frisvad, J.C.; Samson, R.A. Polyphasic Taxonomy of the Genus *Talaromyces*. *Studies in Mycology* **2014**, *78*, 175–341, doi:10.1016/j.simyco.2014.08.001.
48. Lebeau, J.; Petit, T.; Clerc, P.; Dufossé, L.; Caro, Y. Isolation of Two Novel Purple Naphthoquinone Pigments Concomitant with the Bioactive Red Bikaverin and Derivates Thereof Produced by *Fusarium Oxysporum*. *Biotechnol. Prog.* **2019**, *35*, e2738, doi:10.1002/btpr.2738.
49. Lebeau, J.; Petit, T.; Dufossé, L.; Caro, Y. Putative Metabolic Pathway for the Bioproduction of Bikaverin and Intermediates Thereof in the Wild *Fusarium Oxysporum* LCP531 Strain. *AMB Expr* **2019**, *9*, 186, doi:10.1186/s13568-019-0912-4.
50. Studt, L.; Wiemann, P.; Kleigrew, K.; Humpf, H.-U.; Tudzynski, B. Biosynthesis of Fusarubins Accounts for Pigmentation of *Fusarium Fujikuroi* Perithecia. *Appl. Environ. Microbiol.* **2012**, *78*, 4468–4480, doi:10.1128/AEM.00823-12.
51. Lagashetti, A.C.; Dufossé, L.; Singh, S.K.; Singh, P.N. Fungal Pigments and Their Prospects in Different Industries. *Microorganisms* **2019**, *7*, 604, doi:10.3390/microorganisms7120604.
52. Son, S.W.; Kim, H.Y.; Choi, G.J.; Lim, H.K.; Jang, K.S.; Lee, S.O.; Lee, S.; Sung, N.D.; Kim, J.-C. Bikaverin and Fusaric Acid from *Fusarium Oxysporum* Show Antioomycete Activity against *Phytophthora Infestans*. *J Appl Microbiol* **2008**, *104*, 692–698, doi:10.1111/j.1365-2672.2007.03581.x.
53. Stępień, Ł.; Koczyk, G.; Waśkiewicz, A. Diversity of *Fusarium* Species and Mycotoxins Contaminating Pineapple. *J Appl Genetics* **2013**, *54*, 367–380, doi:10.1007/s13353-013-0146-0.
54. Ibrahim, N.F.; Mohd, M.H.; Mohamed Nor, N.M.I.; Zakaria, L. Mycotoxigenic Potential of *Fusarium* Species Associated with Pineapple Diseases. *Archives of Phytopathology and Plant Protection* **2020**, *53*, 217–229, doi:10.1080/03235408.2020.1736971.
55. Venkatesh, N.; Keller, N.P. Mycotoxins in Conversation With Bacteria and Fungi. *Front. Microbiol.* **2019**, *10*, 403, doi:10.3389/fmicb.2019.00403.
56. Zheng, H.; Kim, J.; Liew, M.; Yan, J.K.; Herrera, O.; Bok, J.W.; Kelleher, N.L.; Keller, N.P.; Wang, Y. Redox Metabolites Signal Polymicrobial Biofilm Development via the NapA Oxidative Stress Cascade in *Aspergillus*. *Current Biology* **2015**, *25*, 29–37, doi:10.1016/j.cub.2014.11.018.
57. Baldwin, T.T.; Zitomer, N.C.; Mitchell, T.R.; Zimeri, A.-M.; Bacon, C.W.; Riley, R.T.; Glenn, A.E. Maize Seedling Blight Induced by *Fusarium Verticillioides*: Accumulation of Fumonisin B1 in Leaves without Colonization of the Leaves. *J. Agric. Food Chem.* **2014**, *62*, 2118–2125, doi:10.1021/jf5001106.
58. Camardo Leggieri, M.; Giorni, P.; Pietri, A.; Battilani, P. *Aspergillus Flavus* and *Fusarium Verticillioides* Interaction: Modeling the Impact on Mycotoxin Production. *Front. Microbiol.* **2019**, *10*, 2653, doi:10.3389/fmicb.2019.02653.
59. Lee, K.; Pan, J.J.; May, G. Endophytic *Fusarium Verticillioides* Reduces Disease Severity Caused by *Ustilago Maydis* on Maize. *FEMS Microbiology Letters* **2009**, *299*, 31–37, doi:10.1111/j.1574-6968.2009.01719.x.
60. Rodriguez Estrada, A.E.; Hegeman, A.; Corby Kistler, H.; May, G. In Vitro Interactions between *Fusarium Verticillioides* and *Ustilago Maydis* through Real-Time PCR and Metabolic Profiling. *Fungal Genetics and Biology* **2011**, *48*, 874–885, doi:10.1016/j.fgb.2011.06.006.
61. Jonkers, W.; Rodriguez Estrada, A.E.; Lee, K.; Breakspear, A.; May, G.; Kistler, H.C. Metabolome and Transcriptome of the Interaction between *Ustilago Maydis* and *Fusarium Verticillioides* In Vitro. *Appl. Environ. Microbiol.* **2012**, *78*, 3656–3667, doi:10.1128/AEM.07841-11.

62. Perincherry, L.; Lalak-Kańczugowska, J.; Stepień, Ł. Fusarium-Produced Mycotoxins in Plant-Pathogen Interactions. *Toxins* **2019**, *11*, doi:doi.org/10.3390/toxins11110664.
63. Parnell, S.; Gottwald, T.R.; Gilligan, C.A.; Cunniffe, N.J.; van den Bosch, F. The Effect of Landscape Pattern on the Optimal Eradication Zone of an Invading Epidemic. *Phytopathology*® **2010**, *100*, 638–644, doi:10.1094/PHYTO-100-7-0638.
64. Almaguer, M.; Aira, M.-J.; Rodríguez-Rajo, F.J.; Rojas, T.I. Temporal Dynamics of Airborne Fungi in Havana (Cuba) during Dry and Rainy Seasons: Influence of Meteorological Parameters. *Int J Biometeorol* **2014**, *58*, 1459–1470, doi:10.1007/s00484-013-0748-6.
65. Velásquez, A.C.; Castroverde, C.D.M.; He, S.Y. Plant–Pathogen Warfare under Changing Climate Conditions. *Current Biology* **2018**, *28*, R619–R634, doi:10.1016/j.cub.2018.03.054.
66. Picot, A.; Hourcade-Marcolla, D.; Barreau, C.; Pinson-Gadais, L.; Caron, D.; Richard-Forget, F.; Lannou, C. Interactions between *Fusarium Verticillioides* and *Fusarium Graminearum* in Maize Ears and Consequences for Fungal Development and Mycotoxin Accumulation. *Plant Pathology* **2012**, *12*.
67. Dita, M.; Barquero, M.; Heck, D.; Mizubuti, E.S.G.; Staver, C.P. Fusarium Wilt of Banana: Current Knowledge on Epidemiology and Research Needs Toward Sustainable Disease Management. *Front. Plant Sci.* **2018**, *9*, 1468, doi:10.3389/fpls.2018.01468.
68. Ou, Y.; Penton, C.R.; Geisen, S.; Shen, Z.; Sun, Y.; Lv, N.; Wang, B.; Ruan, Y.; Xiong, W.; Li, R.; et al. Deciphering Underlying Drivers of Disease Suppressiveness Against Pathogenic *Fusarium Oxysporum*. *Front. Microbiol.* **2019**, *10*, 2535, doi:10.3389/fmicb.2019.02535.
69. Barral, B. Maladie des taches noires de l’ananas: Étude des relations hôte-pathogène et compréhension des mécanismes physiologiques de résistance. Thesis, Université de Montpellier: Saint-Pierre, La Réunion, 2017.
70. Bertrand, S.; Azzollini, A.; Schumpp, O.; Bohni, N.; Schrenzel, J.; Monod, M.; Gindro, K.; Wolfender, J.-L. Multi-Well Fungal Co-Culture for de Novo Metabolite-Induction in Time Series Studies Based on Untargeted Metabolomics. *Molecular BioSystems* **2014**, 2–11, doi:10.1039/x0xx00000x.
71. Capodanno, E.; Moreau, S.; Levi, M.; Besse, S.G.; Allende, S. *Rapid Simultaneous Assay of 23 Mycotoxins in a Variety of Food Samples by UHPLC-MS/MS Using Fast Polarity Switching (PO-CON1350E)*; Shimadzu, Tokyo, Japan, 2013; pp. 1–5;.
72. Moreau, S.; Levi, M. *Highly Sensitive and Rapid Simultaneous Method for 45 Mycotoxins in Baby Food Samples by HPLC-MS/MS Using Fast Polarity Switching (PO-CON1480E)*; Shimadzu, Tokyo, Japan, 2014; pp. 1–7;.
73. Abràmoff, D.M.D.; Magalhães, P.J.; Sunanda, J.R. Image Processing with ImageJ. *Biophotonics international* **2004**, *11*, 36–42.
74. Hamzah, T.N.T.; Lee, S.Y.; Hidayat, A.; Terhem, R.; Faridah-Hanum, I.; Mohamed, R. Diversity and Characterization of Endophytic Fungi Isolated From the Tropical Mangrove Species, *Rhizophora Mucronata*, and Identification of Potential Antagonists Against the Soil-Borne Fungus, *Fusarium Solani*. *Front. Microbiol.* **2018**, *9*, 1707, doi:10.3389/fmicb.2018.01707.

Supplementary data – Article 2

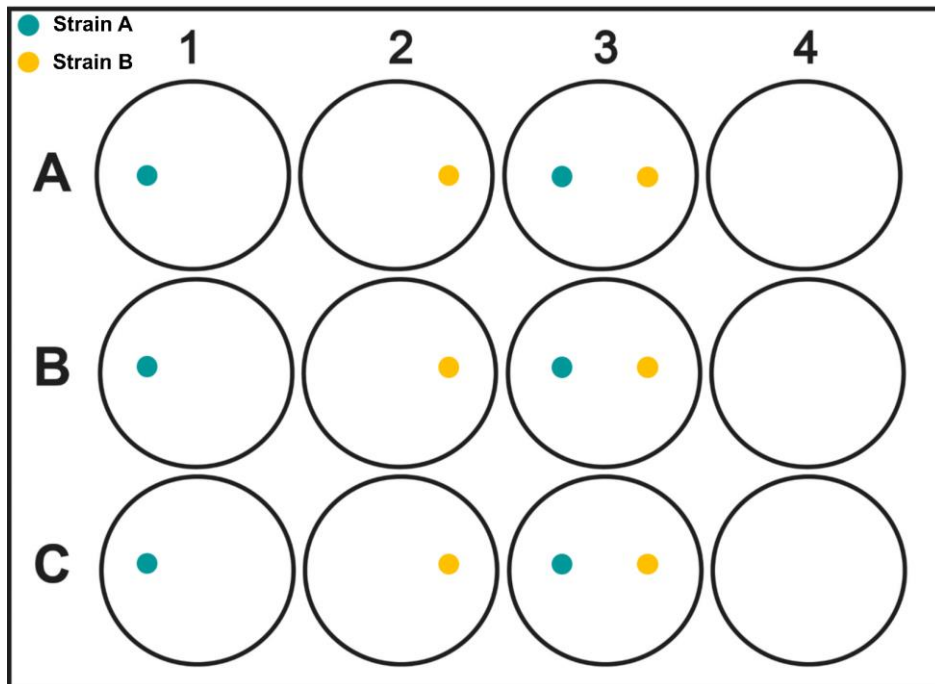


Figure S1. Dual-culture procedure performed with fungal species defined as FCR-pathogens of pineapple on 12-well culture plates. 1 and 2, single-cultures; 3, dual-cultures; 4, Mock.

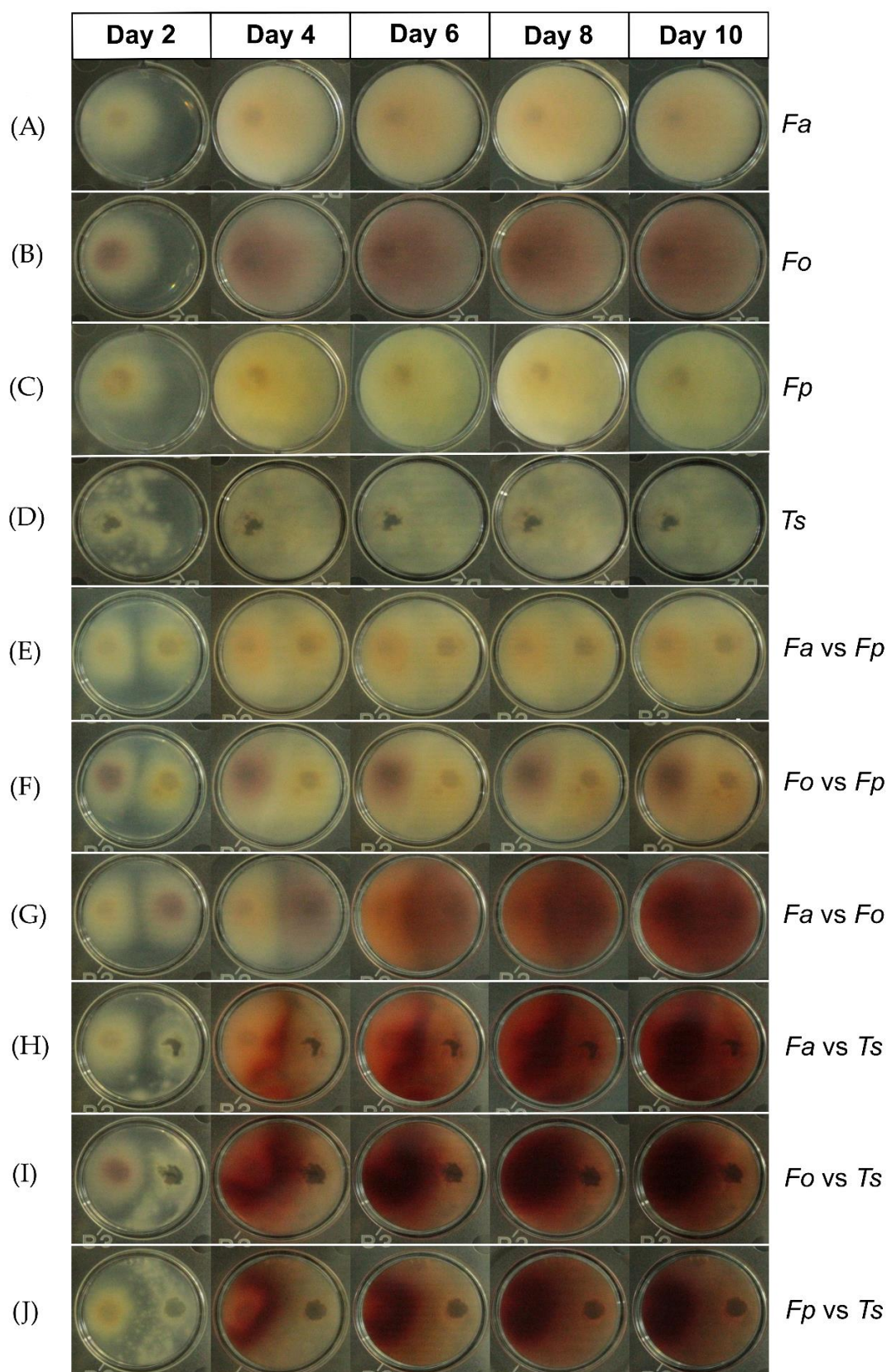


Figure S2. Fungal colony aspects on PDA view from the bottom of plates according to a 10-day kinetics. Conditions are defined as incubation in single-culture of (A) *Fusarium ananatum* (*Fa*), (B) *Fusarium oxysporum* (*Fo*), (C) *Fusarium proliferatum* (*Fp*) and (D) *Talaromyces stollii* (*Ts*) or confrontation by dual-culture of (E) *F. ananatum* versus *F. proliferatum* (*Fa vs Fp*), (F) *F. oxysporum* versus *F. proliferatum* (*Fo vs Fp*), (G) *F. ananatum* versus *F. oxysporum* (*Fa vs Fo*), (H) *F. ananatum* versus *T. stollii* (*Fa vs Ts*), (I) *F. oxysporum* versus *T. stollii* (*Fo vs Ts*), (J) *F. proliferatum* versus *T. stollii* (*Fp vs Ts*).

CHAPITRE III

Réponses moléculaires précoces
conditionnant la sensibilité de
l'ananas 'Queen Victoria' à la tache
noire

**Article 3 - Early genetic markers of 'Queen Victoria'
pineapple susceptibility to Fruitlet Core Rot**

Suite à la définition des facteurs biotiques et abiotiques conditionnant la contamination des fruits simples ainsi que la structuration du cortège pathogène de la tache noire, ce travail de thèse s'est porté sur l'étude des réponses précoces de l'ananas au cours de l'initiation d'un processus infectieux par l'espèce pathogène de référence, *F. ananatum*. En effet, de récents travaux de recherche fondés sur la comparaison des réponses biochimiques de cultivars résistant et sensible ont montré des capacités différentielles d'accumulation constitutive et d'élicitation de composés phénoliques en réponses à une attaque par le pathogène (Barral *et al.*, 2019a, Barral *et al.*, 2019b).

Ainsi, ce troisième chapitre s'appuie sur l'article intitulé « **Early genetic markers of 'Queen Victoria' pineapple susceptibility to Fruitlet Core Rot** » à soumettre dans la revue *Molecular Plant-Microbe Interactions (MPMI)*. Au cours de cette étude, les expressions relatives de huit gènes candidats impliqués dans les voies de signalisation de l'acide salicylique, de l'acide jasmonique et de l'éthylène ont été étudiées chez le cultivar résistant 'MD-2' ainsi que chez le cultivar sensible 'Queen Victoria'. Les profils d'expression des gènes d'intérêt en réponses à l'inoculation du pathogène *F. ananatum* ont été déterminés pour les 96 premières heures de l'interaction hôte – pathogène. Les résultats obtenus pour le cultivar 'Queen Victoria' ont mis en évidence une faible expression constitutive des gènes induits suite à une accumulation d'acide salicylique mais également de certains gènes codant pour des protéines PR. Par ailleurs, il a été démontré que *F. ananatum* restreint l'induction des gènes d'intérêt impliqués dans la transduction des signaux de l'acide salicylique, de l'acide jasmonique et de l'éthylène au cours des 72 heures suivant l'inoculation. Un important basculement des « patterns » d'expression de ces gènes a ensuite été observé à partir de 96 heures post-inoculation. Ces données suggèrent la mise en œuvre d'une stratégie fongique visant à moduler les défenses de l'hôte et assurant dès lors un contexte cellulaire favorable à la croissance ainsi qu'à la colonisation des tissus hôtes par l'agent pathogène.

Early genetic markers of ‘Queen Victoria’ pineapple susceptibility to Fruitlet Core Rot

Manon Vignassa^{1,2*}, Christian Soria^{1,2}, Frédéric Chiroleu³, Sabine Schorr-Galindo², Marc Chillet^{1,2}, Jean-Christophe Meile^{1,2}

¹ CIRAD, UMR Qualisud, F-97410 Saint-Pierre, Réunion, France.

² Qualisud, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, IRD, Université de La Réunion, Montpellier, France.

³ CIRAD, UMR PVBMT, F-97410 Saint-Pierre, Réunion, France.

* Corresponding author

Abstract

Plant-fungi interactions are conditioned by complex signal interplays. Members of the *Fusarium fujikuroi* and *Talaromyces purpureogenus* species complexes are known to be responsible for important quality depreciation of pineapple fruits (*Ananas comosus* (L.) Merr.). Notably, they were identified as the main causing agents of the Fruitlet Core Rot disease (FCR), which symptoms consist of severe browning of the fruitlets flesh. The molecular determinants of the ‘Queen Victoria’ cultivar susceptibility to FCR were explored by monitoring gene expression patterns of the three main fruit defense signals associated with salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) pathways upon infection by the leading FCR-pathogen *Fusarium ananatum*. Infection assays and RT-qPCR analyses targeted on eight different genes were performed on the two cultivars ‘Queen Victoria’ and ‘MD-2’ characterized by their contrasted resistance to FCR over a time course of 96-hours. This study revealed that the major factor supporting ‘Queen Victoria’ susceptibility is the genotype by priming defective basal expression of defense-related genes. Results suggested transcriptional modulations of SA, JA and ET signaling pathways evidencing a shift in host response between 72 and 96 hours after inoculation. Considering the regulation profiles, hijack of pineapple defense signals by *Fusarium ananatum* was hypothesized. According to these findings, we suggest that ‘Queen Victoria’ susceptibility could also result from the various bypass strategies of the FCR-pathogenic fungal set co-occurring in fruitlets.

Keywords : *Ananas comosus*, ethylene, *Fusarium ananatum*, jasmonic acid, pathogenic fungi, salicylic acid, susceptibility

1. Introduction

Tropical crops are exposed to attacks from a broad spectrum of pathogens with numerous infection strategies. The Fruitlet Core Rot (FCR) is one of the most deleterious diseases of pineapple. The pathology is caused by a fungal cortège led by *Fusarium ananatum* (Jacobs *et al.*, 2010; Barral *et al.*, 2017). FCR generate severe fruit quality depreciation due to the development of black spots in the fruitlets. These symptoms result from the oxidation of phenolic compounds accumulated in the cell wall of tissues constituting the blossom cup (Avallone *et al.*, 2003; Barral *et al.*, 2019a). A recent study elucidated that, in Reunion Island, FCR epidemics on 'Queen Victoria' cultivar ('QV') can result from infection by numerous fungal pathogens belonging to the *Fusarium proliferatum*, *Fusarium oxysporum*, *Fusarium sacchari*, *Talaromyces stollii* and *Talaromyces amestolkiae* species (Vignassa *et al.*, 2021). *In vitro*, *T. stollii* was elucidated as the main growth antagonist of *F. proliferatum* and *F. oxysporum*. However, *Fusarium ananatum* was described as the sole pathogenic species able to successfully challenge *T. stollii* demonstrating that specific arrangements in fungal communities could be related to FCR establishment (Vignassa *et al.*, submitted to *Toxins*). Moreover, inoculation of *Fusarium*- and *Talaromyces*-FCR pathogens in pineapple resulted in divergence of fungal network structuration correlated to the increase in biomass of these species (Vignassa *et al.*, 2021). Up to now, control strategies in pineapple are based on the combination of resistant cultivars cultivation (such as the 'MD-2'), suitable agronomic practices and fungicide treatments. Considering current agroecological concern, these methods are therefore precarious in some tropical and subtropical areas where the main cultivar remain the 'QV' appreciated for its sensorial qualities even though exhibiting a high susceptibility to FCR. Despite the rising of molecular tools, so far, no genetic patterns of resistance or susceptibility has been investigated in the context of FCR infection in pineapple. Plant-pathogen interaction start with the detection of molecular patterns from causal agent called PAMPs (Pathogen Associated Molecular Patterns) (Jones and Dangl, 2006). In plant-fungi diseases, most common PAMPs are chitin, as a major compound of fungal cell wall, and xylan (Wan *et al.*, 2008; Kawasaki *et al.*, 2017 ; Escudero *et al.*, 2017). These signal molecules are perceived at the plant cell surface by PRRs (Pattern Recognition Receptors) and lead to the structuration of specific cellular responses (Patin *et al.*, 2019). This initial step is primordial and triggers PAMPs Triggered Immunity (PTI). PTI is an active process allowing the restriction

of host tissues colonization by the fungal pathogen (Nürnberg and Kemmerling, 2018). In these conditions, host plant is therefore considered as resistant. Additionally, the resistance status is also supported by the HR (Hypersensitive Response) which is characterized by the development of necrotic injuries (Künstler *et al.*, 2016). Necrosis specifically appears in the area where the primary dialogue with the pathogen is established (Greenberg and Yao, 2004). HR is mainly set up against biotrophic and hemibiotrophic pathogens. This promotes the initiation of various defense responses associated with salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) pathways. These events define the Systemic Acquired Resistance (SAR). The SA-defense pathway achieves the induction of numerous defense genes coding for the well-characterized pathogenesis-related proteins (PR). Signaling is based on transcriptional co-activators like the *Non-expressor of Pathogenesis-Related gene 1 (NPR1)* which is a key determinant positively regulating both local resistance and SAR (Wang *et al.*, 2018, Castello *et al.*, 2018). Following translocation into the nucleus, *NPR1* interacts with numerous transcription factors of *TGA (TGACG-motif binding factor)* family to induce defense-genes expression like *Pathogenesis-Related gene 1 (PR-1)* (Jin *et al.*, 2018). In contrast, *NPR1* paralogues *NPR3 (Non-expressor of Pathogenesis-Related gene 3)* and *NPR4 (Non-expressor of Pathogenesis-Related gene 4)* also act as SA receptors for immunity (Kaldorf and Naseem, 2013). Playing on their differential binding affinity for SA, *NPR3* and *NPR4* mediate the degradation of *NPR1* by the proteasome (Fu *et al.*, 2012).

Jasmonic acid and ethylene defense pathways are involved against necrotrophic and hemibiotrophic pathogens (Glazebrook, 2005). Actually, both JA and ET are well documented enabling the study of key factors of each signaling cascade. After transduction of primary cellular signals, the COI1 protein interact with (+)-7-iso-jasmonoyl-L-Ile (JA-Ile) which is a master piece of the complex Skp1-Cullin1-Fbox^{COI1} (SCF^{COI1}) (Browse, 2009). Downstream, SCF^{COI1} targets Jasmonate Zim-domain (JAZ) proteins (Mosblech *et al.*, 2011). This interaction results in the action of proteasome on JAZ family proteins alleviating their repressor effect on numerous transcriptional activators of *MYC (Myelocytomatosis oncogene)* family factors (Fernandez-Calvo *et al.*, 2011; Cui *et al.*, 2019). The *MYC2 (Myelocytomatosis oncogene 2)* release resulted in expression induction of JA-defense genes like *PR-3* (coding for chitinase class I) (van Loon *et al.*, 2006; Major *et al.*, 2017). Many signal pathways of plant hormones are controlled in a similar way to JA. In basal conditions, ET pathway is repressed through the

phosphorylative action of MAPK (Mitogen-Activated Protein Kinase) on *Ethylene Insensitive 2* gene (*EIN2*). In presence of ET, repression is abolished leading to *EIN2* dephosphorylation of the C-terminal end and translocation to the nucleus (Qiao *et al.*, 2012). There, *EIN2* and *EIN3* act as regulators of transcription factors such as *Octadecanoid-Responsive Arabidopsis AP2/ERF59 (ORA59)* and *Ethylene Response Factor 1 (ERF1)*. *EIN3* is highly accumulated into the nucleus resulting in downstream induction of ethylene-responsive genes such as *PR-12 (Pathogenesis-Related gene 12)* encoding a plant defensin 1.2 (Pandey *et al.*, 2016). Interestingly, JAZ repressor also interacts with *EIN3* demonstrating synergic effect between JA and ET signaling pathways. The crosstalk between SA and JA/ET is also based on antagonistic interactions (Li *et al.*, 2019). In fact, SA defense pathway can act as a suppressor of JA signal transduction (Leon-Reyes *et al.*, 2010).

The PR proteins encoded by defense-genes are induced through the different signaling pathways previously described (van Loon *et al.*, 1999). PR-1 and PR-5 are SA dependent proteins with an antifungal activity (Breen *et al.*, 2017). Similarly, PR-2 proteins encoding β -1,3-glucanases, are expressed *via* SA signal resulting in cell wall degradation of fungal species (Maglovski *et al.*, 2016). For a broad spectrum of antifungal activity, PR-3 type chitinases accumulate through interaction of JA and ET pathways into apoplast which is considered as a crucial interface in plant-pathogen interactions (van Loon, 2006; Irigoyen *et al.*, 2020). Combining PR-2 and PR-3 lead to an efficient inhibition of fungal growth (Dzhavakhiya *et al.*, 2007). *PR-12* gene coding for plant-defensins, is induced by both JA and ET defense pathways with no dependence to SA (Sels *et al.*, 2008). Under biotic stress, plant-defensins showed plural biological activities such as inhibitors of protein synthesis and antimicrobial activity (mainly against fungi) (Stotz *et al.*, 2009 ; de Oliveira Carvalho and Gomes, 2011). The recent sequencing and annotation of pineapple reference genome promote the search for novel sources of resistance through genomic approach (Zhang *et al.*, 2014; Ming *et al.*, 2015).

Even though FCR epidemics have tried to be elucidate in the past decades, the molecular patterns of pineapple in response to FCR infection still remains a black box. In the present study, we propose to determine the expression profiles of eight genetic key determinants involved in the three major plants defense-pathways (SA, JA and ET) in the context of interaction between pineapple and the pathogen *Fusarium anatum*. Performing RT-qPCR

on two cultivars ('QV' and 'MD-2') with contrasted levels of resistance to FCR, we observed significant differences in gene expression kinetics upon infection according to the cultivar. Results suggest that 'QV' susceptibility to FCR is supported by pathogen modulation strategies and weak host constitutive defenses.

2. Materials and Methods

2.1. Plant material and inoculation

Fusarium ananatum inoculum was prepared using the Clp001 strain isolated on a diseased-fruitlet of pineapple fruit harvested in Reunion Island (Barral *et al.*, 2017). Conidia production was achieved by incubation at 27 °C on Petri dishes containing Potato Dextrose Agar (PDA, BD Difco, Le Pont-de-Claix, France) medium for 7 days in the dark. Conidia solutions were prepared by adding 2 mL of sterile water to the Petri dish and gently scratching the mycelium surface with a sterile spreader. The solution was then adjusted at a concentration of 10³ conidia per mL. Inoculations were conducted on both FCR-susceptible cultivar 'Queen Victoria' and FCR-resistant cultivar 'MD-2'. Both cultivars were grown on the same plot in accordance with the conventional cultural practices locally applied (Fournier, 2012). Experimental plot was located in southwest of Reunion Island at CIRAD Bassin-Plat Experimental Station, Saint-Pierre, 150 meters above sea level (21°19'21''S, 55°29'26''E). The inoculation procedure was carried out on fruits at C1 maturity stage corresponding to 1/4 yellow according to the shell color. The inoculations were performed by injection of 25 µL of conidia solution in the blossom cup of two fruitlets located at the median section for each fruit. Control fruits (H₂O) were inoculated with sterile water in similar conditions. Fruits were harvested at four different time points corresponding to 24, 48, 72 and 96 hours after inoculation. For each of the two cultivars, three control fruits (Mock) were harvested at C1 maturity stage before the inoculation procedure in order to determine basal genes expression levels. After sampling, fruitlets were frozen in liquid nitrogen and lyophilized prior to their storage at -80 °C. Three biological replicates were performed for each cultivar ('MD-2' and 'QV') in each condition of inoculation (*F. ananatum* and H₂O) and for the four kinetic points of infection corresponding to a total of 54 pineapple fruits.

2.2. Total RNA extraction

Two frozen and dehydrated fruitlets collected from the median section of each fruit were pooled and grinded using an A11 analytical mill (IKA, Staufen, Germany) cooled with liquid nitrogen. RNA extractions were performed using the RNeasy® Plant Mini Kit (Qiagen, Courtabœuf, France) with addition of 20 µL of 2M DTT per mL of RLT buffer (2M, DL-Dithiothreitol, Sigma-Aldrich, Darmstadt, Germany) on 100 mg of fine powder in accordance with the manufacturer's instructions. RNA samples were then treated with RNase-free DNase Set (Qiagen, Courtabœuf, France). Subsequently, samples were cleaned and concentrated with the RNeasy® MinElute Cleanup Kit (Qiagen, Courtabœuf, France) following the manufacturer's instructions. Yield and purity were estimated by spectrophotometry using the Qubit® RNA HS Assay Kit and Qubit® 3.0 fluorometer (Life Technologies, Illkirch, France).

2.3. Primers design

Primers were designed with Primer3 software v4.1.0 (Untergasser *et al.*, 2012) on the eight target gene sequences of *Ananas comosus* obtained from the Pineapple Genomics Database (PGD, <http://pineapple.angiosperms.org/pineapple/html/index.html>) (Zhang *et al.*, 2014 ; Ming *et al.*, 2015). Primers design was based on the following criteria: Primer size between 19 bp and 22 bp, primer sequence with a GC content from 40% to 60%, PCR size product between 100 bp and 250 bp and a melting temperature (T_m) between 58°C and 60°C (Table 5). Primers specificity was pre-checked by Primer-BLAST (Ye *et al.*, 2012).

2.4. Primers validation

2.4.1. DNA Extraction

For each tested cultivar ('QV' and 'MD-2'), one sample corresponding to 'Mock' control condition was randomly chosen. DNA extractions were performed on 100 mg of fine powder with the FastDNA SPIN kit and the FastPrep-24 Instrument (MP Biomedicals®, Illkirch, France) using Lysing Matrix A with addition of CLS-VS and PPS as Lysis Buffer in accordance with manufacturer's instructions.

2.4.2. Polymerase Chain Reaction

Primers specificity and their respective optimal annealing temperature were determined by gradient PCR ranging from 51 °C to 60 °C by step of 1 °C. Thus, PCR reactions were performed in a final volume of 50 µL containing 0.6 µM of each primer, all the deoxyribonucleotide triphosphates (dNTPs) at 200 µM, 2 mM of MgCl₂, 10 µL of 5x GoTaq Flexi buffer, 1.25 U of GoTaq[®] G2 Flexi DNA polymerase, and 1 µL of extracted DNA. PCR amplification reactions were carried out as follows: an initial denaturation at 95 °C for 2 min, 40 cycles at 95 °C for 30 s, at [51 – 60] °C for 30 s, and at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR reactions were performed in a thermocycler (Veriti, Applied Biosystems, United Kingdom). PCR products were analyzed with the Qiaxcel[®] Advanced System (Qiagen, Hilden, Germany) using size markers (25 bp - 500 kb).

2.5. Reference gene determination

Five candidate reference genes were tested for their expression stability: *CYC* (*Cyclophilin-like peptidyl-prolyl cistrans isomerase protein CYP37*), *PP2A* (*Protein Phosphatase 2A regulatory subunit A*), *RAN* (*GTP-binding nuclear protein RAN*), *EF1-α* and *60sRP* (*60s Ribosomal Protein*) in accordance with previous studies on pineapple genes expression (Léchaudel *et al.*, 2018 ; Chen *et al.*, 2019). Stability assays were performed using four statistical algorithms independently of each other: geNorm (Vandesompele *et al.*, 2002), BestKeeper (Pfaffl *et al.*, 2004), NormFinder (Andersen *et al.*, 2004) and the Δ Ct method (Silver *et al.*, 2006). These tools were also operated under the RefFinder analytical tool which integrates all of them and allows a consensus determination of the most appropriate reference gene for the study (Xie *et al.*, 2012). Following these different strategies, results were compared and led to the selection of *EF1-α* as reference gene.

Table 5. Primers used in this study.

| Sequence ID | Gene description | PGD gene ID | GenBank Accession Number | 5'→3' Sequence (Forward and Reverse) | Product length (bp) | Optimal annealing temp (°C) | T _m (°C) | References |
|--------------|---|-------------|--------------------------|--|---------------------|-----------------------------|---------------------|---------------------------|
| <i>NPR1</i> | Non-Expressor of Pathogenesis Related protein 1 | Aco029912.1 | XM_020245757.1 | GAAGACACCTCTGCACATCG CTTATTGGGCTCGACACGTG | 190 | 58 | | |
| <i>NPR3</i> | Non-Expressor of Pathogenesis Related protein 3 | Aco005014.1 | XM_020236747.1 | AAGGGCGAAGGACTACAACA TGTGTAGGTCGTGACCCAAT | 152 | 59 | | |
| <i>EIN3</i> | Ethylene Insensitive 3 | Aco001697.1 | XM_020252657.1 | CGCCGAAAGTTTCAGATGGAG AGGCTGGTTTGCGAATTGTT | 155 | 51 | | |
| <i>COI1</i> | Coronatine-insensitive protein 1 | Aco001397.1 | XM_020252102.1 | AGTCCCTCAAGATCAAGGCC GCACCTATCGAGCTTGAACG | 218 | 59 | 59 | This study |
| <i>PR-12</i> | Plant Defensin 1.2 | Aco008353.1 | XM_020254244.1 | TGTCAACACGAGGGCTTCT GCCATCCGTTACCTTTCTCC | 214 | 59 | | |
| <i>PR-3</i> | Chitinase I | Aco018047.1 | XM_020250839.1 | GAATACATTGGAGCCACGGG GGTTTTGGTATCTCGGACGC | 186 | 58 | | |
| <i>PR-2</i> | β-1,3-glucanase | Aco023085.1 | XM_020240396.1 | TACTTTTACGGGAAGGCGGT GATCCCGAAACCAAAGTCCG | 228 | 55 | | |
| <i>PR-1</i> | Pathogenesis-Related 1 protein | Aco008601.1 | XM_020240129.1 | ACTTCTGAAACCGCACAAAC GATGAGTTTGCAGTCTCCGC | 125 | 54 | | |
| <i>EF1-α</i> | Elongation Factor 1-alpha | | XM_020249940.1 | TCTTCTCAGGGAAGGTCTCTAC CTCTGCACACTCTTCACATACA | 100 | 51 | 57 | Chen <i>et al.</i> , 2019 |

2.6. cDNA synthesis and RT-qPCR assays

Reverse transcription was performed in MicroAmp optical 96-well reaction plate (Applied Biosystems, Illkirch, France) using the one-step QuantiTect SYBR[®] Green RT-PCR Kit (Qiagen, Courtabœuf, France) with ROX as a passive reference dye. The cDNA were obtained by addition of 8 ng of total RNA. RT-qPCR were performed in a final volume of 20 µL containing 0.75 µM of each primer, 2.5 mM MgCl₂, 10 µL of 2X QuantiTect SYBR Green Master Mix and 0.2 µL of QuantiTect RT Mix.

RT-qPCR reactions were carried out as follows: Reverse transcription at 50 °C for 30 min, heat-mediated activation of HotStarTaq DNA polymerase at 95 °C during 15 min followed by 40 cycles at 94 °C for 15 sec, optimal annealing temperature (°C) for 30 sec and 72 °C for 15 sec. Control of PCR products quality was achieved by a melting curve starting from 60 to 95 °C by steps of 0.3 °C (15 sec/step). RT-qPCR experiments were conducted with a StepOnePlus Real-Time PCR system (Applied Biosystems, Illkirch, France). PCR efficiencies were calculated from raw data with LinRegPCR program v2020.0 (Heart Failure Research Center, Amsterdam, The Netherlands) following the equation $(1 + E) = 10^{(-1/\text{slope})}$ and are presented in Table S1 (Ramakers *et al.*, 2003 ; Ruijter *et al.*, 2009). Relative expression levels of interest genes were determined by normalization with *EF1-α* expression and 'Mock' condition of each cultivar.

Thus, ΔCt and $\Delta\Delta Ct$ were calculated according to the following equations:

$$\Delta Ct = Ct_{\text{Reference}} - Ct_{\text{Gene of interest}}$$

$$\Delta\Delta Ct_{\text{Experimental-Mock}} = \text{Mean}\Delta Ct_{\text{Experimental}} - \text{Mean}\Delta Ct_{\text{Mock}}$$

The ΔCt formula considers that Ct value is proportional to the $-\log$ of gene expression (Jones *et al.*, 2019). Thus, ΔCt is positively related to the expression of the gene of interest and can be assumed as normally distributed.

2.7. Computational analysis

Data were analyzed with the R statistical language v4.0.3 (R Core Team, 2020). Prior to Principal Component Analysis (PCA), the sampling adequacy of the data was analyzed with the Kaiser-Meyer-Olkin Statistics (Kaiser, 1974). The Bartlett's test of sphericity was then computed in order to determine whether the matrix was significantly different from an identity matrix. The *KMOS* and *bart_spher* functions were implemented with the 'REdaS'

package (REdaS CRAN, 2015). PCA was performed with 'FactoMineR' package on the centered and scaled mean ΔCt values (Lê *et al.*, 2008). Target genes were considered as variables ($n = 8$) while pineapple fruits related to the combinations of cultivar x condition of inoculation x kinetics were defined as individuals ($n = 18$). Fuzzy clustering was computed on dissimilarities between individuals using the *fanny* function from 'cluster' package with Euclidean method (Maechler *et al.*, 2021). Results obtained from PCA and clustering were visualized with 'factoextra' package (factoextra CRAN, 2020).

Significant differences in relative expression ($\Delta\Delta\text{Ct}$) of the eight target genes according to genotype and infection effects were determined by deviance test (three-way ANOVA) considering cultivars ('QV' or 'MD-2'), condition of inoculation (*Fusarium ananatum* or sterile water) and kinetic times of infection (24, 48, 72 and 96 hpi) as factors. For each cultivar, the effect of inoculation on the average relative expression ($\Delta\Delta\text{Ct}$) of the target genes was determined by deviance test (one-way ANOVA) at each time of the studied kinetic. Similarly, effect of kinetic on modulation of relative gene expression ($\Delta\Delta\text{Ct}$) was defined by deviance test (one-way ANOVA) for each cultivar and each conditions of inoculation. Then, multiple comparisons between average of relative gene expressions for the different kinetic times and considering each cultivar and each condition of inoculation were tested using the Tukey's test.

3. Results

3.1. Correlations between gene expression and experimental conditions

Expression profiles of eight pineapple genes acting in three major plant defense-pathways (SA, JA and ET) were monitored upon infection by the FCR pathogen *Fusarium ananatum*. Gene expression measurements (by real-time PCR) were performed on the cultivars 'QV' and 'MD-2' exhibiting contrasted susceptibility status to FCR, at different time points (up to 96 hpi) after *F. ananatum* or H₂O inoculation, or with no inoculation (Mock).

The principal component analysis (PCA) was computed as an exploratory approach in order to summarize the data and define the most influent factors. First, assumptions of Bartlett's test of sphericity evidenced that data were significantly different from an identity matrix ($\text{Chi}^2 = 105.16$, $\text{df} = 28$, $p < 0.001$) and KMO criterion reached a mean index of 0.62 above the suggested threshold of 0.5. These results demonstrate the suitability of the dataset for PCA. The first factorial plane computed on the mean ΔCt of the eight target genes according to

pineapple fruits modalities (cultivar x condition of inoculation x kinetics) represented 74.3% of the variance with 47.2% and 27.1% explained by the Dim1 and Dim2 respectively. Considering the first dimension, individuals corresponding to 'MD-2' pineapple of 'Mock' and H₂O control conditions sampled at 96 hpi, and 'QV' fruits of *F. ananatum*-condition sampled at 48 and 72 hpi were the main contributive individuals with 7.2%, 38.1%, 7.5% and 10.5% respectively (Figure 14B, C, D).

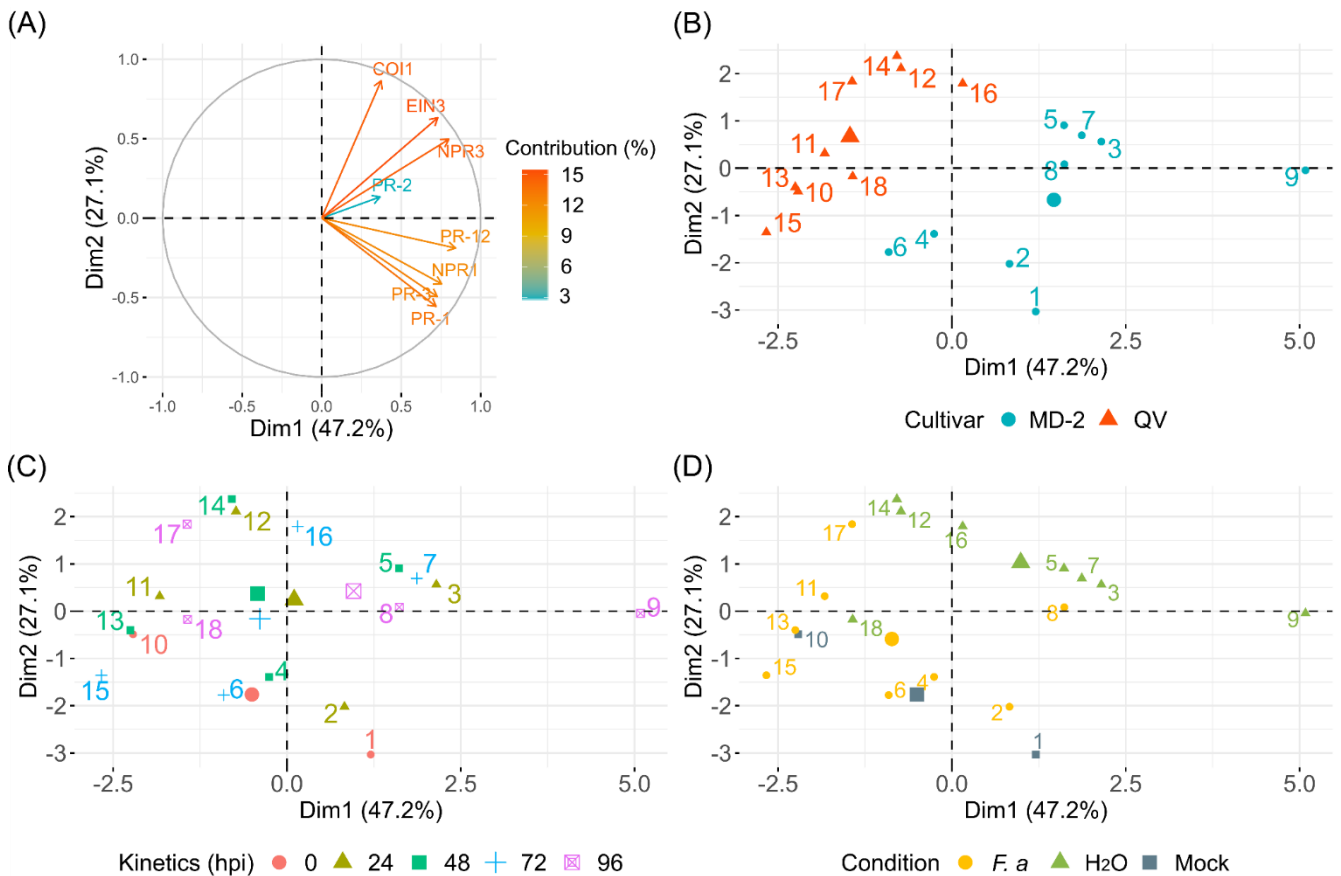


Figure 14. Principal component analysis (PCA) of pineapple fruit experimental conditions according to their mean ΔC_t value. (A) Respective contribution of the genes of interest (variables). (B), (C) and (D) highlight the dispersion of data following cultivar, kinetics and inoculation modality, respectively. *NPR1*, Non-Expressor of Pathogenesis Related gene 1; *NPR3*, Non-Expressor of Pathogenesis Related gene 3; *PR-1*, Pathogenesis-Related gene 1; *PR-2*, Pathogenesis-Related gene 2; *COI1*, Coronatine-insensitive 1; *PR-3*, Pathogenesis-Related gene 3; *PR-12*, Pathogenesis-Related gene 12; *EIN3*, Ethylene Insensitive 3; hpi, hours post-inoculation.

On this first component, variables with the highest contributions were related to both SA, JA and ET pathways and corresponded to the variables *NPR1* (15.1%), *NPR3* (17.0%), *PR-3* (14.0%) and *PR-12* (18.8%) (Figure 14A). The search for correlation between the variables and the first component (Dim1) demonstrated that *NPR1* ($R = 0.76$), *NPR3* ($R = 0.80$), *PR-1* ($R = 0.72$), *PR-3* ($R = 0.73$), *EIN3* ($R = 0.73$) and *PR-12* ($R = 0.84$) were positively correlated to the Dim1 at

$p < 0.001$. Thus, samples belonging to the 'MD-2' cultivar inoculated with H₂O and sampled at 24 and 96 hpi were associated with high expression of *NPR1*, *NPR3*, *PR-3* and *PR-12* by contrast to 'QV' samples corresponding to 'Mock' and *F. ananatum* inoculation at 48 and 72 hpi. Symmetrically, Dim2 was supported by 'QV' fruits of H₂O control condition at 24 and 48 hpi with respective contributions of 11.4% and 14.4%. Samples corresponding to 'MD-2'-Mock condition and *F. ananatum*-inoculated 'MD-2' pineapple at 24 hpi also contributed to Dim2 at 23.6% and 10.5% respectively (Figure 14B, C, D). For this second component, *PR-1* (14.2%), *EIN3* (18.4%) and *COI1* (34.4%) were defined as the most contributive variables (Figure 14A). Additionally, *COI1* ($R = 0.86$) and *EIN3* ($R = 0.63$) were positively correlated to Dim2 at $p < 0.001$ whereas *PR-3* ($R = -0.49$) and *PR-1* ($R = -0.56$) were negatively correlated with this component at $p \leq 0.04$. This revealed that according to normalization with the reference gene (*EF1- α*), 'QV' pineapple inoculated with H₂O of the 24, 48 and 72 hpi times were associated with elevated expression levels of *COI1* and *EIN3*, which were evidenced to be inversely related to expression of *PR-1* and *PR-3*.

3.2. Effects of cultivar and infection by *F. ananatum* on the genetic responses of host defenses

Results obtained from PCA were then used for fuzzy classification of samples. Partitioning evidenced four clusters with a Dunn's partition coefficient of 0.30 (note that a coefficient of 1 correspond to hard clusters). Membership coefficients were ranging from 0.27 to 0.33, 0.32 to 0.64, 0.30 to 0.33 and 0.37 to 0.40 for cluster 1, 2, 3 and 4 respectively (Figure 15). Interestingly, for each cultivar, observations corresponding to the 96 hpi time of a given condition of inoculation was most significantly associated with the cluster related to the opposite condition of inoculation (black trajectories). Moreover, within a cultivar, 'Mock' control condition was related to the cluster constituted of pineapple infected by *F. ananatum* at the kinetic times 24, 48 and 72 hpi. In fact, cluster 3 was composed of 'QV' samples corresponding to *F. ananatum* condition from 24 to 72 hpi with addition of 'Mock' and H₂O control condition at 96 hpi. Similarly, cluster 1 was represented by 'MD-2' observations related to 'Mock' and *F. ananatum*-inoculation at 24, 48 and 72 hpi. These data evidenced differential basal gene expression among cultivars. In fact, according to normalization by reference gene,

constitutive transcripts accumulation of *NPR1*, *NPR3*, *PR-3* and *PR-12* was significantly lower for the susceptible cultivar than for the resistant (Figure S1).

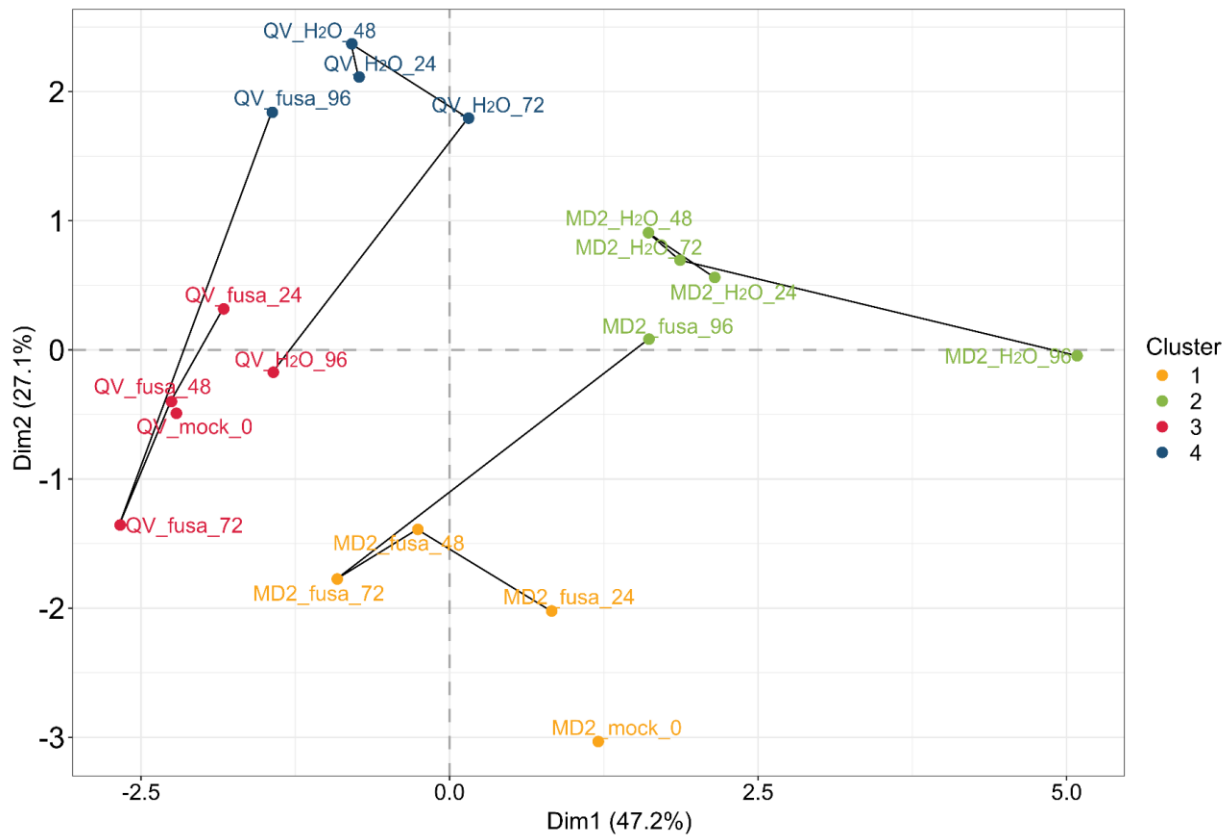


Figure 15. Fuzzy clustering based on principal component analysis (PCA). Conditions are shown as the combination of pineapple fruits of cultivar ‘MD-2’ and ‘Queen Victoria’ (QV) considering no inoculation (Mock), sterile water inoculation (H₂O) or *F. ananatum* inoculation (fusa) and sampled at four kinetic times (24, 48, 72 and 96 hours post-inoculation). Black trajectories illustrate the time line for each condition of inoculation.

Cluster 4 was supported by observations of ‘QV’ cultivar inoculated with H₂O from 24 to 72 hpi and by observations corresponding to *F. ananatum* inoculation at 96 hpi. This pattern was also evidenced for modalities related to ‘MD-2’ pineapple with H₂O inoculation from 24 hpi to 96 hpi and *F. ananatum* inoculation at 96 hpi which together belong to cluster 2. The cluster 2 constituted the unique cluster for which the kinetic point 96 hpi of H₂O inoculation did not belong to the cluster of the opposite condition inoculation (*F. ananatum*).

Following the determination of modalities repartition on the first factorial plane and the definition of the related clusters, the effects of cultivar, condition of inoculation and kinetics were evaluated with a deviance test. In accordance with the results obtained from PCA, the cultivar constituted the most influential factor on transcript levels of the target genes.

After normalization with the 'Mock' condition, the $\Delta\Delta Ct$ of six of the eight genes of interest showed significant difference in relative expression according to the genotype of the studied cultivars (Figure 16). For the SA pathway, *NPR1* was repressed to a lesser extent in 'QV' when compared to 'MD-2' ($p = 0,001$). *NPR3* was defined by a slight induction measured at the highest level in 'QV' by opposition to 'MD-2' ($p = 0.04$). Moreover, profiles of *PR-2* evidenced an induction of its expression in 'MD-2' by contrast to the repressed transcripts levels in 'QV' ($p < 0,001$).

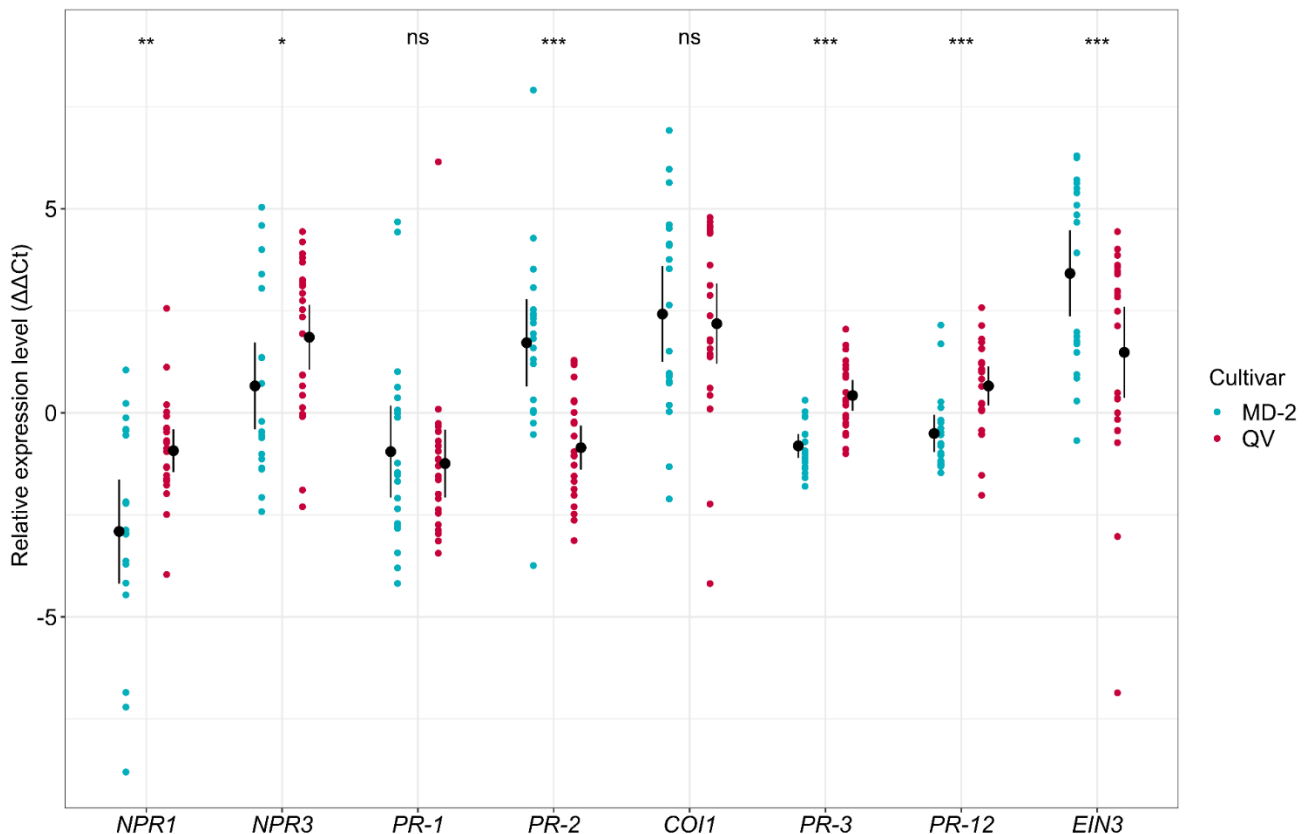


Figure 16. Relative expression of eight defense genes in two pineapple fruit cultivars 'MD-2' and 'Queen Victoria' (QV) characterized by a contrasted susceptibility to FCR in response to *Fusarium ananatum* and sterile water inoculations. Black dots with bars represent average relative expression levels ($\Delta\Delta Ct$) with asymptotic confidence intervals at 95% ($n = 24$). Differences in expression levels between 'MD-2' and 'Queen Victoria' cultivars were either significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) for each gene of interest. *NPR1*, Non-Expressor of Pathogenesis Related gene 1; *NPR3*, Non-Expressor of Pathogenesis Related gene 3; *PR-1*, Pathogenesis-Related gene 1; *PR-2*, Pathogenesis-Related gene 2; *COI1*, Coronatine-insensitive 1; *PR-3*, Pathogenesis-Related gene 3; *PR-12*, Pathogenesis-Related gene 12; *EIN3*, Ethylene Insensitive 3.

PR-1 was characterized by a slight repression of the corresponding transcripts according to the normalization with 'Mock' condition. The comparison of relative expression among cultivar did not evidence any significant difference. Representing one of the first genetic factors activated consecutively to JA accumulation, the expression of *COI1* gene was induced in both 'MD-2' and 'QV' cultivars with no significant variation according to the genotypes. By opposition, *PR-3* described in many studies to be activated by both SA, JA and ET pathways, showed slight repression of its transcripts in 'MD-2' when compared to the low induction in 'QV' ($p < 0,001$). Following ET accumulation, the relative expression of *EIN3* gene was induced in both cultivars considering the initial expression patterns in 'Mock' condition. Moreover, the expression levels were higher in 'MD-2' than in 'QV' at $p < 0,001$. Downstream of JA and ET signaling pathways, *PR-12* transcripts were accumulated at lower levels in 'MD-2' due to a slight repression by contrast to the level quantified in 'QV' ($p < 0,001$).

3.3. Shift of pineapple gene expression between 72- and 96-hours post-inoculation

Relative gene expressions were also significantly affected by the condition of inoculation in interaction with the kinetic times. Among the selected factors, the SA receptors *NPR1* and *NPR3* both showed the highest expression levels in samples corresponding to 'MD-2' pineapple inoculated with H₂O and at the 96 hpi kinetic time (Figure 17 A, C ; Figure S2 A, C). By contrast, their lowest relative expression was observed for 'MD-2' fruits infected by *F. ananatum* at 72 hours post-inoculation. In response to the downstream activation of SA signaling, gene coding for *PR-1* presents the elevated and the lowest expression for modalities corresponding to 'MD-2' inoculated with H₂O after 96 and 72 hpi respectively (Figure 17E, Figure S2 E). Also, *PR-2* coding factor exposed the highest relative expression for 'MD-2' pineapple inoculated with H₂O at 72 hpi while the lowest value was quantified for 'QV' pineapple infected by *F. ananatum* at 48 hpi (Figure 17 G, H ; Figure S2 G, H).

Focusing on the effect of inoculation on transcript levels at each kinetic time, no significant difference was noticed for *NPR1* (Figure 17 A, B ; Figure S2 A, B). A significant downregulation of *NPR3* receptor was highlighted in 'MD-2' pineapple infected with *F. ananatum* by opposition to samples inoculated with H₂O at 24 ($p = 0.02$) and 72 hpi ($p = 0.04$) (Figure 17C, Figure S2 C). Interestingly, a similar pattern was noticed in 'QV' for 24 ($p = 0.004$), 48 ($p = 0.005$) and 72 hpi ($p = 0.04$) with a fall of *NPR3* expression in *F. ananatum* condition by

contrast to H₂O-inoculated (Figure 17D, Figure S2 D). Additionally, the monitoring of *NPR3* evidenced a significantly up-regulated expression for 'QV' in response to *F. ananatum* inoculation between 24, 48, 72 hpi and the final kinetic time 96 hpi ($p = 0.02$, $p = 0.03$ and $p = 0.007$ respectively).

Considering the downstream SA activation of *PR-1*, contrasts in expression levels were noticed at 96 hpi for 'MD-2' pineapple with a significant decrease in *F. ananatum*-inoculated fruits by opposition to H₂O condition ($p < 0.001$, Figure 17E, Figure S2 E). Surprisingly, the comparison of 'QV' profiles evidenced a significant rise of *PR-1* transcript levels in *F. ananatum*-inoculated pineapple compared to H₂O samples for the kinetic time 24 hpi ($p = 0.002$), 48 ($p = 0.02$) and 72 hpi ($p = 0.003$, Figure 17F, Figure S2 F). Along the kinetic assays of each condition of inoculation, changes in *PR-1* transcript levels were measured. Consequently to *F. ananatum* infection of 'MD-2' pineapple, a significant drop of expression was noticed between 48 and 96 hpi ($p = 0.04$). By contrast, for this cultivar, H₂O-inoculated pineapple showed a significant increase of expression from 72 hpi to 96 hpi ($p = 0.02$). *F. ananatum* infection also promoted transcriptional fluctuations in 'QV' between the kinetic points 24, 48, 72 hpi and the later stage 96 hpi characterized by a significant decrease of *PR-1* expression ($p < 0.001$, $p = 0.007$ and $p < 0.001$ respectively). The study of *PR-2* gene coding for β -1,3-glucanase lead to the definition of similar expression profiles for both 'MD-2' and 'QV' cultivar with significantly lowest expression in the *F. ananatum*-inoculated samples than in the H₂O for the studied kinetic stage 48 hpi (at $p = 0.04$ for both cultivars ; Figure 17 G, H ; Figure S2 G, H). Considering the dynamic expression of *PR-2* in 'MD-2' pineapple, no significant variation of transcripts levels was noticed. However, *F. ananatum* inoculation effect on 'QV' cultivar was defined by a significant decrease of *PR-2* relative expression at 48 hpi by opposition to the kinetic stages 24 and 96 hpi ($p = 0.02$ and $p = 0.05$ respectively). Moreover, H₂O inoculation lead to a significantly higher relative expression of *PR-2* at 24 hpi by contrast to levels defined at 48 and 96 hpi ($p = 0.03$ and $p = 0.03$ respectively).

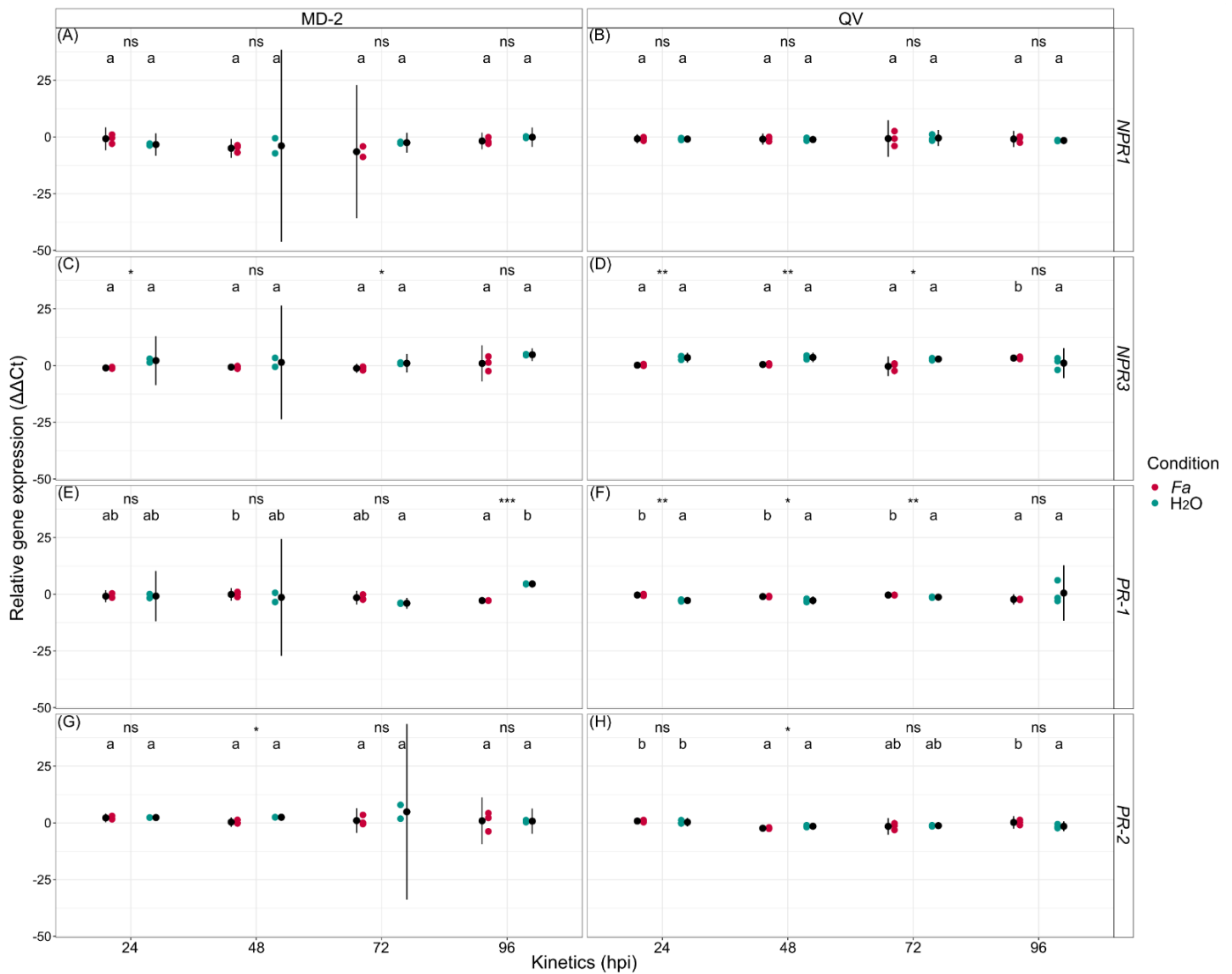


Figure 17. Relative expression dynamics of four defense genes involved in salicylic acid signaling pathway during the early stages of pineapple fruit responses to *Fusarium ananatum* (*Fa*) or sterile water (H_2O) inoculation. Black dots with bars show mean relative expression level ($\Delta\Delta Ct$) with asymptotic confidence intervals at 95% ($n = 3$). Differences in expression following inoculation with *F. ananatum* or sterile water were either significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) for each target gene and each kinetic time. Letters show significant differences in average expression levels between the kinetic points for each condition of inoculation and each cultivar according to Tukey's multiple comparison test at $p < 0.05$. (A) and (B) *NPR1*, Non-Expressor of Pathogenesis Related gene 1; (C) and (D) *NPR3*, Non-Expressor of Pathogenesis Related gene 3; (E) and (F) *PR-1*, Pathogenesis-Related gene 1; (G) and (H) *PR-2*, Pathogenesis-Related gene 2; QV, 'Queen Victoria'.

The phytohormone JA contributed to the activation of defense responses in plant. Trying to evaluate the role of JA pathway in the susceptibility of 'QV' to FCR, relative expression profiles of *COI1*, *PR-3* and *PR-12* factors were studied. The combination of 'QV' pineapple inoculated

with H₂O after 48 hpi and infected by *F. ananatum* at 72 hpi constituted the modalities with the highest and the lowest *COI1* transcript levels respectively (Figure 18B, Figure S3 B).

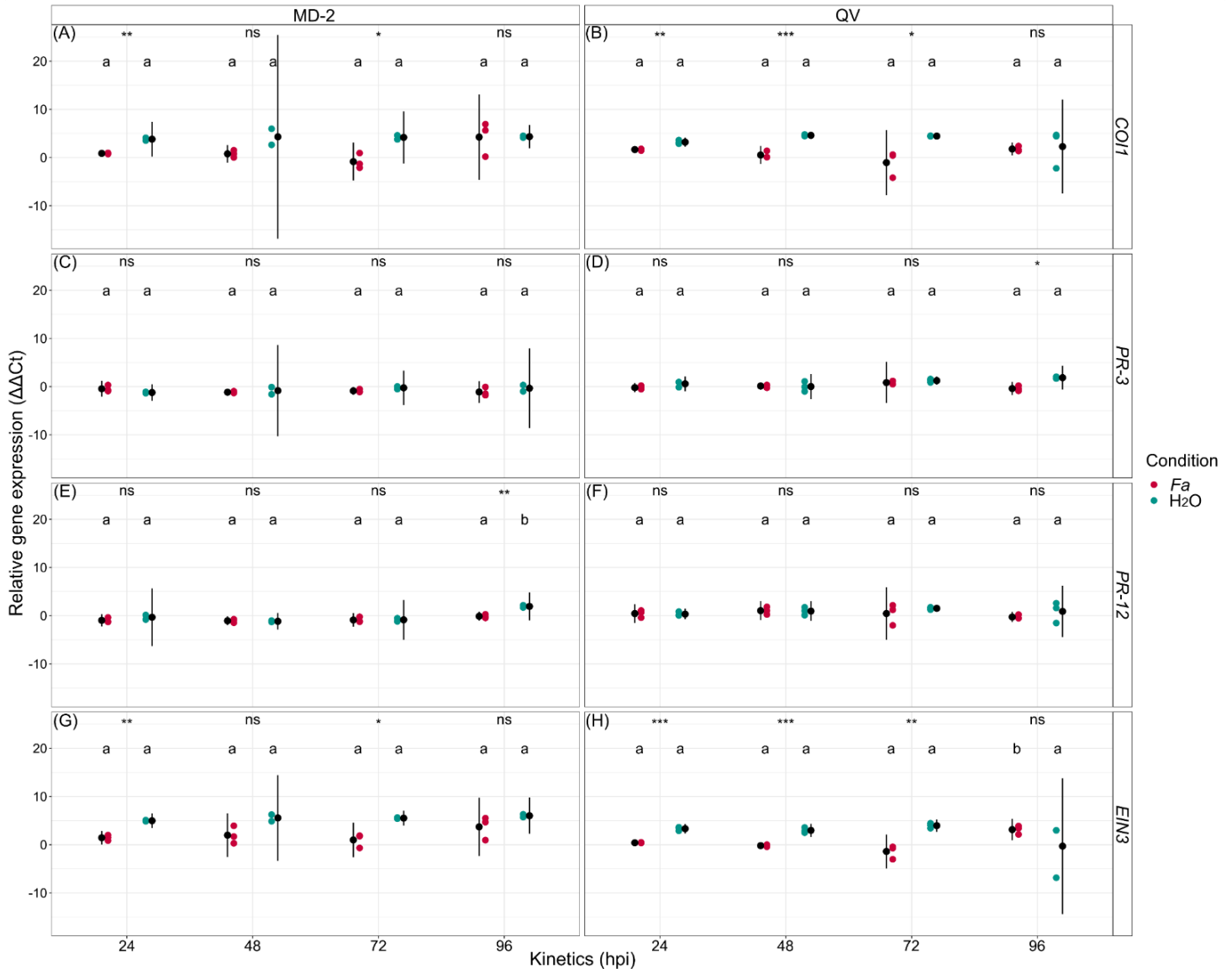


Figure 18. Relative expression dynamics of four defense genes involved in jasmonic acid and ethylene signaling pathways during the early stages of pineapple fruit responses to *Fusarium ananatum* (*Fa*) or sterile water (H₂O) inoculation. Black dots with bars show mean relative expression level ($\Delta\Delta\text{Ct}$) with asymptotic confidence intervals at 95% ($n = 3$). Differences in expression following inoculation with *F. ananatum* or sterile water were either significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) for each target gene and each kinetic time. Letters show significant differences in average expression levels between the kinetic points for each condition of inoculation and each cultivar according to Tukey's multiple comparison test at $p < 0.05$. (A) and (B) *COI1*, *Coronatine-insensitive 1*; (C) and (D) *PR-3*, *Pathogenesis-Related gene 3*; (E) and (F) *PR-12*, *Pathogenesis-Related gene 12*; (G) and (H) *EIN3*, *Ethylene Insensitive 3*; QV, 'Queen Victoria'.

Additionally, the highest and the lowest *PR-3* transcripts levels were respectively supported by 'QV' pineapple at 96 hpi and 'MD-2' pineapple at 24 hpi both inoculated with H₂O (Figure 18 C, D ; Figure S3 C, D). Focusing on 'MD-2' cultivar, a significant downregulation of *COI1* relative expression was determined for *F. ananatum*-inoculated pineapple by contrast to H₂O condition at the kinetic stages 24 ($p = 0.001$) and 72 hpi ($p = 0.03$; Figure 18A ; Figure S3 A). Similar patterns demonstrating the downregulation of *COI1* transcripts were evidenced in 'QV' pineapple inoculated with *F. ananatum* for the kinetic stages 24 ($p = 0.003$), 48 ($p < 0.001$) and 72 hpi ($p = 0.03$; Figure 18B ; Figure S3 B). The JA downstream activated gene *PR-3* also showed variations in its expression according to *F. ananatum* infection condition. By opposition to H₂O-inoculated pineapple, significant repression of *PR-3* was observed at 96 hpi ($p = 0.01$) for cultivar 'QV' (Figure 18D, Figure S3 D). Consequently, the monitoring of *COI1* and *PR-3* relative expression dynamics was explored for each cultivar and considering each condition of inoculation. Statistical analyses showed no significant change between the four kinetic times.

In order to define the involvement of ET in pineapple response to FCR infection, *EIN3* and *PR-12* genes were studied. Results obtained from quantitative real-time PCR showed that 'MD-2' pineapple inoculated with H₂O after 96 hpi and 'QV' pineapple infected with *F. ananatum* and sampled at 72 hpi supported the highest and the lowest relative expression of *EIN3* respectively (Figure 18 G, H ; Figure S3 G, H). Searching for modulation of gene expression, values obtained for each condition of inoculation were compared while considering independently each kinetic time. This approach evidenced a significant repression of *EIN3* relative expression at 24 ($p = 0.004$) and 72 hpi ($p = 0.03$) for 'MD-2' pineapple infected by *F. ananatum* by contrast to the H₂O control condition (Figure 18G, Figure S3 G). This decrease pattern of *EIN3* was also noticed in *Fusarium*-infected 'QV' pineapple for the kinetic stages 24 ($p < 0.001$), 48 ($p < 0.001$) and 72 hpi ($p = 0.004$; Figure 18H ; Figure S3 H). Thus, the dynamic evolution of gene expression was studied considering separately each condition of inoculation. In 'QV' pineapple infected with *F. ananatum*, variations of *EIN3* expression levels were observed during the studied kinetics. In fact, a significant rise in *EIN3* transcripts levels was measured at 96 hpi by contrast to the kinetic stages 24 ($p = 0.02$), 48 ($p = 0.006$) and 72 hpi ($p < 0.001$). Expression profiles established for *PR-12* gene demonstrate that H₂O control condition of 'MD-2' pineapple at 96 hpi and 48 hpi constituted the modalities with the highest

and the lowest relative expression levels respectively (Figure 18E, Figure S3 E). Considering the role of fungal contamination on signal transduction, results evidenced a significantly lower transcript level of *PR-12* in 'MD-2' pineapple infected by *F. ananatum* by comparison to H₂O samples for the kinetic time 96 hpi ($p = 0.008$; Figure 18E ; Figure S3 E). Additionally, according to each condition of inoculation, kinetics highlighted a significant increase of *PR-12* transcripts in H₂O control condition of 'MD-2' pineapple at 96 hpi by contrast to the levels defined at 24 ($p = 0.02$), 48 ($p = 0.008$) and 72 hpi ($p = 0.01$) for the same condition. This pattern was not observed neither for *F. ananatum* nor for H₂O condition in 'QV' pineapple (Figure 18F, Figure S3 F).

4. Discussion

The present study focused on host response related to infection by *F. ananatum* previously described as the leading FCR pathogen in the production areas such as South-Africa, Mauritius and China (Jacobs *et al.*, 2010 ; Barral, 2017 ; Gu *et al.*, 2015). Considering that fungal chemical communication modulates the virulence, this study proposed to explore both SA, JA and ET signaling pathways with the assumption that high biomass of the main FCR causal agent *F. ananatum* may influenced the susceptibility of 'QV' cultivar to FCR.

4.1. Contrasting constitutive expression levels between pineapple cultivars

The data obtained showed significant differences in expression of SA, JA and ET genetic markers related to both cultivar and inoculation effects, which were defined as the key factors influencing host defense responses. First, basal expression levels of the studied genes were contrasted among cultivar as measured with the genetic profiles of 'Mock' condition. In fact, *NPR1*, *NPR3*, *PR-3* and *PR-12* were constitutively express to higher levels in 'MD-2' when compared to 'QV' cultivar. The expression of numerous defense-related genes is induced through the chitin signaling and relied on fungal cell wall degradation by the chitinases promoting detection by the chitin elicitor binding protein (CEBiP) and then signal transduction by this LysM receptor-like-protein (LysM-RLP) (Hayafune *et al.*, 2014 ; Liang *et al.*, 2018). Notably, *PR* genes coding for chitinases showed constitutive expression in flax and were suggested to be up-regulated during the earliest detection of the fungal pathogen *Fusarium oxysporum* f. sp. *lini* (Galindo-Gonzalez and Deyholos, 2016). Additionally, SA secretion

induces cellular redox change leading to *NPR1* conformation changes from oligomers to monomers which can join the nucleus (Backer *et al.*, 2019). Thereby, the low accumulation of *NPR1* in the nucleus leads to a weak induction of *PR* genes such as *PR-1* and *PR-2*. For example, *Arabidopsis thaliana npr1* mutants revealed an incapacity to bind SA molecules resulting in low defense responses potentially counterbalanced by other SA receptors (Canet *et al.*, 2010; Castello *et al.*, 2018). In 'QV' cultivar, both *NPR1* and *NPR3* receptors were constitutively expressed at low levels thus suggesting a defective transduction of SA signal. Within pineapple cultivars, these contrasted levels of constitutive gene expression were previously demonstrated on fruits harvested at C1 stage and established that *PAL* gene coding for phenylalanine ammonia-lyase was expressed at higher levels in 'QV' compared to 'MD-2' cultivar (Léchaudel *et al.*, 2018). Considering the role of *PAL* in the shikimate pathway leading to SA biosynthesis, one can hypothesize an insufficient SA binding by *NPR1* and *NPR3* explaining the low constitutive expression of *PR-1*. Moreover, transcript expression profiles defined according to normalization with reference gene demonstrated that expression levels of *COI1/EIN3* and *PR-1/PR-3* were inversely correlated in both cultivars. However, accumulation of *PR-3* transcripts is induced through JA and ET pathways which priming is triggered by *COI1* and *EIN3* respectively (Sheard *et al.*, 2010 ; Yang *et al.*, 2017). Pineapple of 'MD-2' cultivar constitutively presented a balance in *COI1*, *EIN3* and *PR-3* expression which is not observed in 'QV' presenting high transcript levels of *COI1* and *EIN3* and low accumulation of *PR-3*. For the FCR-resistant 'MD-2' cultivar, this pattern suggested a control of transcriptional activities in order to support sufficient basal expression of genes encoding for chitinase I and antifungal proteins. Similarly, it was evidenced that basal expression of *NPR1* and *NPR3* was relatively elevated in 'MD-2'. This is consistent with the constitutive expression of *PR-1* in 'MD-2' which was quantified at slightly higher levels than for 'QV' (Mock) cultivar. Similar profiles were depicted for *PR-12* coding for the salicylic-independent plant defensin 1.2 (PDF1.2) which showed a higher basal expression level in 'MD-2' than in 'QV' cultivar. Thus, the balanced expression of *COI1* and *EIN3* in the FCR-resistant cultivar ('MD-2') may be inherent to multiple loops of negative feedback as demonstrated for the modulation of JA and ET signals (Howe *et al.*, 2018; Azhar *et al.*, 2020). Taking together, these elements evidenced that genes coding for both *NPR1*, *NPR3*, *PR-3* and *PR-12* were constitutively more expressed in 'MD-2' than in 'QV' cultivar and could thus constitute key factors for basal resistance of pineapple to FCR.

4.2. *Fusarium ananatum* repressed host defenses until 72 hours post-inoculation

Deciphering the dynamics of pineapple defense gene expression demonstrated specific response patterns related to the infection by *F. ananatum*. For both 'MD-2' and 'QV' cultivars, inoculation with *F. ananatum* did not promote changes in *NPR3*, *PR-1*, *PR-3* and *PR-12* expression when compared to constitutive levels up to 72 hpi. By contrast, the profiling of H₂O condition in 'MD-2' and 'QV' cultivars showed that *NPR3*, *COI1* and *EIN3* were up-regulated during the first 72 hours following inoculation according to their respective basal expression levels. This is consistent with previous studies demonstrating that ET and JA pathways promote plant regeneration after mechanical injuries or wounding (Li *et al.*, 2018, Zhang *et al.*, 2019). Additionally, *Arabidopsis COI1* knock-out mutants revealed a complete deficiency in JA perception leading to high susceptibility condition against *Botrytis cinerea* (Xie *et al.*, 1998; He *et al.*, 2012; An *et al.*, 2018). Similar up-regulation was evidenced for *PR-2* encoding gene in 'MD-2' at 48 hpi consecutively to sterile water injection. This shows that both cultivars responded to the wounding of the apical zone (where bract and sepals fused) which is necessary for the injection procedure (Barral *et al.*, 2017). Considering *F. ananatum*-inoculated pineapples, only three of the eight studied genes were up-regulated in response to infection: *COI1* in both 'MD-2' and 'QV' samples and *PR-2/EIN3* in 'MD-2'. These results showed effective ET signal transduction in 'MD-2' cultivar consequently to infection by the pathogen. In fact, without the binding with ET, *EIN3* remains targeted by the complex SCF^{EBF1/2} ubiquitin ligase (*EIN3*-Binding-Fbox 1 and 2) and targeted by the proteasome (He *et al.*, 2017). Völz and his collaborators demonstrated the ET-hyposensitivity of *Arabidopsis ein3* mutants during infection by *Cochliobolus miyabeanus* resulted in higher levels of susceptibility (Völz *et al.*, 2020). Despite being more expressed from 24 to 72 hpi in 'QV' samples following H₂O injection, levels of *NPR1*, *NPR3*, *PR-3* and *PR-12* were related to those obtained with *F. ananatum* inoculation in 'MD-2' resistant cultivar (at similar time points) and were thus significantly lower than their respective expression levels in 'MD-2' samples of H₂O control condition (according to reference gene normalization). Interestingly, defense-related genes such as *PR* genes were shown to be the main drivers determining the balance between tolerance and susceptibility of grapevine cultivars to infection by the fungal pathogen *Eutypa lata* (Cardot *et al.*, 2019). In addition to its high susceptibility to FCR, 'QV' cultivar also appear

to manage the wounding to a lesser extent than 'MD-2' potentially due to the low basal expression of both *NPR1* and *NPR3*. As an example, *NPR1* is required for the priming of *Arabidopsis thaliana* responses to pathogens and wounding (Kohler *et al.*, 2002). Proteasome-mediated protein degradation increased the turnover of the transcription initiation complex. Thus, an accumulation of *NPR1* in nucleus led to basal defense gene expression and resistance, while its recycling is necessary for SAR establishment (Fu *et al.*, 2012).

Interestingly, the comparison of transcript levels according to conditions of inoculation from 24 to 72 hpi showed that *NPR3*, *COI1* and *EIN3* were significantly less expressed during *F. ananatum* infection when compared to H₂O condition in both cultivars. Thus, inoculation of *F. ananatum* did not induce the same responses to wounding than those observed following injection of H₂O. Similarly, *F. ananatum* infection also promoted a downregulated expression of *PR-2* in both susceptible and resistant cultivars at time point 48 hpi when compared to its expression in H₂O control condition. These data suggested the capacity of the FCR-pathogen to maintain genes expression at their basal levels through to the concomitant bypass of SA, JA and ET signaling pathways activated by both wounding (injection) and detection of PAMPs. As an example, host-defenses manipulation has been evidenced during the interaction of tomato with the necrotrophic fungal pathogen *Botrytis cinerea* that takes advantage of the antagonism between SA and JA pathways. To this end, *B. cinerea* secretes an exopolysaccharide eliciting the SA pathway thus allowing the colonization of senescent or dead host tissues and the establishment of the gray mold (El Oirdi *et al.*, 2011). Such strategy could explain that no change in expression levels was evidenced for these genes during *F. ananatum* infection contrarily to the levels quantified upon H₂O control samples. Thereby, host responses only depended on the constitutive expression of the defense-related genes. Signaling of defense pathways could be considered as sufficient for challenging the pathogen and lead to host resistance in 'MD-2'. On the contrary, the expression of basal defenses is relatively low in 'QV' cultivar explaining therefore its susceptibility and establishment of FCR.

4.3. Shift of pineapple responses to *F. ananatum* and wounding between 72 and 96 hpi

The data partitioning led to the definition of four clusters that were mainly supported by the cultivar ('MD-2' or 'QV) and the condition of inoculation (*F. ananatum* or H₂O). According to the expression of the defense-related genes, the clustering demonstrated that within a cultivar, transcript profiles related to the time point 96 hpi were significantly opposed to the patterns measured from 24 to 72 hpi for a same condition of inoculation. The dynamic approach suggested an important shift in expression between 72 and 96 hpi for the defense-promoting genes of both SA, JA and ET pathways as evidenced with *NPR3*, *PR-2* and *EIN3* kinetic profiles in the susceptible cultivar. Exploration of gene expression during the earliest stages of a plant-pathogen interaction enabled in some pathosystems to highlight host transcriptional reprogramming associated with infection and disease establishment. For example, the transcriptomic analyses of tomato in response to *Phytophthora capsici* infection suggested a dramatic shift in expression during the first 8 hours post-inoculation characterized by either up-regulation and downregulation of 3,720 genes (Jupe *et al.*, 2013). In pineapple, this tendency was observed on the overall experimental conditions (except for 'MD2_H2O_96hpi') and could be explained according to two differential host response patterns. First, as previously mentioned, controlled inoculation in pineapple required injection with a needle leading to the wounding of the fruit. The results demonstrated that both cultivars managed the wounding at different levels by signal induction of SA, JA and ET pathways from 24 to 72 hpi. The decrease of host responses to wounding after 72 hpi may depict the establishment of defense mechanisms and the potential initiation of plant tissues regeneration. Notably, *PINPPO1* (*Polyphenol Oxidase 1*) and *PINPPO2* (*Polyphenol Oxidase 2*) were found to be induced in the pineapple cultivar 'Smooth Cayenne' in response to mechanical injuries and during the transduction of methyl jasmonate signal hypothesizing their role in host response defenses (Stewart *et al.*, 2001). However, Léchaudel *et al.* showed that *PINPPO1* and *PINPPO2* genes were constitutively less expressed in 'QV' when compared to 'MD-2' or 'RL41' cultivars at both C1 and full ripening maturity stages (Léchaudel *et al.*, 2018). Second, the present study revealed that the infection by *F. ananatum* restrain the setup of host defense signal pathways during the first 72 hours of interaction. Indeed, it was observed in both 'MD-2' and 'QV' cultivars that consecutively to *F. ananatum* infection,

expression levels of defense-related genes were lower or similar to the basal levels. This demonstrates that *F. ananatum* could be able to modulate the host response during the early stages of the host-pathogen interaction. Particularly, it was shown that during precocious stages of cotton root infection by the pathogen *Fusarium oxysporum* f. sp. *vasinfectum*, defense-related genes were repressed leading to few changes according to basal expression levels (Dowd *et al.*, 2004). In pineapple, after 72 hpi, the cellular metabolism of *F. ananatum* could be sufficiently adjusted enabling the lift of repression performed on host defense-related genes. Fabre *et al.* have evidenced significant regulations in abundance of *Fusarium graminearum* proteins involved in fungal development between the 48 and 72 first hours of the *Fusarium* head blight establishment in wheat. They also hypothesized that the regulated *Fusarium* proteins may condition the evolution of this host-pathogen interaction (Fabre *et al.*, 2019). Such strategy could explain that the expression of genes involved in SA, JA and ET pathways reach expression levels similar to inoculation with H₂O only after 96 hpi. From this stage, the metabolism of *F. ananatum* might be able to challenge the newly expressed host defenses and *de facto* to promote host susceptibility. Thus, it is suggested that 'QV' susceptibility to FCR mainly relies on the low basal expression of defense-related genes. The repression of signal transduction induced by *F. ananatum* is the second key factor of FCR establishment in 'QV' cultivar. In fact, even if SA, JA and ET pathways were inhibited by the pathogen, their constitutive expression lead to a higher basal defense level in 'MD-2' which appeared to counterbalance the colonization of host tissues and the development of black spots.

5. Conclusion

The present study provided new understanding on the genetic factors that contributed to the susceptibility of the pineapple cultivar 'Queen Victoria' to FCR. We evidenced that constitutive expression of defense-related genes involved in SA, JA and ET was defective in 'Queen Victoria' cultivar compared to the resistant cultivar of reference 'MD-2'. Infection by *Fusarium ananatum* caused strong transcriptional reprogramming in host responses through repression of both SA, JA and ET receptors and *PR* genes. A major transcriptional shift was observed between the 72 and 96 first hours of the interaction suggesting a modulation of pineapple defenses by the pathogen. Considering the diversity and the concomitance of FCR-pathogens, fungal associations may result in complex strategies for the bypass of pineapple

immunity. Determining the influence of co-occurring pathogens on fruit transcriptome would help to accurately defined susceptibility factors in pineapple. Future transcriptomic prospects on both host and pathogen(s) in interaction may also be useful for the management of FCR epidemics.

Funding

Funding for this research, including M.V.'s PhD grant, was provided by the European Regional Development Fund (FEDER Réunion, PO-COSAQ).

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Conceptualization, M.V. and J.-C.M.; methodology, M.V. and J.-C.M.; formal analysis, M.V. and F.C.; investigation, M.V. and C.S.; data curation, M.V.; writing—original draft preparation, M.V.; writing—review and editing, M.V., F.C., C.S., S.S.-G., M.C. and J.-C.M.; visualization, M.V. and F.C.; supervision, J.-C.M. All authors have read and agreed to the published version of the manuscript.

6. References

- An, L., Ahmad, R. M., Ren, H., Qin, J., and Yan, Y. (2019). Jasmonate signal receptor gene family ZmCOIs restore male fertility and defense response of *Arabidopsis* mutant coi1-1. *J Plant Growth Regul* 38, 479–493. doi:10.1007/s00344-018-9863-2.
- Andersen, C. L., Jensen, J. L., and Ørntoft, T. F. (2004). Normalization of Real-Time Quantitative Reverse Transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res* 64, 5245–5250. doi:10.1158/0008-5472.CAN-04-0496.
- Avallone, S., Guiraud, J.-P., Brillouet, J.-M., and Teisson, C. (2003). Enzymatic browning and biochemical alterations in black spots of pineapple [*Ananas comosus* (L.) Merr.]. *Current Microbiology* 47, 113–118. doi:10.1007/s00284-002-3958-4.
- Azhar, B. J., Zulfiqar, A., Shakeel, S. N., and Schaller, G. E. (2020). Amplification and adaptation in the ethylene signaling pathway. *Small Methods* 4, 1900452. doi:10.1002/smt.201900452.

- Backer, R., Naidoo, S., and van den Berg, N. (2019). The NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1) and related family: Mechanistic insights in plant disease resistance. *Front. Plant Sci.* 10, 102. doi:10.3389/fpls.2019.00102.
- Barral, B. (2017). Maladie des taches noires de l'ananas: Étude des relations hôte-pathogène et compréhension des mécanismes physiologiques de résistance. Available at: <http://www.theses.fr/2017MONTT116> [Accessed January 20, 2018].
- Barral, B., Chillet, M., Léchaudel, M., Lugan, R., and Schorr-Galindo, S. (2019a). Coumaroyl-isocitric and caffeoyl-isocitric acids as markers of pineapple fruitlet core rot disease. *Fruits* 74, 11–17. doi:10.17660/th2019/74.1.2.
- Barral, B., Chillet, M., Léchaudel, M., Lartaud, M., Verdeil, J.-L., Conéjéro, G., et al. (2019b). An imaging approach to identify mechanisms of resistance to pineapple Fruitlet Core Rot. *Front. Plant Sci.* 10, 1065. doi:10.3389/fpls.2019.01065.
- Barral, B., Chillet, M., Minier, J., Léchaudel, M., and Schorr-Galindo, S. (2017). Evaluating the response to *Fusarium ananatum* inoculation and antifungal activity of phenolic acids in pineapple. *Fungal Biology* 121, 1045–1053. doi:10.1016/j.funbio.2017.09.002.
- Breen, S., Williams, S. J., Outram, M., Kobe, B., and Solomon, P. S. (2017). Emerging insights into the functions of Pathogenesis-Related protein 1. *Trends in Plant Science* 22, 871–879. doi:10.1016/j.tplants.2017.06.013.
- Browse, J. (2009). Jasmonate passes muster: A receptor and targets for the defense hormone. *Annu. Rev. Plant Biol.* 60, 183–205. doi:10.1146/annurev.arplant.043008.092007.
- Canet, J. V., Dobón, A., Roig, A., and Tornero, P. (2010). Structure-function analysis of npr1 alleles in *Arabidopsis* reveals a role for its paralogs in the perception of salicylic acid: NPR1 alleles and NPR1 paralogs. *Plant, Cell & Environment* 33, 1911–1922. doi:10.1111/j.1365-3040.2010.02194.x.
- Cardot, C., Mappa, G., La Camera, S., Gaillard, C., Vriet, C., Lecomte, P., et al. (2019). Comparison of the molecular responses of tolerant, susceptible and highly susceptible grapevine cultivars during interaction with the pathogenic fungus *Eutypa lata*. *Front. Plant Sci.* 10, 991. doi:10.3389/fpls.2019.00991.
- Castelló, M. J., Medina-Puche, L., Lamilla, J., and Tornero, P. (2018). NPR1 paralogs of *Arabidopsis* and their role in salicylic acid perception. *PLoS ONE* 13, e0209835. doi:10.1371/journal.pone.0209835.
- Chen, H., Hu, B., Zhao, L., Shi, D., She, Z., Huang, X., et al. (2019). Differential expression analysis of reference genes in pineapple (*Ananas comosus* L.) during reproductive development and response to abiotic stress, hormonal stimuli. *Tropical Plant Biol.* 12, 67–77. doi:10.1007/s12042-019-09218-2.
- Cui, Y., Mao, R., Chen, J., and Guo, Z. (2019). Regulation mechanism of MYC family transcription factors in jasmonic acid signalling pathway on taxol biosynthesis. *Int. J. Mol. Sci.*, 46.

- de Oliveira Carvalho, A., and Moreira Gomes, V. (2011). Plant defensins and defensin-Like peptides - Biological activities and biotechnological applications. *CPD* 17, 4270–4293. doi:10.2174/138161211798999447.
- Dowd, C., Wilson, I. W., and McFadden, H. (2004). Gene expression profile changes in cotton root and hypocotyl tissues in response to infection with *Fusarium oxysporum* f. sp. *vasinfectum*. *MPMI* 17, 654–667. doi:10.1094/MPMI.2004.17.6.654.
- Dzhavakhiya, V. G., Ozeretskoykaya, O. L., and Zinovyeva, S. V. (2007). “Immune response,” in *Comprehensive and Molecular Phytopathology*, 265–314.
- El Oirdi, M., El Rahman, T. A., Rigano, L., El Hadrami, A., Rodriguez, M. C., Daayf, F., et al. (2011). *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato. *Plant Cell* 23, 2405–2421. doi:10.1105/tpc.111.083394.
- Escudero, V., Jordá, L., Sopena-Torres, S., Mérida, H., Miedes, E., Muñoz-Barrios, A., et al. (2017). Alteration of cell wall xylan acetylation triggers defense responses that counterbalance the immune deficiencies of plants impaired in the β -subunit of the heterotrimeric G-protein. *Plant J* 92, 386–399. doi:10.1111/tpj.13660.
- Fabre, F., Vignassa, M., Urbach, S., Langin, T., and Bonhomme, L. (2019). Time-resolved dissection of the molecular crosstalk driving *Fusarium* head blight in wheat provides new insights into host susceptibility determinism. *Plant Cell Environ* 42, 2291–2308. doi:10.1111/pce.13549.
- Fernández-Calvo, P., Chini, A., Fernández-Barbero, G., Chico, J.-M., Gimenez-Ibanez, S., Geerinck, J., et al. (2011). The *Arabidopsis* bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. *Plant Cell* 23, 701–715. doi:10.1105/tpc.110.080788.
- Fournier, P. (2012). La culture de l’ananas Victoria à la Réunion pour l’exportation : Recueil de bonnes pratiques. CIRAD, Chambre d’Agriculture de la Réunion.
- Fu, Z. Q., Yan, S., Saleh, A., Wang, W., Ruble, J., Oka, N., et al. (2012). NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. *Nature* 486, 228–232. doi:10.1038/nature11162.
- Galindo-González, L., and Deyholos, M. K. (2016). RNA-seq transcriptome response of flax (*Linum usitatissimum* L.) to the pathogenic fungus *Fusarium oxysporum* f. sp. *lini*. *Front. Plant Sci.* 7. doi:10.3389/fpls.2016.01766.
- Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43, 205–227. doi:10.1146/annurev.phyto.43.040204.135923.
- Greenberg, J. T., and Yao, N. (2004). The role and regulation of programmed cell death in plant–pathogen interactions. *Cellular Microbiology*, 11.

- Gu, Zhan, R. L., Zhan, L. B., Gong, D. Q., and Jia, Z. W. (2015). First report of *Fusarium ananatum* causing pineapple fruitlet core rot in China. *Plant Disease* 99, 1653–16. doi:10.1094/PDIS-03-15-0279-PDN.
- Hayafune, M., Berisio, R., Marchetti, R., Silipo, A., Kayama, M., Desaki, Y., et al. Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. *Plant Biology*, 10.
- He, X., Jiang, J., Wang, C.-Q., and Dehesh, K. (2017). ORA59 and EIN3 interaction couples jasmonate-ethylene synergistic action to antagonistic salicylic acid regulation of PDF expression: ORA59 is a coordinator of JA/SA antagonism. *J. Integr. Plant Biol.* 59, 275–287. doi:10.1111/jipb.12524.
- He, Y., Chung, E.-H., Hubert, D. A., Tornero, P., and Dangl, J. L. (2012). Specific missense alleles of the *Arabidopsis* jasmonic acid co-receptor COI1 regulate innate immune receptor accumulation and function. *PLOS Genetics* 8, 12.
- Howe, G. A., Major, I. T., and Koo, A. J. (2018). Modularity in jasmonate signaling for multistress resilience. *Annu. Rev. Plant Biol.* 69, 387–415. doi:10.1146/annurev-arplant-042817-040047.
- Irigoyen, M. L., Garceau, D. C., Bohorquez-Chaux, A., Lopez-Lavalle, L. A. B., Perez-Fons, L., Fraser, P. D., et al. (2020). Genome-wide analyses of cassava pathogenesis-related (PR) gene families reveal core transcriptome responses to whitefly infestation, salicylic acid and jasmonic acid. *BMC Genomics* 21, 93. doi:10.1186/s12864-019-6443-1.
- Jacobs, A., Van Wyk, P. S., Marasas, W. F. O., Wingfield, B. D., Wingfield, M. J., and Coutinho, T. A. (2010). *Fusarium ananatum* sp. nov. in the *Gibberella fujikuroi* species complex from pineapples with fruit rot in South Africa. *Fungal Biology* 114, 515–527. doi:10.1016/j.funbio.2010.03.013.
- Jin, H., Choi, S.-M., Kang, M.-J., Yun, S.-H., Kwon, D.-J., Noh, Y.-S., et al. (2018). Salicylic acid-induced transcriptional reprogramming by the HAC–NPR1–TGA histone acetyltransferase complex in *Arabidopsis*. *Nucleic Acids Research*. doi:10.1093/nar/gky847.
- Jones, J. D. G., and Dangl, J. L. (2006). The plant immune system. *Nature* 444, 323–329. doi:10.1038/nature05286.
- Jones, M. R., Dilai, S., Lingampally, A., Chao, C.-M., Danopoulos, S., Carraro, G., et al. (2019). A comprehensive analysis of fibroblast growth factor receptor 2b signaling on epithelial tip progenitor cells during early mouse lung branching morphogenesis. *Front. Genet.* 9, 746. doi:10.3389/fgene.2018.00746.
- Jupe, J., Stam, R., Howden, A. J., Morris, J. A., Zhang, R., Hedley, P. E., et al. (2013). *Phytophthora capsici*-tomato interaction features dramatic shifts in gene expression associated with a hemi-biotrophic lifestyle. *Genome Biol* 14, R63. doi:10.1186/gb-2013-14-6-r63.

- Kaiser, H. F. (1974). An index of factorial simplicity. *Psychometrika* 39, 31–36. doi:10.1007/BF02291575.
- Kaltdorf, M., and Naseem, M. (2013). How many salicylic acid receptors does a plant cell need? *Science Signaling* 6, jc3–jc3. doi:10.1126/scisignal.2003944.
- Kassambara, A., and Mundt, F. (2020). *factoextra*. Available at: <http://www.sthda.com/english/rpkgs/factoextra> [Accessed February 14, 2021].
- Kawasaki, T., Yamada, K., Yoshimura, S., and Yamaguchi, K. (2017). Chitin receptor-mediated activation of MAP kinases and ROS production in rice and *Arabidopsis*. *Plant Signaling & Behavior* 12, e1361076. doi:10.1080/15592324.2017.1361076.
- Kohler, A., Schwindling, S., and Conrath, U. (2002). Benzothiadiazole-induced priming for potentiated responses to pathogen infection, wounding, and infiltration of water into leaves requires the NPR1/NIM1 gene in *Arabidopsis*. *Plant Physiol.* 128, 1046–1056. doi:10.1104/pp.010744.
- Künstler, A. (2016). Staying alive - is cell death dispensable for plant disease resistance during the hypersensitive response? *Physiological and Molecular Plant Pathology*, 10.
- Lê, S., Josse, J., and Husson, F. FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*, 18.
- Léchaudel, M., Darnaudery, M., Joët, T., Fournier, P., and Joas, J. (2018). Genotypic and environmental effects on the level of ascorbic acid, phenolic compounds and related gene expression during pineapple fruit development and ripening. *Plant Physiology and Biochemistry* 130, 127–138. doi:10.1016/j.plaphy.2018.06.041.
- Leon-Reyes, A., Van der Does, D., De Lange, E. S., Delker, C., Wasternack, C., Van Wees, S. C. M., et al. (2010). Salicylate-mediated suppression of jasmonate-responsive gene expression in *Arabidopsis* is targeted downstream of the jasmonate biosynthesis pathway. *Planta* 232, 1423–1432. doi:10.1007/s00425-010-1265-z.
- Li, N., Han, X., Feng, D., Yuan, D., and Huang, L.-J. (2019). Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: Do we understand what they are whispering? *Int. J. Mol. Sci.*, 15.
- Li, S., Han, X., Yang, L., Deng, X., Wu, H., Zhang, M., et al. (2018). Mitogen-activated protein kinases and calcium-dependent protein kinases are involved in wounding-induced ethylene biosynthesis in *Arabidopsis thaliana*: MAPK and CPK pathways in wounding-induced ethylene production. *Plant Cell Environ* 41, 134–147. doi:10.1111/pce.12984.
- Liang, X., and Zhou, J.-M. (2018). Receptor-like cytoplasmic kinases: Central players in plant receptor kinase-mediated signaling. *Annu. Rev. Plant Biol.* 69, 267–299. doi:10.1146/annurev-arplant-042817-040540.
- Maechler, M. (2021). *cluster*. Available at: <https://svn.r-project.org/R-packages/trunk/cluster/> [Accessed November 20, 2020].

- Maglovski, M., Gregorová, Z., Rybanský, L., Mészáros, P., Moravčíková, J., Hauptvogel, P., et al. (2017). Nutrition supply affects the activity of pathogenesis-related β -1,3-glucanases and chitinases in wheat. *Plant Growth Regul* 81, 443–453. doi:10.1007/s10725-016-0222-7.
- Maier, M. J. (2015). *REdaS*. Available at: <https://cran.r-project.org/web/packages/REdaS/index.html> [Accessed February 22, 2021].
- Major, I. T., Yoshida, Y., Campos, M. L., Kapali, G., Xin, X.-F., Sugimoto, K., et al. (2017). Regulation of growth-defense balance by the JASMONATE ZIM-DOMAIN (JAZ)-MYC transcriptional module. *New Phytol* 215, 1533–1547. doi:10.1111/nph.14638.
- Ming, R., VanBuren, R., Wai, C. M., Tang, H., Schatz, M. C., Bowers, J. E., et al. (2015). The pineapple genome and the evolution of CAM photosynthesis. *Nat Genet* 47, 1435–1442. doi:10.1038/ng.3435.
- Mosblech, A., Thurow, C., Gatz, C., Feussner, I., and Heilmann, I. (2011). Jasmonic acid perception by COI1 involves inositol polyphosphates in *Arabidopsis thaliana*: Perception of JA involves inositol polyphosphates. *The Plant Journal* 65, 949–957. doi:10.1111/j.1365-313X.2011.04480.x.
- Nurnberger, T., and Kemmerling, B. (2008). Pathogen-Associated Molecular Patterns (PAMP) and PAMP-Triggered Immunity. *Molecular Aspects of Plant Disease Resistance*, 32.
- Pandey, D., Rajendran, S. R. C. K., Gaur, M., Sajeesh, P. K., and Kumar, A. (2016). Plant defense signaling and responses against necrotrophic fungal pathogens. *J Plant Growth Regul* 35, 1159–1174. doi:10.1007/s00344-016-9600-7.
- Patin, E. C., Thompson, A., and Orr, S. J. (2019). Pattern recognition receptors in fungal immunity. *Seminars in Cell & Developmental Biology* 89, 24–33. doi:10.1016/j.semcdb.2018.03.003.
- Pfaffl, M. W., Tichopad, A., Prgomet, C., and Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations. *Biotechnology Letters* 26, 509–515. doi:10.1023/B:BILE.0000019559.84305.47.
- Qiao, H., Shen, Z., Huang, S. -s. C., Schmitz, R. J., Urich, M. A., Briggs, S. P., et al. (2012). Processing and subcellular trafficking of ER-Tethered EIN2 control response to ethylene gas. *Science* 338, 390–393. doi:10.1126/science.1225974.
- Ramakers, C., Ruijter, J. M., Deprez, R. H. L., and Moorman, A. F. M. (2003). Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters* 339, 62–66. doi:10.1016/S0304-3940(02)01423-4.
- Ruijter, J. M., Ramakers, C., Hoogaars, W. M. H., Karlen, Y., Bakker, O., van den Hoff, M. J. B., et al. (2009). Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research* 37, e45–e45. doi:10.1093/nar/gkp045.

- Sels, J., Mathys, J., De Coninck, B. M. A., Cammue, B. P. A., and De Bolle, M. F. C. (2008). Plant pathogenesis-related (PR) proteins: A focus on PR peptides. *Plant Physiology and Biochemistry* 46, 941–950. doi:10.1016/j.plaphy.2008.06.011.
- Sheard, L. B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T. R., et al. (2010). Jasmonate perception by inositol-phosphate-potentiated COI1–JAZ co-receptor. *Nature* 468, 400–405. doi:10.1038/nature09430.
- Silver, N., Best, S., Jiang, J., and Thein, S. (2006). Selection of housekeeping genes for gene expression studies in human reticulocytes using real-time PCR. *BMC Mol Biol* 7, 33. doi:10.1186/1471-2199-7-33.
- Stewart, R. J., Sawyer, B. J. B., Bucheli, C. S., and Robinson, S. P. (2001). Polyphenol oxidase is induced by chilling and wounding in pineapple. *Functional Plant Biol.* 28, 181. doi:10.1071/PP00094.
- Stotz, H. U., Thomson, J., and Wang, Y. (2009). Plant defensins: Defense, development and application. *Plant Signaling & Behavior* 4, 1010–1012. doi:10.4161/psb.4.11.9755.
- Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., et al. (2012). Primer3—new capabilities and interfaces. *Nucleic Acids Research* 40, e115–e115. doi:10.1093/nar/gks596.
- van Loon, L. C., Rep, M., and Pieterse, C. M. J. (2006). Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* 44, 135–162. doi:10.1146/annurev.phyto.44.070505.143425.
- van Loon, L. C., and van Strien, E. A. (1999). The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiological and Molecular Plant Pathology* 55, 85–97. doi:10.1006/pmpp.1999.0213.
- Vandesompele, J., Preter, K. D., Roy, N. V., and Paepe, A. D. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology* 3, 12.
- Vignassa, M., Meile, J.-C., Chiroleu, F., Soria, C., Leneveu-Jenvrin, C., Schorr-Galindo, S., et al. (2021). Pineapple mycobiome related to Fruitlet Core Rot occurrence and the influence of fungal species dispersion patterns. *J. Fungi* 7, 175. doi:10.3390/jof7030175.
- Vignassa, M., Soria, C., Durand, N., Poss, C., Meile, J.-C., Chillet, M., et al. (Submitted). Interactions between fungal pathogens of pineapple Fruitlet Core Rot modulate their mycotoxigenic potential. *Toxins*.
- Völz, R., Park, J., Kim, S., Park, S., Harris, W., Chung, H., et al. (2020). The rice/maize pathogen *Cochliobolus* spp. infect and reproduce on *Arabidopsis* revealing differences in defensive phytohormone function between monocots and dicots. *Plant J* 103, 412–429. doi:10.1111/tbj.14743.

- Wan, J., Zhang, X.-C., and Stacey, G. (2008). Chitin signaling and plant disease resistance. *Plant Signaling & Behavior* 3, 831–833. doi:10.4161/psb.3.10.5916.
- Wang, X., Bi, W., Gao, J., Yu, X., Wang, H., and Liu, D. (2018). Systemic acquired resistance, NPR1, and pathogenesis-related genes in wheat and barley. *Journal of Integrative Agriculture* 17, 2468–2477. doi:10.1016/S2095-3119(17)61852-5.
- Xie, D.-X., Feys, B. F., James, S., Nieto-Rostro, M., and Turner, J. G. (1998). COI1: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science* 280, 1091–1094.
- Xie, F., Xiao, P., Chen, D., Xu, L., and Zhang, B. (2012). miRDeepFinder: a miRNA analysis tool for deep sequencing of plant small RNAs. *Plant Mol Biol* 80, 75–84. doi:10.1007/s11103-012-9885-2.
- Yang, C., Li, W., Cao, J., Meng, F., Yu, Y., Huang, J., et al. (2017). Activation of ethylene signaling pathways enhances disease resistance by regulating ROS and phytoalexin production in rice. *Plant J* 89, 338–353. doi:10.1111/tpj.13388.
- Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., and Madden, T. L. (2012). Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 13, 134. doi:10.1186/1471-2105-13-134.
- Zhang, G., Zhao, F., Chen, L., Pan, Y., Sun, L., Bao, N., et al. (2019). Jasmonate-mediated wound signalling promotes plant regeneration. *Nat. Plants* 5, 491–497. doi:10.1038/s41477-019-0408-x.
- Zhang, J., Liu, J., and Ming, R. (2014). Genomic analyses of the CAM plant pineapple. *Journal of Experimental Botany* 65, 3395–3404. doi:10.1093/jxb/eru101.

Supplementary data – Article 3

Table S1. Mean qPCR primers efficiency for genes of interest and reference gene estimated for each experimental conditions with LinRegPCR program. 10, 1/10 RNA dilution.

| Group | Mean PCR efficiency | | | | | | | | |
|----------------------|--------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|-------------|
| | <i>EF-1α</i> | <i>PR-2</i> | <i>PR-3</i> | <i>COI1</i> | <i>EIN3</i> | <i>NPR1</i> | <i>NPR3</i> | <i>PR-12</i> | <i>PR-1</i> |
| Mock - MD2 | 1.996 | 1.924 | 1.945 | 1.925 | 1.937 | 1.935 | 1.965 | 1.936 | 1.907 |
| Mock - MD2 - 10 | 2.038 | 1.931 | 1.979 | 1.922 | 1.946 | 1.925 | 1.963 | 1.960 | 1.922 |
| Fusa - MD2 - 24 | 1.989 | 1.907 | 1.952 | 1.939 | 1.950 | 1.924 | 1.970 | 1.922 | 1.926 |
| Fusa - MD2 - 24 - 10 | 2.015 | 1.921 | 1.929 | 1.914 | 1.949 | 1.922 | 1.973 | 1.947 | 1.940 |
| Fusa - MD2 - 48 | 1.985 | 1.916 | 1.935 | 1.927 | 1.974 | 1.931 | 1.989 | 1.911 | 1.922 |
| Fusa - MD2 - 48 - 10 | 1.936 | 1.924 | 1.919 | 1.920 | 1.925 | 1.905 | 1.997 | 1.962 | 1.918 |
| Fusa - MD2 - 72 | 1.959 | 1.936 | 1.947 | 1.923 | 1.938 | 1.901 | 1.962 | 1.933 | 1.915 |
| Fusa - MD2 - 72 - 10 | 1.945 | 1.917 | 1.976 | 1.920 | 1.916 | 1.936 | 1.991 | 1.914 | 1.917 |
| Mock - QV | 1.934 | 1.898 | 1.946 | 1.914 | 1.941 | 1.925 | 1.967 | 1.906 | 1.933 |
| Mock - QV - 10 | 1.908 | 1.898 | 1.955 | 1.922 | 1.862 | 1.934 | 2.003 | 1.928 | 1.919 |
| Fusa - QV - 24 | 1.948 | 1.904 | 1.943 | 1.911 | 1.961 | 1.928 | 1.935 | 1.912 | 1.933 |
| Fusa - QV - 24 - 10 | 1.937 | 1.899 | 1.966 | 1.910 | 1.921 | 1.897 | 1.988 | 1.921 | 1.917 |
| Fusa - QV - 48 | 1.982 | 1.893 | 1.950 | 1.917 | 1.969 | 1.930 | 1.955 | 1.908 | 1.941 |
| Fusa - QV - 48 - 10 | 1.994 | 1.912 | 1.943 | 1.929 | 1.919 | 1.899 | 1.968 | 1.939 | 1.917 |
| Fusa - QV - 72 | 1.990 | 1.894 | 1.940 | 1.929 | 1.935 | 1.916 | 1.935 | 1.908 | 1.908 |
| Fusa - QV - 72 - 10 | 1.984 | 1.921 | 1.964 | 1.917 | 1.936 | 1.905 | 1.981 | 1.920 | 1.925 |
| Fusa - MD2 - 96 | 1.898 | 1.933 | 1.960 | 1.927 | 1.895 | 1.909 | 1.888 | 1.926 | 1.941 |
| Fusa - MD2 - 96 - 10 | 1.973 | 1.908 | 1.946 | 1.928 | 1.937 | 1.923 | 1.984 | 1.940 | 1.920 |
| H2O - MD2 - 24 | 2.031 | 1.930 | 1.932 | 1.927 | 1.936 | 1.882 | 1.922 | 1.915 | 1.914 |
| H2O - MD2 - 24 - 10 | 1.984 | 1.894 | 1.942 | 1.932 | 1.920 | 1.936 | 1.956 | 1.997 | 1.911 |
| H2O - MD2 - 48 | 1.926 | 1.905 | 1.877 | 1.913 | 1.936 | 1.916 | 1.966 | 1.913 | 1.929 |
| H2O - MD2 - 48 - 10 | 1.950 | 1.918 | 1.945 | 1.928 | 1.933 | 1.956 | 1.945 | 1.903 | 1.918 |
| H2O - MD2 - 72 | 1.934 | 1.936 | 1.886 | 1.929 | 1.965 | 1.902 | 1.968 | 1.899 | 1.894 |
| H2O - MD2 - 72 - 10 | 1.931 | 1.917 | 1.924 | 1.908 | 1.904 | 1.914 | 1.951 | 1.954 | 1.928 |
| H2O - MD2 - 96 | 1.952 | 1.914 | 1.925 | 1.947 | 1.977 | 1.908 | 1.937 | 1.905 | 1.925 |
| H2O - MD2 - 96 - 10 | 1.925 | 1.917 | 1.965 | 1.914 | 1.899 | 1.910 | 1.981 | 1.920 | 1.907 |
| Fusa - QV - 96 | 1.922 | 1.935 | 1.937 | 1.936 | 1.939 | 1.921 | 1.963 | 1.906 | 1.914 |
| Fusa - QV - 96 - 10 | 1.917 | 1.911 | 1.960 | 1.929 | 1.894 | 1.929 | 1.953 | 1.902 | 1.913 |
| H2O - QV - 24 | 1.914 | 1.921 | 1.929 | 1.919 | 1.953 | 1.909 | 1.936 | 1.908 | 1.931 |
| H2O - QV - 24 - 10 | 1.938 | 1.908 | 1.944 | 1.917 | 1.916 | 1.912 | 1.951 | 1.915 | 1.928 |
| H2O - QV - 48 | 1.954 | 1.916 | 1.922 | 1.919 | 1.916 | 1.889 | 1.979 | 1.927 | 1.928 |
| H2O - QV - 48 - 10 | 1.934 | 1.899 | 1.954 | 1.907 | 1.905 | 1.902 | 1.954 | 1.901 | 1.925 |
| H2O - QV - 72 | 1.966 | 1.913 | 1.932 | 1.921 | 1.955 | 1.913 | 1.984 | 1.913 | 1.917 |
| H2O - QV - 72 - 10 | 1.968 | 1.899 | 1.955 | 1.925 | 1.925 | 1.948 | 2.006 | 1.932 | 1.926 |
| H2O - QV - 96 | 1.911 | 1.906 | 1.930 | 1.915 | 1.946 | 1.946 | 1.971 | 1.918 | 1.904 |
| H2O - QV - 96 - 10 | 1.915 | 1.901 | 1.946 | 1.916 | 1.911 | 1.929 | 1.968 | 1.913 | 1.924 |

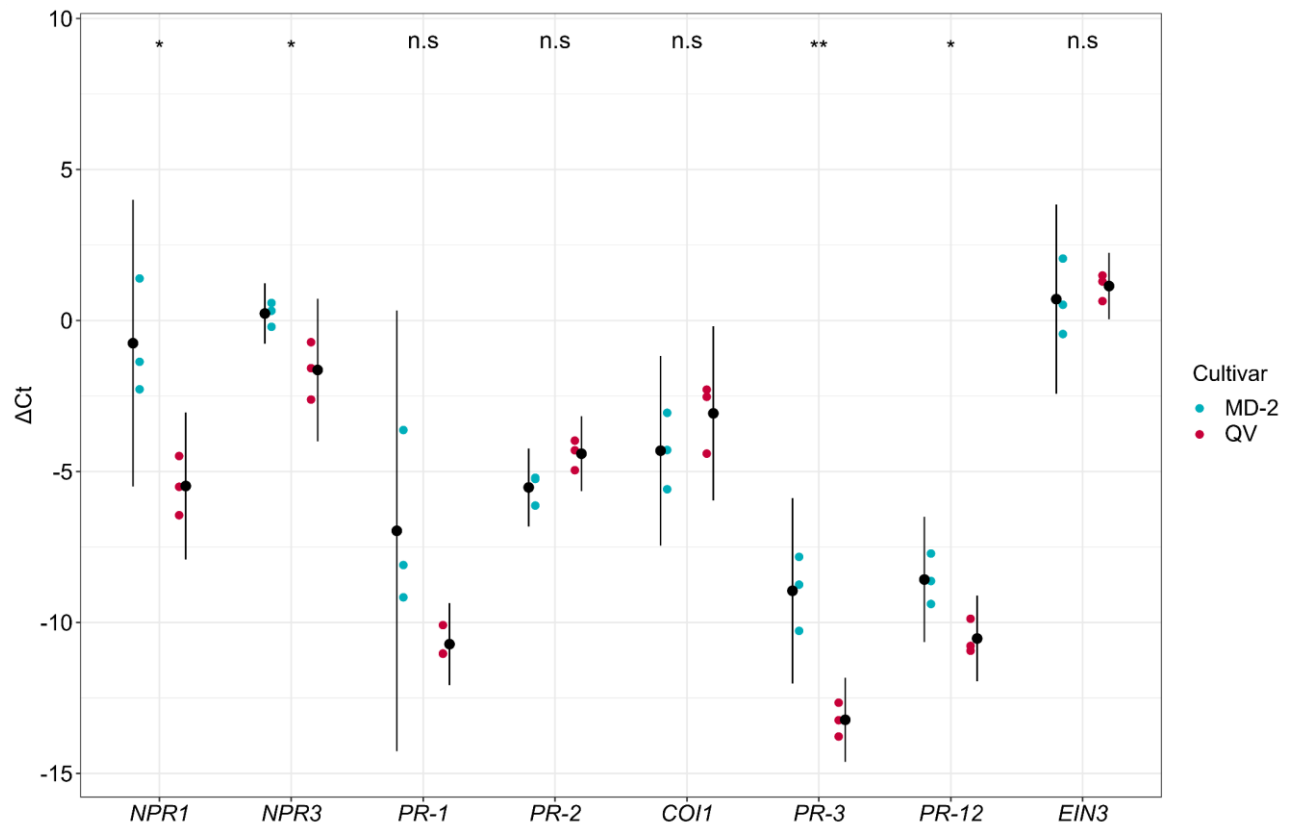


Figure S1. Constitutive relative expression of eight defense genes according to normalization with reference gene (*EF1- α*) in two pineapple cultivars ‘MD-2’ and ‘Queen Victoria’ (QV) characterized by their contrasted susceptibility to FCR. Black dots with bars represent average relative expression levels (ΔCt) with asymptotic confidence intervals at 95% ($n = 3$). Differences in constitutive expression levels (Mock condition) between ‘MD-2’ and ‘QV’ cultivars were either significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) for each gene of interest. *NPR1*, Non-Expressor of Pathogenesis Related gene 1; *NPR3*, Non-Expressor of Pathogenesis Related gene 3; *PR-1*, Pathogenesis-Related gene 1; *PR-2*, Pathogenesis-Related gene 2; *COI1*, Coronatine-insensitive 1; *PR-3*, Pathogenesis-Related gene 3; *PR-12*, Pathogenesis-Related gene 12; *EIN3*, Ethylene Insensitive 3.

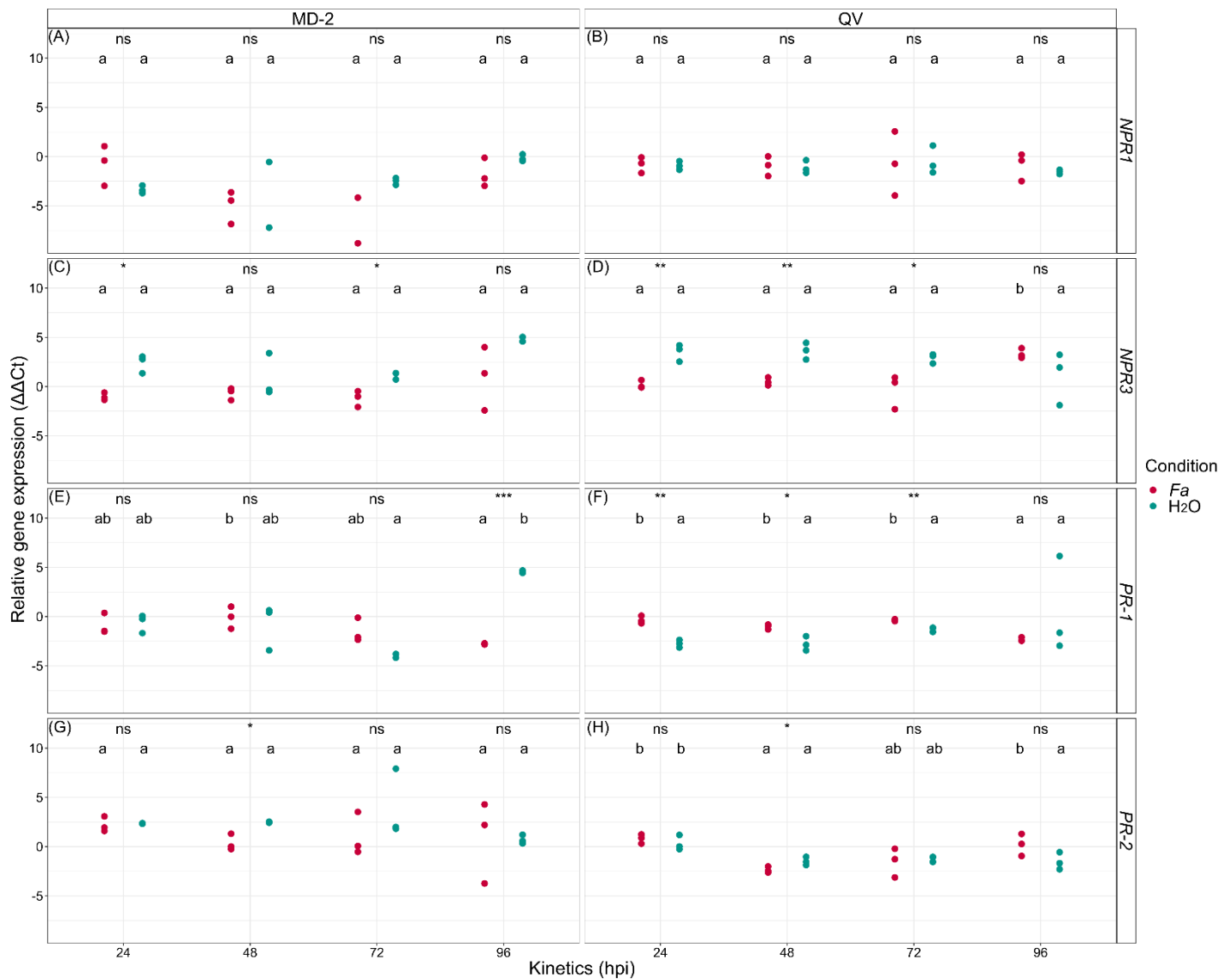


Figure S2. Relative expression dynamics of four defense genes involved in salicylic acid signaling pathway during the early stages of pineapple fruit responses to *Fusarium ananatum* (*Fa*) or sterile water (H₂O) inoculation. Differences in expression following inoculation with *F. ananatum* or sterile water were either significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) for each target gene and each kinetic time. Letters show significant differences in average expression levels between the kinetic points for each condition of inoculation and each cultivar according to Tukey's multiple comparison test at $p < 0.05$. (A) and (B) *NPR1*, Non-Expressor of Pathogenesis Related gene 1; (C) and (D) *NPR3*, Non-Expressor of Pathogenesis Related gene 3; (E) and (F) *PR-1*, Pathogenesis-Related gene 1; (G) and (H) *PR-2*, Pathogenesis-Related gene 2; QV, 'Queen Victoria'.

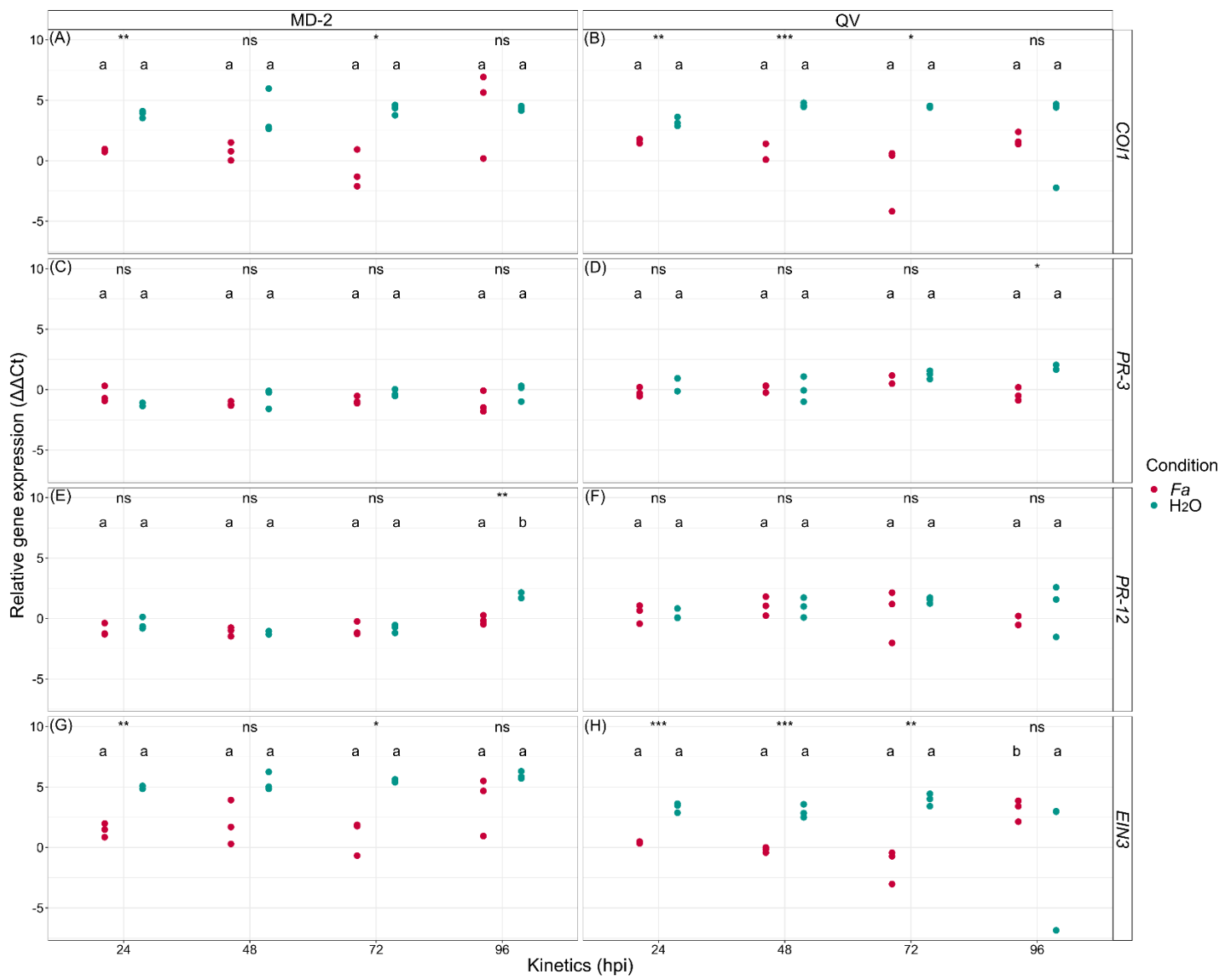


Figure S3. Relative expression dynamics of four defense genes involved in jasmonic acid and ethylene signaling pathways during the early stages of pineapple fruit responses to *Fusarium ananatum* (*Fa*) or sterile water (H₂O) inoculation. Differences in expression following inoculation with *F. ananatum* or sterile water were either significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) for each target gene and each kinetic time. Letters show significant differences in average expression levels between the kinetic points for each condition of inoculation and each cultivar according to Tukey's multiple comparison test at $p < 0.05$. (A) and (B) *COI1*, *Coronatine-insensitive 1*; (C) and (D) *PR-3*, *Pathogenesis-Related gene 3*; (E) and (F) *PR-12*, *Pathogenesis-Related gene 12*; (G) and (H) *EIN3*, *Ethylene Insensitive 3*; QV, 'Queen Victoria'.

CHAPITRE IV

Discussion générale et perspectives

1. Distribution spatiale et détermination de nouveaux agents pathogènes

Le premier axe de recherche de ce travail de thèse visait à évaluer l'influence des paramètres environnementaux et du contexte parcellaire sur l'occurrence de la tache noire. Pour cela, deux parcelles expérimentales d'ananas 'Queen Victoria' présentant des billons d'orientation contraire ont été sélectionnées. Cette étude a été fondée sur des approches de microbiologie classique et de génétique d'identification. La flore fongique cultivable de fruits simples sains et de fruits simples naturellement infectés a été isolée puis identifiée grâce au criblage des gènes et régions TEF-1 α , β -tubuline et ITS. Les résultats obtenus ont permis d'établir la prédominance des espèces fongiques : *Fusarium proliferatum* (14 %), *Fusarium ananatum* (10.8 %), *Fusarium oxysporum* (5.3 %), *Talaromyces stollii* (7 %) et *Talaromyces amestolkiae* (6.4 %) dans les tissus infectés mais également dans les tissus sains (non symptomatiques). Ces abondances sont en accord avec une précédente étude menée sur plusieurs régions productrices de la Réunion au cours de laquelle *F. ananatum* fut décrite comme l'espèce dominante devant *F. oxysporum*, *F. proliferatum* et *T. stollii* (Barral *et al.*, 2020). Ces quatre espèces constituent les composantes majoritaires du mycobiome de l'ananas 'Queen Victoria'. Les espèces *Fusarium temperatum*, *Fusarium polyphialidicum* et *Fusarium concentricum* isolées de fruits cultivés au Costa Rica semblent absentes des productions d'ananas de la Réunion (Stępień *et al.*, 2013). Conjointement, ces travaux établissent que les abondances relatives varient à la fois selon les zones géographiques considérées et selon le contexte biotique des parcelles. En effet, notre étude démontre l'influence des cultures périphériques sur l'occurrence de la tache noire. La configuration spatiale d'une parcelle et son paysage sont notamment définis comme des paramètres clés de la propagation des épidémies au sein des cultures (Parnell *et al.*, 2010). Parmi les parcelles expérimentales étudiées, un gradient de symptômes caractéristiques de la tache noire a été observé montrant que les fruits cultivés à proximité du verger de manguiers présentaient une occurrence de symptômes supérieure aux fruits cultivés en centre de parcelle ou à proximité de jacquiers. Ce gradient d'infection a par la suite été positivement corrélé aux variations d'abondances relatives des espèces *F. proliferatum*, *F. ananatum*, *F. oxysporum* et *T. stollii*. Les profils de dispersion de ces espèces sont en effet analogues au gradient de tache noire.

En considérant les données météorologiques et l'environnement parcellaire, il a été démontré que la contamination des fruits résulte de la dispersion des spores fongiques par les vents

dominants. En effet, lors de notre étude, les stades phénologiques de l'induction florale à la récolte furent soumis au flux d'alizés. Au sein des vergers de pommiers et de manguiers, des profils de dispersion sur de courtes distances montrent que les infections sévères dépendent de la distribution spatiale des pathogènes (Charest *et al.*, 2002, Gamliel-Atinsky *et al.*, 2008). Ainsi, il est aujourd'hui établi que l'occurrence et la sévérité d'une maladie cryptogamique sont d'autant plus élevées que la proximité de la source d'inoculum primaire est faible (Holb *et al.*, 2004 ; Rieux *et al.*, 2014). Ce travail de thèse rapporte pour la première fois l'implication d'une dispersion aérienne des spores de pathogènes responsables de la maladie de la tache noire. Jusqu'alors, cette pathologie était considérée comme d'origine tellurique (Mourichon, 1997). De ce fait, l'actuel itinéraire technique de référence préconise l'enfouissement des résidus de culture et le dépôt de bois raméal fragmenté entre les billons favorisant le maintien des formes latentes de pathogènes et notamment des périthèces de *Fusarium* (Fournier, 2012). Ces éléments expliquent en partie les difficultés rencontrées dans la maîtrise de cette maladie.

Tenant compte de la direction des vents dominants, la canopée du verger de manguiers semble constituer la source d'inoculum à l'origine de la contamination des parcelles expérimentales. Pour cette culture arboricole, *F. proliferatum* et *F. sacchari* sont définis comme les agents pathogènes responsables du développement de malformations des inflorescences (Lima *et al.*, 2009 ; Zhan *et al.*, 2010). De plus, *F. proliferatum* est impliqué dans l'établissement de lésions sur les feuilles de manguiers (Omar *et al.*, 2018). Ces travaux suggèrent donc que les risques d'occurrence de la tache noire dépendent de contaminations croisées entre ces cultures. Cette typicité de contamination constitue un résultat essentiel pour la gestion de la maladie. En effet, le paysage agricole réunionnais est en grande partie représenté par la culture de la canne à sucre. Cette espèce végétale est notamment impactée par la maladie du Pokkah Boeng causée par les espèces *F. verticillioides*, *F. fujikuroi* et *F. proliferatum* (Lin *et al.*, 2014). Cette pathologie a été ponctuellement observée sur les cultures de canne à sucre du sud de la Réunion (Saint-Pierre et Petite-Île) en fin d'année 2016 (Bulletin de Santé du Végétal, 2016). Dès lors, de nombreuses contaminations croisées peuvent être envisagées.

Suite aux travaux de thèse de Bastien Barral, l'implication des espèces *F. proliferatum*, *F. oxysporum* et *T. stollii* dans le pathosystème de la tache noire était suspectée de par leur

représentation sur les cultures d'ananas 'Queen Victoria' de la Réunion et de l'île Maurice (Barral, 2017 ; Barral *et al.*, 2020). La présente étude a permis de démontrer l'implication de *F. proliferatum*, *F. oxysporum*, *F. sacchari*, *T. stollii* et de *T. amestolkiae* dans l'expression de la maladie. Il est intéressant de noter que ces espèces appartiennent respectivement aux complexes *Fusarium fujikuroi* et *Talaromyces purpureogenus*. Il est ainsi supposé que de nombreuses espèces rattachées à ces complexes puissent être impliquées dans l'établissement de la tache noire de l'ananas. Ces données peuvent expliquer l'ambiguïté rencontrée au cours des décennies pour la définition des pathogènes responsables de cette pathologie (Rohrbach and Pfeiffer, 1976 ; Lim and Rohrbach, 1980, Jacobs *et al.*, 2010).

D'autre part, l'étude des relations phylogénétiques a montré une importante proximité entre les isolats issus de fruits simples sains et naturellement tachés. Ces résultats permettent d'établir que l'expression de la maladie n'est pas supportée par un polymorphisme génétique favorisant la virulence de certaines souches isolées à la Réunion.

L'influence des associations entre les différentes composantes des complexes d'espèces sur la pathogénicité a ensuite été étudiée. La caractérisation des mycobiomes a ainsi permis de mettre en évidence la présence d'un inoculum pathogène complexe dans l'ensemble des fruits simples. La contamination des fruits est ainsi globale sans pour autant être associée au développement systématique de symptômes de la tache noire. L'analyse par approche combinatoire a démontré une corrélation significative entre les profils de co-occurrences d'espèces au sein des tissus sains et infectés. Des profils distinctifs d'associations par paires ont alors pu être caractérisés. Ainsi, les tissus sains présentaient une fréquence élevée de co-occurrences entre *F. proliferatum* et *T. amestolkiae* ainsi qu'entre *F. anatum* et *T. amestolkiae*. Par opposition, ces espèces de *Fusarium* étaient plus fréquemment observées en association avec *T. stollii* dans les tissus infectés. L'expression de la tache noire a déjà pu être corrélée à ce type d'association *Fusarium-Talaromyces*. En effet, *F. verticillioides* et *T. funiculosus* ont été simultanément isolées à partir de fruits simples infectés appartenant au cultivar 'Cayenne Lisse' (Lim and Rohrbach, 1980). L'espèce *T. funiculosus* n'est toutefois pas identifiée comme prévalente à la Réunion. Il est donc suggéré que la tache noire puisse plus généralement être induite par la co-occurrence d'espèces appartenant aux complexes *Fusarium fujikuroi* et *Talaromyces purpureogenus*.

Enfin, les postulats de Koch ont permis de mettre en évidence des divergences de profils de diversité au sein du mycobiome suite à l'inoculation des cinq pathogènes nouvellement identifiés. En effet, les fruits ayant subi une inoculation avec les *Fusarium* spp. pathogènes montrent une signature fongique particulière. De telles données n'avaient jusqu'alors jamais été rapportées dans la littérature pour ce pathosystème. Dès lors, ces profils suggèrent un réarrangement des communautés fongiques induit par une surreprésentation de la biomasse des espèces pathogènes appartenant au complexe *Fusarium fujikuroi*.

2. Influence des interactions inter-pathogènes sur la croissance et la toxigenicité

La détermination des potentiels mycotoxinogènes d'espèces de *Fusarium* isolées d'ananas en provenance de plusieurs régions productrices du monde montre des profils contrastés. Ainsi, *F. ananatum* est décrit pour sa production majoritaire de beauvericine bien que certains isolats présentent des capacités d'accumulation de fumonisines B₁, B₂, B₃ et de moniliformine. *F. proliferatum* est caractérisé pour son importante production de fumonisines B₁, B₂ et B₃ mais également pour sa biosynthèse de moniliformine et de beauvericine à de plus faibles concentrations. De plus, *F. proliferatum* montre une importante stabilité dans la biosynthèse de fumonisines chez des plantes hôtes telles que l'ananas, l'ail (*Allium sativum*) ou l'asperge (*Asparagus officinalis*) (Stępień *et al.*, 2011a ; Stępień *et al.*, 2011b). A l'inverse, les isolats de *F. oxysporum* issus de l'ananas produisent exclusivement de la moniliformine et de la beauvericine (Stępień *et al.*, 2013).

Lors d'une première étude sur la flore fongique d'ananas 'Queen Victoria' issus de dix exploitations de la Réunion, les capacités toxigènes des espèces *F. proliferatum*, *F. ananatum* et *F. oxysporum* furent démontrées (Barral *et al.*, 2020). *In vitro*, les souches étudiées sont en effet capables d'accumuler des fumonisines B₁, B₂ ainsi que de la beauvericine. Par ailleurs, aucune mycotoxine n'a été détectée ou quantifiée pour les isolats appartenant à l'espèce *T. stollii*. L'analyse des profils toxigènes a également mis en évidence des concentrations significativement supérieures en fumonisine B₁ et en beauvericine dans des fruits simples naturellement infectés par comparaison à des tissus sains (Barral *et al.*, 2020).

Considérant les résultats obtenus avec le premier axe de recherche de cette thèse et ces éléments de littérature, il a ainsi été postulé que les mycotoxines pourraient constituer des

paramètres clés de la structuration du mycobiome de l'ananas. De surcroît, les mycotoxines sont définies en tant que facteurs de virulence et pourraient favoriser l'établissement de la maladie de la tache noire chez l'ananas 'Queen Victoria'.

Mon travail de thèse a donc, dans un second temps, tenter de déterminer l'influence des interactions entre les pathogènes sur leurs capacités de colonisation et de toxinogénèse. Pour ce faire, une approche *in vitro* a été menée sur la base de confrontations suivies sur une cinétique de dix jours permettant la caractérisation dynamique des productions de mycotoxines. Les résultats obtenus ont tout d'abord montré le potentiel antagoniste de *T. stollii* sur la croissance de *F. proliferatum* et *F. oxysporum*. *F. ananatum* a été caractérisée comme l'unique espèce pathogène capable de restreindre la colonisation du milieu par *T. stollii*. Il est donc suggéré, que pour des biomasses initiales équivalentes, *T. stollii* constitue le compétiteur dominant du cortège pathogène de la tache noire. Ces résultats peuvent expliquer l'importante concentration de conidies ellipsoïdales caractéristiques du genre *Talaromyces* observée dans les cavités florales du cultivar résistant 'MD-2' (Barral *et al.*, 2019b). Par ailleurs, une coloration rouge a été notée pour toutes les confrontations *Fusarium* spp. – *T. stollii*, pouvant correspondre à une production de pigment. De façon intéressante, cette pigmentation a également été rapportée *in planta* suite aux procédures d'inoculation avec deux isolats distincts de *T. stollii*. Toutefois, notre étude n'a pas permis d'identifier la molécule impliquée et donc de confirmer s'il s'agissait d'une pigmentation d'origine fongique. De façon analogue aux mycotoxines, les pigments sont des métabolites secondaires possédant des activités antimicrobienne et antifongique (Sánchez-Muñoz *et al.*, 2020). Les hypothèses sur l'origine de cette pigmentation sont donc multiples. En effet, *T. stollii* appartient au complexe d'espèces *Talaromyces purpureogenus* au sein duquel *T. amestolkiae* et *T. purpureogenus* sont décrites comme sécrétrices d'azaphilones, des polycétides de coloration rouge (Yilmaz *et al.*, 2012 ; Yilmaz *et al.*, 2014). Cependant, un exsudat de coloration rouge a été distingué à la surface du mycélium de *F. proliferatum* au cours de sa confrontation avec *T. stollii*. En effet, certaines composantes du complexe d'espèces *Fusarium fujikuroi* possèdent des capacités de sécrétion de pigments rouges tels que la bostrycoïdine, la norjavanicine ou la 8-O-méthylfusarubine (Studt *et al.*, 2012 ; Lagashetti *et al.*, 2019). De plus, *F. oxysporum* est étudiée pour sa production de bikavérine définie comme une mycotoxine de pigmentation rouge (Lebeau *et al.*, 2019a ; Lebeau *et al.*, 2019b).

La détection et la quantification des mycotoxines issues des cultures simples ont démontré que *F. ananatum* produisait exclusivement de la beauvericine. Les espèces *F. proliferatum* et *F. oxysporum* ont été caractérisées pour leurs potentiels de biosynthèse de fumonisines B₁, B₂ et de beauvericine. De nouveau, aucune toxinogénèse n'a été mise en évidence pour *T. stollii*. Ces données sont en accord avec une étude antérieure menée par Barral *et al.* (2020). Il est ainsi démontré que les pathogènes responsables de la tache noire à la Réunion ne sont pas producteurs de fumonisine B₃ ou de moniliformine comme observé pour les souches provenant d'Amérique du Sud ou de Hawaï (Stępień *et al.*, 2013). Ces données montrent donc l'existence de chémotypes divergents menant pourtant tous au développement d'une infection. Il est donc suggéré que la virulence des espèces *F. ananatum*, *F. proliferatum* et *F. oxysporum* puisse en partie reposer sur la biosynthèse de fumonisines B₁, B₂ et de beauvericine.

A l'issue de l'établissement des profils dynamiques de production de toxines, une diminution des concentrations en fumonisines B₁, B₂ et en beauvericine au cours des confrontations entre les pathogènes a été mise en évidence. Ceci est établi tant pour les confrontations *Fusarium* – *Fusarium* que pour les confrontations *Fusarium* – *T. stollii* à l'exception de *F. proliferatum*, caractérisée comme la seule espèce pour laquelle la confrontation avec *T. stollii* n'a pas impacté la production de beauvericine. D'après ces résultats, il semble donc que les potentiels toxigènes des pathogènes de la tache noire soient impactés par leurs interactions. *In situ*, la proportion de biomasse de ces espèces reste inconnue. Cette variable semble pourtant essentielle pour une détermination robuste des profils antagonistes intervenant au cours du processus infectieux. Il a notamment été démontré que le développement de la fusariose de l'épi chez le maïs résulte d'interactions compétitives et facilitatrices entre les pathogènes *F. graminearum* et *F. verticillioides* (Picot *et al.*, 2012). D'après la prédominance des espèces toxigènes de *Fusarium* parmi le mycobiome du cultivar 'Queen Victoria', il est ainsi suggéré que les toxines constituent des métabolites indispensables au contournement et/ou à l'atténuation des réponses de défense de l'hôte. Notamment, l'implication des fumonisines est démontrée lors de l'infection du maïs par le pathogène *F. verticillioides* (Glenn *et al.*, 2008). Bien que des contaminations par la beauvericine aient été rapportées parmi des cultures telles que le melon (*Cucumis melo*), la banane (*Musa spp.*), le dattier (*Phoenix dactylifera* L.) ou le

blé (*Triticum* spp.), son rôle dans les pathosystèmes reste encore méconnu (Moretti *et al.*, 2002 ; Li *et al.*, 2013, Abdalla *et al.*, 2000 ; Logrieco *et al.*, 2002).

Enfin, mes travaux de thèse montrent que l'ananas constitue un excellent modèle pour l'étude des contaminations des fruits en toxines fusariennes. En effet, les risques mycotoxinogènes associés aux fruits et aux légumes se rapportent exclusivement aux concentrations en patuline et en ochratoxine A. Ces toxines sont d'ailleurs les seules pour lesquelles des seuils limites sont définis par la commission des communautés européennes pour les fruits et leurs produits transformés (Règlement (CE) N°1881/2006, 2006).

3. Transduction et modulation des signaux de défense

Les modifications anatomiques mises en œuvre durant les stades post-anthèse montrent d'importantes dissimilarités structurelles entre les cultivars d'ananas. Le cultivar résistant ('MD-2') présente une fusion parfaite entre les sépales et la bractée mais également entre les trois loges carpellaires qui n'est pas observée chez le cultivar 'Queen Victoria' (Barral *et al.*, 2019b). Ces caractéristiques anatomiques favorisent la contamination mais également la colonisation des tissus hôtes par les pathogènes. Ces divergences phénotypiques sont en partie attribuables aux génotypes de ces cultivars. De plus, la mise en évidence de potentiels facteurs de virulence suggère l'établissement d'un dialogue moléculaire complexe menant à la sensibilité de l'ananas 'Queen Victoria'. Le troisième objectif de ce travail de thèse a donc été de caractériser les voies de signalisation de l'acide salicylique, de l'acide jasmonique et de l'éthylène en réponse à une infection par le pathogène de référence décrit dans la littérature, *F. ananatum*.

Pour cet axe de recherche, huit gènes d'intérêt impliqués dans la transduction des signaux de défense des voies de l'acide salicylique, de l'acide jasmonique et de l'éthylène ont été ciblés. L'expression de ces gènes a été étudiée par RT-qPCR sur la base d'une comparaison variétale entre le cultivar résistant 'MD-2' et le cultivar sensible 'Queen Victoria'. Les réponses de ces cultivars à une infection par *F. ananatum* ont été étudiées durant les stades précoces de l'interaction hôte - pathogène. Cette approche a été établie sur la base du génome de l'ananas récemment séquencé et annoté, se trouvant disponible sur la base de données 'Pineapple Genomics Database' (Zhang *et al.*, 2014 ; Ming *et al.*, 2015).

Les résultats ont montré d'importants contrastes dans l'expression constitutive des gènes d'intérêt. Une faible expression des gènes codant pour une chitinase de classe I et une défensine (deux protéines PR) a été observée chez le cultivar 'Queen Victoria' par opposition aux transcrits quantifiés chez le cultivar 'MD-2'. De façon analogue, les facteurs *NPR1* et *NPR3* induits consécutivement à l'accumulation du SA, sont également faiblement exprimés chez le cultivar 'Queen Victoria'. Une précédente étude a pourtant démontré une forte expression du gène codant pour la PAL (Phénylalanine Ammonia-Lyase) chez le cultivar sensible par comparaison au cultivar résistant induite au cours de la maturation des fruits (Léchaudel *et al.*, 2018). Cette enzyme est notamment impliquée dans la biosynthèse du SA *via* la voie des shikimate-phénylpropanoïdes (Wildermuth *et al.*, 2001). Ces résultats suggèrent donc une faible capacité de perception du SA accumulé chez le cultivar 'Queen Victoria'.

Par ailleurs, les gènes *COI1* et *EIN3* respectivement impliqués dans la signalisation en réponse à une accumulation du JA et de l'éthylène ont présenté des niveaux moyens de transcrits plus élevés pour le cultivar sensible. Cependant, ces voies sont impliquées dans l'induction des gènes codant pour la chitinase de classe I et la défensine. La présence de rétro-contrôles négatifs chez le 'MD-2' a donc été supposée pour l'accumulation de ces protéines PR chez l'ananas. En effet, ces profils de contrôle transcriptionnel sont démontrés d'après la dégradation par le protéasome de facteurs intervenant dans la signalisation des voies du JA et de l'éthylène (Howe *et al.*, 2018 ; Azhar *et al.*, 2020). Par ailleurs, ces résultats concordent avec l'accumulation constitutive de coumaroylisocitrate et d'acides hydroxybenzoïques observée à des concentrations significativement plus élevées chez le cultivar 'MD-2' par opposition au cultivar 'Queen Victoria' (Barral *et al.*, 2019b). D'après ces données, il a donc été suggéré que la sensibilité de l'ananas 'Queen Victoria' soit en partie conditionnée par une faible accumulation des métabolites constituant les défenses basales de l'hôte.

La dynamique des profils d'expression des gènes d'intérêt a démontré une répression des réponses de défense tant chez le cultivar sensible que chez le cultivar résistant. En effet, les « patterns » observés au cours des 72 heures suivant l'inoculation montrent des niveaux d'expression analogues à ceux déterminés sur des fruits non infectés. Cette stratégie d'inhibition des défenses est également établie chez le pathogène *Fusarium oxysporum* f. sp. *vasinfectum* lors de l'infection du système racinaire du cotonnier (*Gossypium* spp.).

Dans ces conditions, cette plante hôte présente une incapacité d'induction des gènes *R* codant pour des chitinases de classes I et IV ainsi que pour une glucanase (Dowd *et al.*, 2004).

Chez l'ananas, un basculement des profils d'expression est distingué entre les 72 et 96 premières heures suivant l'inoculation de *F. ananatum*. Les niveaux d'expression observés sont alors similaires à ceux rapportés au cours de 72 heures pour les fruits inoculés avec de l'eau stérile et ayant subi une blessure due à la procédure d'inoculation. Le pathogène *F. ananatum* pourrait alors procéder à la levée de son pouvoir inhibiteur des défenses après les 72 premières heures de l'interaction. Cette stratégie lui permettrait ainsi d'assurer sa croissance et la colonisation des tissus hôtes. De telles modulations des signaux cellulaires de l'hôte ont notamment été mises en évidence chez la tomate (*Solanum lycopersicum*) en réponse au pathogène *Phytophthora capsici*. En effet, l'étude transcriptomique de ce pathosystème a démontré une inversion des profils d'expression de 3720 gènes au cours des huit premières heures de l'interaction (Jupe *et al.*, 2013). Chez l'ananas, cette répression ne permet toutefois pas au pathogène d'induire un état sensible chez le cultivar 'MD-2'. En effet, ces éléments semblent montrer que la résistance du cultivar 'MD-2' repose sur son importante accumulation de défenses constitutives dont ne disposent pas le cultivar sensible ('Queen Victoria').

4. Perspectives de recherche et d'application pour la filière de production

Les résultats des travaux réalisés démontrent l'implication de trois composantes majeures dans la sensibilité de l'ananas 'Queen Victoria' à la maladie de la tache noire. Tout d'abord, il est à présent établi que la combinaison de l'environnement paysager et des conditions météorologiques constitue le premier déterminant de la contamination des fruits au champ *via* une dispersion aérienne des pathogènes. Dans une démarche de gestion agroécologique des productions d'ananas 'Queen Victoria', la caractérisation des flux d'inoculum et l'évaluation des contaminations croisées représentent des perspectives d'intérêt. En effet, les cultures de canne à sucre, de banane ou de mangue (comme illustrées dans ce manuscrit) peuvent représenter des niches écologiques pour des espèces telles que *F. proliferatum* et *F. oxysporum* à présent définies comme des agents pathogènes de la tache noire. Un diagnostic estimant l'influence de la proximité de ces cultures sur l'occurrence de la tache noire en corrélation avec différents contextes pédoclimatiques permettrait aux

producteurs de mettre en place des solutions adaptées pour la maîtrise de cette pathologie. Des telles données pourraient être incrémentées à des outils d'aide à la décision tels que la base de données VICTORIA développée au sein du CIRAD par l'Unité de Recherche Aïda (<https://smartis.re/VICTORIA>). Cet outil mis à la disposition des cultivateurs, permet la simulation de plusieurs itinéraires techniques en fonction des zones de production et des conditions climatiques dans le but d'optimiser les rendements et la qualité des productions d'ananas. La prédiction des risques épidémiologiques permettrait ainsi aux producteurs d'adapter leurs pratiques culturales afin d'optimiser la qualité des récoltes au travers d'une prise en compte spécifique du contexte parcellaire et des facteurs abiotiques.

D'autre part, la compréhension des interactions inter-pathogènes semblent également indispensable pour la maîtrise des risques associés à cette pathologie cryptogamique. Certaines interactions deux à deux semblent réduire le risque mycotoxique mais *in vivo* les interactions microbiennes sont complexes et pourraient dans certains cas, conduire à des concentrations dangereuses pour les consommateurs. Les fumonisines produites par les espèces de *Fusarium* étudiées (FB₁ et FB₂) sont en effet suspectées par l'Organisation Mondiale de la Santé d'importants préjudices sur la santé humaine tels que des effets carcinogènes ou le développement de pathologies congénitales (OMS, 2018). De plus, la mise en évidence d'une pigmentation rouge suggère l'implication de régulations complexes du métabolisme secondaire des pathogènes. L'identification de la ou des molécule(s) impliquée(s) permettrait d'en déterminer l'impact tant sur l'expression de la maladie de la tache noire que sur sa/leur potentielle nocivité pour les consommateurs. En effet, les fruits ne présentant pas une qualité suffisante pour la vente en frais (hors calibre export ou maturité trop avancée) sont valorisés en jus ou en compotes par le biais de processus de transformation qui ne permettent pas de réduire les concentrations en molécules toxiques. La toxicité des mycotoxines associée à une toxicité avérée d'un métabolite pigmentaire nécessiteraient dès lors l'établissement de concentrations limites afin d'exclure tout risque sanitaire pour les consommateurs.

Par ailleurs, une évaluation fine des risques associés aux potentiels toxigènes des pathogènes pourrait être menée *via* la définition *in planta* de ces interactions interspécifiques. Pour cela, il conviendrait de quantifier la biomasse de chacune des espèces fongiques constituant le mycobiome des cavités florales suite à une contamination naturelle.

Une telle approche peut être envisagée grâce à l'émergence des outils de métabarcoding fondés sur du séquençage à haut débit. Cette technologie ouvre également le champ des possibles quant au rôle du microbiome (champignons, bactéries et levures) dans l'expression de la tache noire. D'autre part, ces données pourraient faciliter le développement de méthodes alternatives de protection des cultures grâce à la définition de potentiels agents de biocontrôle issus de la flore endophyte de l'ananas.

De surcroît, la considération conjointe de l'étude histologique de Barral *et al.* (2019b) et des nouveaux agents pathogènes identifiés génère de nouvelles hypothèses quant à l'influence des interactions inter-pathogènes sur la colonisation des tissus vivants. Il serait intéressant d'effectuer un suivi microscopique *in situ* des profils de propagation sur la base d'un marquage par fluorescence des espèces pathogènes. Ce type d'approche a notamment permis la détermination des modèles de colonisation mis en place pour des pathosystèmes tels que tomate – *F. oxysporum* f. sp. *radicis-lycopersici*, blé – *F. graminearum* ou encore orge – *F. graminearum* (Lagopodi *et al.*, 2002 ; Boenisch and Schäfer, 2011 ; Skadsen and Hohn, 2004).

Enfin, les profils génétiques et métaboliques de l'ananas 'Queen Victoria' en réponse à une infection par *F. ananatum* respectivement établis au cours de cette thèse et de travaux précédents, démontrent que la sensibilité de cet hôte est en partie conditionnée par ses faibles niveaux de défenses constitutives (Barral *et al.*, 2017 ; Barral *et al.*, 2019a). Des recherches portant sur l'élicitation des défenses mises en œuvre parmi les voies de signalisation de l'acide salicylique, de l'acide jasmonique et de l'éthylène pourraient ainsi permettre une stimulation accrue de la résistance systémique chez l'ananas 'Queen Victoria'. De plus, la détermination des modèles d'expression menant à la synthèse de protéines PR a démontré d'importantes modulations des défenses de l'hôte au cours des stades précoces de l'interaction ananas – *F. ananatum*. Il serait intéressant de rechercher les molécules fongiques à l'origine de ces mécanismes de manipulation. Cette démarche est aujourd'hui abordable grâce au développement d'outils protéomiques permettant de définir les interactions entre les protéomes fongique et végétal dans un contexte de relation hôte - pathogène. Particulièrement, une telle approche rend possible la détermination de molécules effectrices ainsi que de leurs cibles au sein du métabolisme de l'hôte. Ceci a notamment permis d'identifier 152 effecteurs putatifs sécrétés dans les tissus hôtes par le pathogène

F. graminearum au cours des stades précoces de l'infection du blé tendre (Fabre *et al.*, 2019). Des perspectives tant fondamentales qu'appliquées sont ainsi possibles pour la poursuite de travaux destinés à une meilleure gestion de la maladie de la tache noire.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Abdalla, M. Y., Al-Rokibah, A., Moretti, A., and Mulè, G. (2000). Pathogenicity of toxigenic *Fusarium proliferatum* from date palm in Saudi Arabia. *Plant Disease* 84, 321–324. doi:10.1094/PDIS.2000.84.3.321.
- Acevedo-Garcia, J., Kusch, S., and Panstruga, R. (2014). Magical mystery tour : MLO proteins in plant immunity and beyond. *New Phytol* 204, 273–281. doi:10.1111/nph.12889.
- Adnan, M., Zheng, W., Islam, W., Arif, M., Abubakar, Y., Wang, Z., et al. (2017). Carbon catabolite repression in filamentous fungi. *IJMS* 19, 48. doi:10.3390/ijms19010048.
- AFSSA (2009). Évaluation des risques liés à la présence de mycotoxines dans les chaînes alimentaires humaine et animale. Agence Française de Sécurité Sanitaire des Aliments.
- Ahmed, E., and Holmström, S. J. M. (2014). Siderophores in environmental research: roles and applications: Siderophores in environmental research. *Microbial Biotechnology* 7, 196–208. doi:10.1111/1751-7915.12117.
- Akiyama, T., Pillai, M. A., and Sentoku, N. (2004). Cloning, characterization and expression of OsGLN2, a rice endo-1,3- β -glucanase gene regulated developmentally in flowers and hormonally in germinating seeds. *Planta* 220, 129–139. doi:10.1007/s00425-004-1312-8.
- Alexander, N. J., Proctor, R. H., and McCormick, S. P. (2009). Genes, gene clusters, and biosynthesis of trichothecenes and fumonisins in *Fusarium*. *Toxin Reviews* 28, 198–215. doi:10.1080/15569540903092142.
- Ali, S., Ganai, B. A., Kamili, A. N., Bhat, A. A., Mir, Z. A., Bhat, J. A., et al. (2018). Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiological Research* 212–213, 29–37. doi:10.1016/j.micres.2018.04.008.
- Anal, A. K. D., Rai, S., Singh, M., and Solanki, M. K. (2020). “Plant Mycobiome: Current Research and Applications,” in *Phytobiomes: Current Insights and Future Vistas*, eds. M. K. Solanki, P. L. Kashyap, and B. Kumari (Singapore: Springer Singapore), 81–104. doi:10.1007/978-981-15-3151-4_4.
- Annis, S. L., and Goodwin, P. H. (1997). Recent advances in the molecular genetics of plant cell wall-degrading enzymes produced by plant pathogenic fungi. *Eur J Plant Pathol* 103, 1–14. doi:10.1023/A:1008656013255.
- Apel, K., and Hirt, H. (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55, 373–399. doi:10.1146/annurev.arplant.55.031903.141701.

- Ashraf, N., Ghai, D., Barman, P., Basu, S., Nagaraju, G., Mandal, M. K., et al. (2009). Comparative analyses of genotype dependent expressed sequence tags and stress-responsive transcriptome of chickpea wilt illustrates predicted and unexpected genes and novel regulators of plant immunity. *BMC Genomics* 10, 415. doi:10.1186/1471-2164-10-415.
- Audenaert, K., Vanheule, A., Höfte, M., and Haesaert, G. (2013). Deoxynivalenol: A major player in the multifaceted response of *Fusarium* to its environment. *Toxins* 6, 1–19. doi:10.3390/toxins6010001.
- Azhar, B. J., Zulfiqar, A., Shakeel, S. N., and Schaller, G. E. (2020). Amplification and adaptation in the ethylene signaling pathway. *Small Methods* 4, 1900452. doi:10.1002/smt.201900452.
- Backer, R., Naidoo, S., and van den Berg, N. (2019). The NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1) and related family: Mechanistic insights in plant disease resistance. *Front. Plant Sci.* 10, 102. doi:10.3389/fpls.2019.00102.
- Bai, Y., Müller, D. B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., et al. (2015). Functional overlap of the Arabidopsis leaf and root microbiota. *Nature* 528, 364–369. doi:10.1038/nature16192.
- Barda, O., Maor, U., Sadhasivam, S., Bi, Y., Zakin, V., Prusky, D., et al. (2020). The pH-responsive transcription factor PacC governs pathogenicity and ochratoxin A biosynthesis in *Aspergillus carbonarius*. *Front. Microbiol.* 11, 210. doi:10.3389/fmicb.2020.00210.
- Barral, B. (2017). Maladie des taches noires de l’ananas: Étude des relations hôte-pathogène et compréhension des mécanismes physiologiques de résistance. Available at: <http://www.theses.fr/2017MONTT116> [Accessed January 20, 2018].
- Barral, B., Chillet, M., Doizy, A., Grassi, M., Ragot, L., Léchaudel, M., et al. (2020). Diversity and toxigenicity of fungi that cause pineapple Fruitlet Core Rot. *Toxins* 12, 16. doi:10.3390/toxins12050339.
- Barral, B., Chillet, M., Léchaudel, M., Lugan, R., and Schorr-Galindo, S. (2019a). Coumaroyl-isocitric and caffeoyl-isocitric acids as markers of pineapple fruitlet core rot disease. *Fruits* 74, 11–17. doi:10.17660/th2019/74.1.2.
- Barral, B., Chillet, M., Léchaudel, M., Lartaud, M., Verdeil, J.-L., Conéjéro, G., et al. (2019b). An imaging approach to identify mechanisms of resistance to pineapple Fruitlet Core Rot. *Front. Plant Sci.* 10, 1065. doi:10.3389/fpls.2019.01065.
- Barral, B., Chillet, M., Minier, J., Léchaudel, M., and Schorr-Galindo, S. (2017). Evaluating the response to *Fusarium ananatum* inoculation and antifungal activity of phenolic acids in pineapple. *Fungal Biology* 121, 1045–1053. doi:10.1016/j.funbio.2017.09.002.
- Barriuso, J., Hogan, D. A., Keshavarz, T., and Martínez, M. J. (2018). Role of quorum sensing and chemical communication in fungal biotechnology and pathogenesis. *FEMS Microbiology Reviews* 42, 627–638. doi:10.1093/femsre/fuy022.

- Bartholomew, D. P., Hawkins, R. A., and Lopez, J. A. (2012). Hawaii Pineapple: The rise and fall of an industry. *horts* 47, 1390–1398. doi:10.21273/HORTSCI.47.10.1390.
- Benjamin, C. R. (1955). Ascocarps of *Aspergillus* and *Penicillium*. *Mycologia* 47, 669–687. doi:10.1080/00275514.1955.12024485.
- Bentham, A. R., De la Concepcion, J. C., Mukhi, N., Zdrzałek, R., Draeger, M., Gorenkin, D., et al. (2020). A molecular roadmap to the plant immune system. *Journal of Biological Chemistry* 295, 14916–14935. doi:10.1074/jbc.REV120.010852.
- Bisson, M. M. A., Bleckmann, A., Allekotte, S., and Groth, G. (2009). EIN2, the central regulator of ethylene signalling, is localized at the ER membrane where it interacts with the ethylene receptor ETR1. *Biochemical Journal* 424, 1–6. doi:10.1042/BJ20091102.
- Boatwright, J. L., and Pajerowska-Mukhtar, K. (2013). Salicylic acid: An old hormone up to new tricks. *Molecular Plant Pathology* 14, 623–634. doi:10.1111/mpp.12035.
- Boenisch, M. J., and Schäfer, W. (2011). *Fusarium graminearum* forms mycotoxin producing infection structures on wheat. *BMC Plant Biol* 11, 110. doi:10.1186/1471-2229-11-110.
- Boutigny, A.-L., Barreau, C., Atanasova-Penichon, V., Verdal-Bonnin, M.-N., Pinson-Gadais, L., and Richard-Forget, F. (2009). Ferulic acid, an efficient inhibitor of type B trichothecene biosynthesis and Tri gene expression in *Fusarium* liquid cultures. *Mycological Research* 113, 746–753. doi:10.1016/j.mycres.2009.02.010.
- Bowman, S. M., and Free, S. J. (2006). The structure and synthesis of the fungal cell wall. *Bioessays* 28, 799–808. doi:10.1002/bies.20441.
- Braga, G. U. L., Rangel, D. E. N., Fernandes, É. K. K., Flint, S. D., and Roberts, D. W. (2015). Molecular and physiological effects of environmental UV radiation on fungal conidia. *Curr Genet* 61, 21. doi:10.1007/s00294-015-0483-0.
- Brennan, J. M., Fagan, B., van Maanen, A., Cooke, B. M., and Doohan, F. M. (2003). Studies on in vitro growth and pathogenicity of European *Fusarium* fungi. *European Journal of Plant Pathology* 109, 11. doi:10.1023/A:1024712415326.
- Brodhagen, M., and Keller, N. P. (2006). Signalling pathways connecting mycotoxin production and sporulation. *Molecular Plant Pathology* 7, 285–301. doi:10.1111/j.1364-3703.2006.00338.x.
- Camardo Leggieri, M., Giorni, P., Pietri, A., and Battilani, P. (2019). *Aspergillus flavus* and *Fusarium verticillioides* interaction: Modeling the impact on mycotoxin production. *Front. Microbiol.* 10, 10. doi:10.3389/fmicb.2019.02653.
- Cardoza, R. E., Malmierca, M. G., Hermosa, M. R., Alexander, N. J., McCormick, S. P., Proctor, R. H., et al. (2011). Identification of loci and functional characterization of trichothecene biosynthesis genes in filamentous fungi of the genus *Trichoderma*. *Appl. Environ. Microbiol.* 77, 4867–4877. doi:10.1128/AEM.00595-11.

- Chambre d'agriculture, Direction de l'Alimentation de l'Agriculture et de la Forêt, FDGDON, and ANSES - LSV (2016). Bulletin de Santé du Végétal - Canne à sucre. St-Denis, La Réunion: Chambre d'agriculture, Direction de l'Alimentation de l'Agriculture et de la Forêt, Fédération Départementale des Groupements de Défense contre les Organismes Nuisibles, Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'environnement et du travail – Laboratoire de la Santé des Végétaux. Available at: www.bsv-reunion.fr.
- Chanclud, E., and Morel, J. (2016). Plant hormones: a fungal point of view. *Molecular Plant Pathology* 17, 1289–1297. doi:10.1111/mpp.12393.
- Charest, J., Dewdney, M., Paulitz, T., Phillion, V., and Carisse, O. (2002). Spatial distribution of *Venturia inaequalis* airborne ascospores in orchards. *Phytopathology*® 92, 769–779. doi:10.1094/PHYTO.2002.92.7.769.
- Checker, V. G., Kushwaha, H. R., Kumari, P., and Yadav, S. (2018). “Role of phytohormones in plant defense: Signaling and cross talk,” in *Molecular Aspects of Plant-Pathogen Interaction*, eds. A. Singh and I. K. Singh (Singapore: Springer Singapore), 159–184. doi:10.1007/978-981-10-7371-7_7.
- Chen, T., Nomura, K., Wang, X., Sohrabi, R., Xu, J., Yao, L., et al. (2020). A plant genetic network for preventing dysbiosis in the phyllosphere. *Nature* 580, 653–657. doi:10.1038/s41586-020-2185-0.
- Coll, N. S., Epple, P., and Dangl, J. L. (2011). Programmed cell death in the plant immune system. *Cell Death Differ* 18, 1247–1256. doi:10.1038/cdd.2011.37.
- Connolly, J., Wayne, P., and Bazzaz, F. A. (2001). Interspecific competition in plants: How well do current methods answer fundamental questions? *The American Naturalist* 157, 107–125. doi:10.1086/318631.
- Cordo, C. A., Mónaco, C. I., Altamirano, R., Perelló, A. E., Larrán, S., Kripelz, N. I., et al. (2017). Weather conditions associated with the release and dispersal of *Zymoseptoria tritici* spores in the Argentine Pampas region. *International Journal of Agronomy* 2017, 1–13. doi:10.1155/2017/1468580.
- Crandall, S. G., and Gilbert, G. S. (2017). Meteorological factors associated with abundance of airborne fungal spores over natural vegetation. *Atmospheric Environment* 162, 87–99. doi:10.1016/j.atmosenv.2017.05.018.
- DAAF de la Réunion (2014). La production fruitière à la Réunion. Direction de l'alimentation, de l'agriculture et de la forêt de la Réunion Available at: www.daaf974.agriculture.gouv.fr.
- Darnaudery, M., Fournier, P., and Léchaudel, M. (2018). Low-input pineapple crops with high quality fruit: Promising impacts of locally integrated and organic fertilisation compared to chemical fertilisers. *Ex. Agric.* 54, 286–302. doi:10.1017/S0014479716000284.

- del Río, L. A. (2015). ROS and RNS in plant physiology: an overview. *Journal of Experimental Botany* 66, 2827–2837. doi:10.1093/jxb/erv099.
- DeYoung, B. J., and Innes, R. W. (2006). Plant NBS-LRR proteins in pathogen sensing and host defense. *Nat Immunol* 7, 1243–1249. doi:10.1038/ni1410.
- Diamond, M., Reape, T. J., Rocha, O., Doyle, S. M., Kacprzyk, J., Doohan, F. M., et al. (2013). The Fusarium mycotoxin deoxynivalenol can inhibit plant apoptosis-like programmed cell death. *PLoS ONE* 8, 8. doi:10.1371/journal.pone.0069542.
- Dita, M., Barquero, M., Heck, D., Mizubuti, E. S. G., and Staver, C. P. (2018). Fusarium wilt of banana: Current knowledge on epidemiology and research needs toward sustainable disease management. *Front. Plant Sci.* 9, 21. doi:10.3389/fpls.2018.01468.
- Dixon, R. A., and Barros, J. (2019). Lignin biosynthesis: old roads revisited and new roads explored. *Open Biol.* 9, 14. doi:10.1098/rsob.190215.
- Dmitriev, A. A., Krasnov, G. S., Rozhmina, T. A., Novakovskiy, R. O., Snezhkina, A. V., Fedorova, M. S., et al. (2017). Differential gene expression in response to Fusarium oxysporum infection in resistant and susceptible genotypes of flax (*Linum usitatissimum* L.). *BMC Plant Biol* 17, 253. doi:10.1186/s12870-017-1192-2.
- Doohan, F. M., Brennan, J., and Cooke, B. M. (2003). “Influence of climatic factors on Fusarium species pathogenic to cereals,” in *Epidemiology of Mycotoxin Producing Fungi*, eds. X. Xu, J. A. Bailey, and B. M. Cooke (Dordrecht: Springer Netherlands), 755–768. doi:10.1007/978-94-017-1452-5_10.
- Dowd, C., Wilson, I. W., and McFadden, H. (2004). Gene expression profile changes in cotton root and hypocotyl tissues in response to infection with Fusarium oxysporum f. sp. vasinfectum. *MPMI* 17, 654–667. doi:10.1094/MPMI.2004.17.6.654.
- Duran, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P., et al. (2018). Microbial interkingdom interactions in roots promote Arabidopsis survival. *Cell* 175, 11. doi:10.1016/j.cell.2018.10.020.
- Durrant, W. E., and Dong, X. (2004). Systemic Acquired Resistance. *Annu. Rev. Phytopathol.* 42, 185–209. doi:10.1146/annurev.phyto.42.040803.140421.
- Eranthodi, A., Schneiderman, D., Harris, L. J., Witte, T. E., Sproule, A., Hermans, A., et al. (2020). Enniatin production influences Fusarium avenaceum virulence on potato tubers, but not on durum wheat or peas. *Pathogens* 9, 75. doi:10.3390/pathogens9020075.
- Espeso, E. A., and Peñalva, M. A. (1996). Three binding sites for the Aspergillus nidulans PacC zinc-finger transcription factor are necessary and sufficient for regulation by ambient pH of the isopenicillin N synthase gene promoter. *Journal of Biological Chemistry* 271, 28825–28830. doi:10.1074/jbc.271.46.28825.

- Fabre, F., Vignassa, M., Urbach, S., Langin, T., and Bonhomme, L. (2019). Time-resolved dissection of the molecular crosstalk driving Fusarium head blight in wheat provides new insights into host susceptibility determinism. *Plant Cell Environ* 42, 2291–2308. doi:10.1111/pce.13549.
- FAO (2020). Major tropical fruits - Preliminary market results 2019. Rome: Food and Agriculture Organization of the United Nations.
- Fasoyin, O. E., Wang, B., Qiu, M., Han, X., Chung, K.-R., and Wang, S. (2018). Carbon catabolite repression gene creA regulates morphology, aflatoxin biosynthesis and virulence in *Aspergillus flavus*. *Fungal Genetics and Biology* 18, 41. doi:10.1016/j.fgb.2018.04.008.
- Fitzpatrick, C. R., Salas-González, I., Conway, J. M., Finkel, O. M., Gilbert, S., and Russ, D. (2020). The plant microbiome: From ecology to reductionism and beyond. *Annu. Rev. Microbiol.*, 20. doi:10.1146/annurev-micro-022620-014327.
- Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9, 275–296. doi:10.1146/annurev.py.09.090171.001423.
- Fournier, P. (2012). La culture de l’ananas Victoria à la Réunion pour l’exportation : Recueil de bonnes pratiques. CIRAD, Chambre d’Agriculture de la Réunion.
- Fournier, P., Benneveau, A., Hardy, C., Chillet, M., and Léchaudel, M. (2015). A predictive model based on a pluviothermic index for leathery pocket and fruitlet core rot of pineapple cv. ‘Queen.’ *Eur J Plant Pathol* 142, 449–460. doi:10.1007/s10658-015-0625-8.
- Fox, E. M., and Howlett, B. J. (2008). Secondary metabolism: regulation and role in fungal biology. *Current Opinion in Microbiology* 11, 7. doi:10.1016/j.mib.2008.10.007.
- Foyer, C. H., and Noctor, G. (2011). Ascorbate and glutathione: The heart of the redox hub. *Plant Physiol.* 155, 2–18. doi:10.1104/pp.110.167569.
- Freeman, B. C., and Beattie, G. A. (2008). An overview of plant defenses against pathogens and herbivores. *PHI*. doi:10.1094/PHI-I-2008-0226-01.
- Fu, J., and Wang, S. (2011). Insights into auxin signaling in plant-pathogen interactions. *Front. Plant Sci.* 2, 7. doi:10.3389/fpls.2011.00074.
- Fu, Z. Q., and Dong, X. (2013). Systemic Acquired Resistance: Turning local infection into global defense. *Annu. Rev. Plant Biol.* 64, 839–863. doi:10.1146/annurev-arplant-042811-105606.
- Fumero, M. V., Villani, A., Susca, A., Haidukowski, M., Cimmarusti, M. T., Toomajian, C., et al. (2020). Fumonisin and beauvericin chemotypes and genotypes of the sister species *Fusarium subglutinans* and *Fusarium temperatum*. *Appl Environ Microbiol* 86, 15. doi:10.1128/AEM.00133-20.

- Galeana-Sánchez, E., Sánchez-Rangel, D., de la Torre-Hernández, M. E., Nájera-Martínez, M., Ramos-Villegas, P., and Plasencia, J. (2017). Fumonisin B1 produced in planta by *Fusarium verticillioides* is associated with inhibition of maize β -1,3-glucanase activity and increased aggressiveness. *Physiological and Molecular Plant Pathology* 100, 75–83. doi:10.1016/j.pmpp.2017.07.003.
- Gamir, J., Darwiche, R., van't Hof, P., Choudhary, V., Stumpe, M., Schneiter, R., et al. (2017). The sterol-binding activity of PATHOGENESIS-RELATED PROTEIN 1 reveals the mode of action of an antimicrobial protein. *Plant J* 89, 502–509. doi:10.1111/tpj.13398.
- Gamliel-Atinsky, E., Szejnberg, A., Maymon, M., Shtienberg, D., and Freeman, S. (2009). Inoculum availability and conidial dispersal patterns of *Fusarium mangiferae*, the causal agent of mango malformation disease. *Phytopathology*® 99, 160–166. doi:10.1094/PHYTO-99-2-0160.
- García-Estrada, C., Domínguez-Santos, R., Kosalková, K., and Martín, J.-F. (2018). Transcription factors controlling primary and secondary metabolism in filamentous fungi: The β -lactam paradigm. *Fermentation* 4, 47. doi:10.3390/fermentation4020047.
- Geoghegan, I., Steinberg, G., and Gurr, S. (2017). The role of the fungal cell wall in the infection of plants. *Trends in Microbiology* 25, 957–967. doi:10.1016/j.tim.2017.05.015.
- Ghoul, M., and Mitri, S. (2016). The ecology and evolution of microbial competition. *Trends in Microbiology* 24, 13. doi:10.1016/j.tim.2016.06.011.
- Giorni, P., Bertuzzi, T., and Battilani, P. (2019). Impact of fungi co-occurrence on mycotoxin contamination in maize during the growing season. *Front. Microbiol.* 10, 1265. doi:10.3389/fmicb.2019.01265.
- Gladieux, P., Byrnes, E. J., Aguileta, G., C. Fisher, M., Heitman, J., and Giraud, T. (2011). “Epidemiology and evolution of fungal pathogens in plants and animals,” in *Genetics and Evolution of Infectious Disease* (Elsevier), 59–132. doi:10.1016/B978-0-12-384890-1.00004-2.
- Glenn, A. E., Zitomer, N. C., Zimeri, A. M., Williams, L. D., Riley, R. T., and Proctor, R. H. (2008). Transformation-mediated complementation of a FUM gene cluster deletion in *Fusarium verticillioides* restores both fumonisin production and pathogenicity on maize seedlings. *MPMI* 21, 87–97. doi:10.1094/MPMI-21-1-0087.
- Golan, J. J., and Pringle, A. (2017). “Long-distance dispersal of fungi,” in *The Fungal Kingdom*, eds. J. Heitman, B. J. Howlett, P. W. Crous, E. H. Stukenbrock, T. Y. James, and N. A. R. Gow (Washington, DC, USA: ASM Press), 309–333. doi:10.1128/9781555819583.ch14.
- Gonçalves, A. P., Heller, J., Rico-Ramírez, A. M., Daskalov, A., Rosenfield, G., and Glass, N. L. (2020). Conflict, competition, and cooperation regulate social interactions in filamentous fungi. *Annu. Rev. Microbiol.* 74, 693–712. doi:10.1146/annurev-micro-012420-080905.

- Goncalves, B. L., Sengling Cebin Coppa, C. F., Valganon de Neeff, D., Corassin, C. H., and Fernandes Oliveira, C. A. (2018). Mycotoxins in fruits and fruit-based products: occurrence and methods for decontamination. *Toxin Reviews*, 1–10. doi:10.1080/15569543.2018.1457056.
- Gow, N. A. R., Latge, J.-P., and Munro, C. A. (2017). “The fungal cell wall: Structure, biosynthesis, and function,” in *The Fungal Kingdom*, eds. J. Heitman, B. J. Howlett, P. W. Crous, E. H. Stukenbrock, T. Y. James, and N. A. R. Gow (Washington, DC, USA: ASM Press), 267–292. doi:10.1128/9781555819583.ch12.
- Großkinsky, D. K., Naseem, M., Abdelmohsen, U. R., Plickert, N., Engelke, T., Griebel, T., et al. (2011). Cytokinins mediate resistance against *Pseudomonas syringae* in tobacco through increased antimicrobial phytoalexin synthesis independent of salicylic acid signaling. *Plant Physiol.* 157, 815–830. doi:10.1104/pp.111.182931.
- Gu, Zhan, R. L., Zhan, L. B., Gong, D. Q., and Jia, Z. W. (2015). First report of *Fusarium ananatum* causing pineapple fruitlet core rot in China. *Plant Disease* 99, 1653–16. doi:10.1094/PDIS-03-15-0279-PDN.
- Han, X., and Kahmann, R. (2019). Manipulation of phytohormone pathways by effectors of filamentous plant pathogens. *Front. Plant Sci.* 10, 822. doi:10.3389/fpls.2019.00822.
- Hawksworth, D. L. (2015). Naming fungi involved in spoilage of food, drink, and water. *Current Opinion in Food Science* 5, 23–28. doi:10.1016/j.cofs.2015.07.004.
- Hawksworth, D. L., and Lücking, R. (2016). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiol Spectrum* 5, 17. doi:10.1128/microbiolspec.FUNK-0052-2016.
- Heil, M., and Ton, J. (2008). Long-distance signalling in plant defence. *Trends in Plant Science* 13, 264–272. doi:10.1016/j.tplants.2008.03.005.
- Heinl, S., Hartinger, D., Thamhesl, M., Schatzmayr, G., Moll, W.-D., and Grabherr, R. (2011). An aminotransferase from bacterium ATCC 55552 deaminates hydrolyzed fumonisin B. *Biodegradation* 22, 25–30. doi:10.1007/s10532-010-9371-y.
- Heinl, S., Hartinger, D., Thamhesl, M., Vekiru, E., Krska, R., Schatzmayr, G., et al. (2010). Degradation of fumonisin B1 by the consecutive action of two bacterial enzymes. *Journal of Biotechnology* 145, 120–129. doi:10.1016/j.jbiotec.2009.11.004.
- Holb, I. J., Heijne, B., Withagen, J. C. M., and Jeger, M. J. (2004). Dispersal of *Venturia inaequalis* ascospores and disease gradients from a defined inoculum source. *J Phytopathol* 152, 639–646. doi:10.1111/j.1439-0434.2004.00910.x.
- Horberg, H. M. (2002). Patterns of splash dispersed conidia of *Fusarium poae* and *Fusarium culmorum*. *European Journal of Plant Pathology* 108, 8. doi:10.1023/A:1013936624884.

- Hou, Y.-H., Hsu, L.-H., Wang, H.-F., Lai, Y.-H., and Chen, Y.-L. (2020). Calcineurin regulates conidiation, chlamydospore formation and virulence in *Fusarium oxysporum* f. sp. *lycopersici*. *Front. Microbiol.* 11, 539702. doi:10.3389/fmicb.2020.539702.
- Howe, G. A., Major, I. T., and Koo, A. J. (2018). Modularity in jasmonate signaling for multistress resilience. *Annu. Rev. Plant Biol.* 69, 387–415. doi:10.1146/annurev-arplant-042817-040047.
- Huchelmann, A., Boutry, M., and Hachez, C. (2017). Plant glandular trichomes: Natural cell factories of high biotechnological interest. *Plant Physiol.* 175, 6–22. doi:10.1104/pp.17.00727.
- Irieda, H., Inoue, Y., Mori, M., Yamada, K., Oshikawa, Y., Saitoh, H., et al. (2018). Conserved fungal effector suppresses PAMP-triggered immunity by targeting plant immune kinases. *PNAS* 116, 10. doi:10.1073/pnas.1807297116.
- Jacobs, A., Van Wyk, P. S., Marasas, W. F. O., Wingfield, B. D., Wingfield, M. J., and Coutinho, T. A. (2010). *Fusarium ananatum* sp. nov. in the *Gibberella fujikuroi* species complex from pineapples with fruit rot in South Africa. *Fungal Biology* 114, 515–527. doi:10.1016/j.funbio.2010.03.013.
- Jacobs, S., Zechmann, B., Molitor, A., Trujillo, M., Petutschnig, E., Lipka, V., et al. (2011). Broad-spectrum suppression of innate immunity is required for colonization of *Arabidopsis* roots by the fungus *Piriformospora indica*. *Plant Physiol.* 156, 726–740. doi:10.1104/pp.111.176446.
- Jansen, C., von Wettstein, D., Schafer, W., Kogel, K.-H., Felk, A., and Maier, F. J. (2005). Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. *Proceedings of the National Academy of Sciences* 102, 16892–16897. doi:10.1073/pnas.0508467102.
- Jones, J. D. G., and Dangl, J. L. (2006). The plant immune system. *Nature* 444, 323–329. doi:10.1038/nature05286.
- Jonkers, W., Rodriguez Estrada, A. E., Lee, K., Breakspear, A., May, G., and Kistler, H. C. (2012). Metabolome and transcriptome of the interaction between *Ustilago maydis* and *Fusarium verticillioides* in vitro. *Appl. Environ. Microbiol.* 78, 3656–3667. doi:10.1128/AEM.07841-11.
- Journal officiel de l'Union européenne (2006). Règlement (CE) No 1881/2006 de la commission du 19 décembre 2006 portant fixation de teneurs maximales pour certains contaminants dans les denrées alimentaires. Commission des communautés Européennes.
- Jupe, J., Stam, R., Howden, A. J., Morris, J. A., Zhang, R., Hedley, P. E., et al. (2013). *Phytophthora capsici*-tomato interaction features dramatic shifts in gene expression associated with a hemi-biotrophic lifestyle. *Genome Biol* 14, R63. doi:10.1186/gb-2013-14-6-r63.

- Kamle, M., and Kumar, P. (2016). "Colletotrichum gloeosporioides: Pathogen of anthracnose disease in mango (*Mangifera indica* L.)," in *Current Trends in Plant Disease Diagnostics and Management Practices Fungal Biology.*, eds. P. Kumar, V. Gupta, A. Tiwari, and M. Kamle (Springer, Cham), 13.
- Kamoun, S. (2006). A catalogue of the effector secretome of plant pathogenic oomycetes. *Annu. Rev. Phytopathol.* 44, 41–60. doi:10.1146/annurev.phyto.44.070505.143436.
- Kang, X., Kirui, A., Muszyński, A., Widanage, M. C. D., Chen, A., Azadi, P., et al. (2018). Molecular architecture of fungal cell walls revealed by solid-state NMR. *Nat Commun* 9, 2747. doi:10.1038/s41467-018-05199-0.
- Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D., and Bresta, P. (2020). Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. *J. For. Res.* 31, 1–12. doi:10.1007/s11676-019-01034-4.
- Kazan, K., Gardiner, D. M., and Manners, J. M. (2012). On the trail of a cereal killer: Recent advances in *Fusarium graminearum* pathogenomics and host resistance. *Molecular Plant Pathology* 13, 399–413. doi:10.1111/j.1364-3703.2011.00762.x.
- Keller, N. P. (2019). Fungal secondary metabolism: regulation, function and drug discovery. *Nat Rev Microbiol* 17, 167–180. doi:10.1038/s41579-018-0121-1.
- Khan, M. R., and Doohan, F. M. (2009). Bacterium-mediated control of *Fusarium* head blight disease of wheat and barley and associated mycotoxin contamination of grain. *Biological Control* 48, 42–47. doi:10.1016/j.biocontrol.2008.08.015.
- Khosravi, C., Benocci, T., Battaglia, E., Benoit, I., and de Vries, R. P. (2015). "Sugar catabolism in *Aspergillus* and other fungi related to the utilization of plant biomass," in *Advances in Applied Microbiology* (Elsevier), 1–28. doi:10.1016/bs.aambs.2014.09.005.
- Kim, J.-Y., Park, S.-C., Hwang, I., Cheong, H., Nah, J.-W., Hahm, K.-S., et al. (2009). Protease inhibitors from plants with antimicrobial activity. *IJMS* 10, 2860–2872. doi:10.3390/ijms10062860.
- Kimura, S., Waszczak, C., Hunter, K., and Wrzaczek, M. (2017). Bound by fate: The role of reactive oxygen species in receptor-like kinase signaling. *Plant Cell* 29, 638–654. doi:10.1105/tpc.16.00947.
- Kovacs, I., Durner, J., and Lindermayr, C. (2015). Crosstalk between nitric oxide and glutathione is required for NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1)-dependent defense signaling in *Arabidopsis thaliana*. *New Phytol* 208, 860–872. doi:10.1111/nph.13502.
- Lacerda, A. F., Vasconcelos, E. A. R., Pelegrini, P. B., and Grossi de Sa, M. F. (2014). Antifungal defensins and their role in plant defense. *Front. Microbiol.* 5. doi:10.3389/fmicb.2014.00116.

- Lagashetti, A. C., Dufossé, L., Singh, S. K., and Singh, P. N. (2019). Fungal pigments and their prospects in different industries. *Microorganisms* 7, 604. doi:10.3390/microorganisms7120604.
- Lagomarsino Oneto, D., Golan, J., Mazzino, A., Pringle, A., and Seminara, A. (2020). Timing of fungal spore release dictates survival during atmospheric transport. *Proc Natl Acad Sci USA* 117, 5134–5143. doi:10.1073/pnas.1913752117.
- Lagopodi, A. L., Ram, A. F. J., Lamers, G. E. M., and Punt, P. J. (2002). Novel aspects of tomato root colonization and infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. *MPMI* 15, 172–179. doi:10.1094/MPMI.2002.15.2.172.
- Lam, T. B. T., Kadoya, K., and Iiyama, K. (2001). Bonding of hydroxycinnamic acids to lignin: ferulic and p-coumaric acids are predominantly linked at the benzyl position of lignin, not the *b*-position, in grass cell walls. *Phytochemistry* 57, 987–992. doi:10.1016/s0031-9422(01)00052-8.
- Lanver, D., Tollot, M., Schweizer, G., Lo Presti, L., Reissmann, S., Ma, L.-S., et al. (2017). *Ustilago maydis* effectors and their impact on virulence. *Nat Rev Microbiol* 15, 409–421. doi:10.1038/nrmicro.2017.33.
- Lebeau, J., Petit, T., Clerc, P., Dufossé, L., and Caro, Y. (2019a). Isolation of two novel purple naphthoquinone pigments concomitant with the bioactive red bikaverin and derivatives thereof produced by *Fusarium oxysporum*. *Biotechnol. Prog.* 35, e2738. doi:10.1002/btpr.2738.
- Lebeau, J., Petit, T., Dufossé, L., and Caro, Y. (2019b). Putative metabolic pathway for the bioproduction of bikaverin and intermediates thereof in the wild *Fusarium oxysporum* LCP531 strain. *AMB Expr* 9, 186. doi:10.1186/s13568-019-0912-4.
- Léchaudel, M., Darnaudery, M., Joët, T., Fournier, P., and Joas, J. (2018). Genotypic and environmental effects on the level of ascorbic acid, phenolic compounds and related gene expression during pineapple fruit development and ripening. *Plant Physiology and Biochemistry* 130, 127–138. doi:10.1016/j.plaphy.2018.06.041.
- Lee, H.-A., Lee, H.-Y., Seo, E., Lee, J., Kim, S.-B., Oh, S., et al. (2017). Current understandings of plant nonhost resistance. *MPMI* 30, 5–15. doi:10.1094/MPMI-10-16-0213-CR.
- Leneveu-Jenvrin, C., Quentin, B., Assemat, S., Hoarau, M., Meile, J.-C., and Remize, F. (2020). Changes of quality of minimally-processed pineapple (*Ananas comosus*, var. 'Queen Victoria') during cold storage: fungi in the leading role. *Microorganisms* 8, 185. doi:10.3390/microorganisms8020185.
- LeNoble, M. E., Spollen, W. G., and Sharp, R. E. (2003). Maintenance of shoot growth by endogenous ABA: genetic assessment of the involvement of ethylene suppression. *Journal of Experimental Botany* 55, 237–245. doi:10.1093/jxb/erh031.

- Leslie, J. F., and Summerell, B. A. (2006). *The Fusarium laboratory manual*. 1st ed. Ames, Iowa: Blackwell Pub.
- Leyronas, C., and Nicot, P. C. (2013). Monitoring viable airborne inoculum of *Botrytis cinerea* in the South-East of France over 3 years: relation with climatic parameters and the origin of air masses. *Aerobiologia* 29, 291–299. doi:10.1007/s10453-012-9280-0.
- Li, C., Zuo, C., Deng, G., Kuang, R., Yang, Q., Hu, C., et al. (2013). Contamination of bananas with beauvericin and fusaric acid produced by *Fusarium oxysporum* f. sp. *cubense*. *PLoS ONE* 8, 11. doi:10.1371/journal.pone.0070226.
- Li, N., Han, X., Feng, D., Yuan, D., and Huang, L.-J. (2019). Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: Do we understand what they are whispering? *IJMS* 20, 671. doi:10.3390/ijms20030671.
- Lim, P. O., Kim, H. J., and Gil Nam, H. (2007). Leaf senescence. *Annu. Rev. Plant Biol.* 58, 115–136. doi:10.1146/annurev.arplant.57.032905.105316.
- Lim, T.-K., and Rohrbach, K. G. (1980). Role of *Penicillium funiculosum* strains in the development of pineapple fruit diseases. *Phytopathology* 70, 663. doi:10.1094/Phyto-70-663.
- Lima, C. S., Pfenning, L. H., Costa, S. S., Campos, M. A., and Leslie, J. F. (2009). A new *Fusarium* lineage within the *Gibberella fujikuroi* species complex is the main causal agent of mango malformation disease in Brazil. *Plant Pathology* 58, 33–42. doi:10.1111/j.1365-3059.2008.01946.x.
- Lin, Z., Xu, S., Que, Y., Wang, J., Comstock, J. C., Wei, J., et al. (2014). Species-specific detection and identification of *Fusarium* Species complex, the causal agent of sugarcane Pokkah Boeng in China. *PLoS ONE* 9, e104195. doi:10.1371/journal.pone.0104195.
- Liu, K., Newman, M., McInroy, J. A., Hu, C.-H., and Kloepper, J. W. (2017). Selection and assessment of plant growth-promoting rhizobacteria for biological control of multiple plant diseases. *Phytopathology*® 107, 928–936. doi:10.1094/PHYTO-02-17-0051-R.
- Liu, Y., Yang, K., Qin, Q., Lin, G., Hu, T., Xu, Z., et al. (2018). G protein α subunit GpaB is required for asexual development, aflatoxin biosynthesis and pathogenicity by regulating cAMP signaling in *Aspergillus flavus*. *Toxins* 10, 15. doi:10.3390/toxins10030117.
- Logrieco, A., Rizzo, A., Ferracane, R., and Ritieni, A. (2002). Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. *Appl Environ Microbiol* 68, 82–85. doi:10.1128/AEM.68.1.82–85.2002.
- Lord, K. M., and Read, N. D. (2011). Perithecial morphogenesis in *Sordaria macrospora*. *Fungal Genetics and Biology* 48, 388–399. doi:10.1016/j.fgb.2010.11.009.
- Lu, Y., and Tsuda, K. (2021). Intimate association of PRR- and NLR-mediated signaling in plant immunity. *MPMI* 34, 3–14. doi:10.1094/MPMI-08-20-0239-IA.

- Luengwilai, K., Beckles, D. M., and Siriphanich, J. (2016). Postharvest internal browning of pineapple fruit originates at the phloem. *Journal of Plant Physiology* 202, 121–133. doi:10.1016/j.jplph.2016.07.011.
- Lutz, M. P., Feichtinger, G., Défago, G., and Duffy, B. (2003). Mycotoxigenic *Fusarium* and deoxynivalenol production repress chitinase gene expression in the biocontrol agent *Trichoderma atroviride* P1. *Appl. Environ. Microbiol.* 69, 3077–3084. doi:10.1128/AEM.69.6.3077-3084.2003.
- Matsuura, H. N., and Fett-Neto, A. G. (2015). “Plant alkaloids: Main features, toxicity, and mechanisms of action,” in *Plant Toxins*, eds. P. Gopalakrishnakone, C. R. Carlini, and R. Ligabue-Braun (Dordrecht: Springer Netherlands), 1–15. doi:10.1007/978-94-007-6728-7_2-1.
- McHale, L., Tan, X., Koehl, P., and Michelmore, R. W. (2006). Plant NBS-LRR proteins: adaptable guards. *Genome Biol* 7, 212. doi:10.1186/gb-2006-7-4-212.
- Michielse, C. B., Pfanmüller, A., Macios, M., Rengers, P., Dzikowska, A., and Tudzynski, B. (2014). The interplay between the GATA transcription factors AreA, the global nitrogen regulator and AreB in *Fusarium fujikuroi* : Interplay between AreA and AreB. *Molecular Microbiology* 91, 472–493. doi:10.1111/mmi.12472.
- Mikulič Petkovšek, M., Štampar, F., and Veberič, R. (2009). Accumulation of phenolic compounds in apple in response to infection by the scab pathogen, *Venturia inaequalis*. *Physiological and Molecular Plant Pathology* 74, 60–67. doi:10.1016/j.pmpp.2009.09.003.
- Mina, D., Pereira, J. A., Lino-Neto, T., and Baptista, P. (2020). Impact of plant genotype and plant habitat in shaping bacterial pathobiome: a comparative study in olive tree. *Sci Rep* 10, 3475. doi:10.1038/s41598-020-60596-0.
- Ming, R., VanBuren, R., Wai, C. M., Tang, H., Schatz, M. C., Bowers, J. E., et al. (2015). The pineapple genome and the evolution of CAM photosynthesis. *Nat Genet* 47, 1435–1442. doi:10.1038/ng.3435.
- Mondal, S. N., Gottwald, T. R., and Timmer, L. W. (2003). Environmental factors affecting the release and dispersal of ascospores of *Mycosphaerella citri*. *Phytopathology*® 93, 1031–1036. doi:10.1094/PHYTO.2003.93.8.1031.
- Mondal, S. N., and Hyakumachi, M. (1998). Carbon loss and germinability, viability, and virulence of chlamydospores of *Fusarium solani* f. sp. *phaseoli* after exposure to soil at different pH levels, temperatures, and matric potentials. *Phytopathology* 88, 8. doi:10.1094/PHYTO.1998.88.2.148.
- Moretti, A., Belisario, A., Tafuri, A., Ritieni, A., Corazza, L., and Logrieco, A. (2002). Production of beauvericin by different races of *Fusarium oxysporum* f. sp. *melonis*, the *Fusarium* wilt agent of muskmelon. *European Journal of Plant Pathology* 108, 6. doi:10.1023/A:1020674929084.

- Moretti, A., Pascale, M., and Logrieco, A. F. (2019). Mycotoxin risks under a climate change scenario in Europe. *Trends in Food Science & Technology* 84, 38–40. doi:10.1016/j.tifs.2018.03.008.
- Mourichon, X. (1983). Contribution à l'étude des taches noires (Fruitlet core rot) et leathery pocket de l'ananas causés par *Penicillium funiculosum* Thom. en Côte d'Ivoire. *Fruits* 38, 601–609.
- Mourichon, X. (1997). Pineapple Fruitlet Core Rot (black spot) and Leathery Pocket: Review and prospects. *Acta Horticulturae* 425, 8. doi:10.17660/ActaHortic.1997.425.54.
- Mourichon, X., Perrier, X., and Thibaud, B. (1987). Fruitlet core rot and leathery pocket of pineapple in Cote d'Ivoire. Research into the main explanatory factors. *Fruits* 42, 343–352.
- Mousa, W. K., Shearer, C. R., Limay-Rios, V., Zhou, T., and Raizada, M. N. (2015). Bacterial endophytes from wild maize suppress *Fusarium graminearum* in modern maize and inhibit mycotoxin accumulation. *Front. Plant Sci.* 6. doi:10.3389/fpls.2015.00805.
- Müllenborn, C., Steiner, U., Ludwig, M., and Oerke, E.-C. (2008). Effect of fungicides on the complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels. *Eur J Plant Pathol* 120, 157–166. doi:10.1007/s10658-007-9204-y.
- Müller, M. E. H., Steier, I., Köppen, R., Siegel, D., Proske, M., Korn, U., et al. (2012). Cocultivation of phytopathogenic *Fusarium* and *Alternaria* strains affects fungal growth and mycotoxin production. *J Appl Microbiol* 113, 874–887. doi:10.1111/j.1365-2672.2012.05388.x.
- Musser, S. M., and Plattner, R. D. (1997). Fumonisin composition in cultures of *Fusarium moniliforme*, *Fusarium proliferatum*, and *Fusarium nygami*. *J. Agric. Food Chem.* 45, 1169–1173. doi:10.1021/jf960663t.
- Nantapong, N., Murata, R., Trakulnaleamsai, S., Kataoka, N., Yakushi, T., and Matsushita, K. (2019). The effect of reactive oxygen species (ROS) and ROS-scavenging enzymes, superoxide dismutase and catalase, on the thermotolerant ability of *Corynebacterium glutamicum*. *Appl Microbiol Biotechnol* 103, 5355–5366. doi:10.1007/s00253-019-09848-2.
- Nascimento, E., da Silva, S. H., dos Reis Marques, E., Roberts, D. W., and Braga, G. U. L. (2010). Quantification of cyclobutane pyrimidine dimers induced by UVB radiation in conidia of the fungi *Aspergillus fumigatus*, *Aspergillus nidulans*, *Metarhizium acridum* and *Metarhizium robertsii*. *Photochem. Photobiol.* 86, 8. doi:10.1111/j.1751-1097.2010.00793.x.
- Nawrath, C., and Métraux, J.-P. (1999). Salicylic acid induction-deficient mutants of *Arabidopsis* express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. *The Plant Cell*, 13.

- Nelson, S. C. (2008). Mango anthracnose (*Colletotrichum gloeosporioides*). *Plant Disease* 48, 9.
- Niehaus, E.-M., Studt, L., von Bargen, K. W., Kummer, W., Humpf, H.-U., Reuter, G., et al. (2016). Sound of silence: the beauvericin cluster in *Fusarium fujikuroi* is controlled by cluster-specific and global regulators mediated by H3K27 modification: Regulation of beauvericin biosynthesis in *Fusarium fujikuroi*. *Environ Microbiol* 18, 4282–4302. doi:10.1111/1462-2920.13576.
- Nirlane da Costa Souza, P., Luiza Bim Grigoletto, T., Alberto Beraldo de Moraes, L., Abreu, L. M., Henrique Souza Guimarães, L., Santos, C., et al. (2016). Production and chemical characterization of pigments in filamentous fungi. *Microbiology* 162, 12–22. doi:10.1099/mic.0.000168.
- Niu, L., and Liao, W. (2016). Hydrogen peroxide signaling in plant development and abiotic responses: crosstalk with nitric oxide and calcium. *Front. Plant Sci.* 7. doi:10.3389/fpls.2016.00230.
- Nürnberg, T., and Kemmerling, B. (2009). “Pathogen-Associated Molecular Patterns (PAMP) and PAMP-Triggered Immunity,” in *Annual Plant Reviews online*, ed. J. A. Roberts (Chichester, UK: John Wiley & Sons, Ltd), 16–47. doi:10.1002/9781119312994.apr0362.
- O’Brien, J. A., and Benková, E. (2013). Cytokinin cross-talking during biotic and abiotic stress responses. *Front. Plant Sci.* 4. doi:10.3389/fpls.2013.00451.
- Oide, S., Bejai, S., Staal, J., Guan, N., Kaliff, M., and Dixelius, C. (2013). A novel role of PR2 in abscisic acid (ABA) mediated, pathogen-induced callose deposition in *Arabidopsis thaliana*. *New Phytol* 200, 1187–1199. doi:10.1111/nph.12436.
- Oirdi, M. E., Rahman, T. A. E., Rigano, L., Hadrami, A. E., Rodriguez, M. C., Daayf, F., et al. (2011). *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato. *The Plant Cell* 23, 18.
- Omar, N. H., Mohd, M., Mohamed Nor, N. M. I., and Zakaria, L. (2018). Characterization and pathogenicity of *Fusarium* species associated with leaf spot of mango (*Mangifera indica* L.). *Microbial Pathogenesis* 114, 362–368. doi:10.1016/j.micpath.2017.12.026.
- OMS (2018). Note de sécurité sanitaire des aliments - Les fumonisines. Organisation Mondiale de la Santé Available at: www.who.int/foodsafety/areas_work/chemical-risks/en/.
- Osborne, L. E., and Stein, J. M. (2007). Epidemiology of *Fusarium* head blight on small-grain cereals. *International Journal of Food Microbiology* 119, 6. doi:10.1016/j.ijfoodmicro.2007.07.032.
- Paccanaro, M. C., Sella, L., Castiglioni, C., Giacomello, F., Martínez-Rocha, A. L., D’Ovidio, R., et al. (2017). Synergistic effect of different plant cell wall-degrading enzymes is important for virulence of *Fusarium graminearum*. *MPMI* 30, 886–895. doi:10.1094/MPMI-07-17-0179-R.

- Palmer, T. M., Riginos, C., Damiani, R. E., Morgan, N., Lemboi, J. S., Lengingiro, J., et al. (2017). Influence of neighboring plants on the dynamics of an ant-acacia protection mutualism. *Ecology* 98, 3034–3043. doi:10.1002/ecy.2008.
- Parnell, S., Gottwald, T. R., Gilligan, C. A., Cunniffe, N. J., and van den Bosch, F. (2010). The effect of landscape pattern on the optimal eradication zone of an invading epidemic. *Phytopathology*® 100, 638–644. doi:10.1094/PHYTO-100-7-0638.
- Patkar, R. N., Benke, P. I., Qu, Z., Constance Chen, Y. Y., Yang, F., Swarup, S., et al. (2015). A fungal monooxygenase-derived jasmonate attenuates host innate immunity. *Nat Chem Biol* 11, 733–740. doi:10.1038/nchembio.1885.
- Patkar, R. N., and Naqvi, N. I. (2017). Fungal manipulation of hormone-regulated plant defense. *PLoS Pathog* 13, e1006334. doi:10.1371/journal.ppat.1006334.
- Pearce, R. B., Strange, R. N., and Smith, H. (1976). Glycinebetaine and choline in wheat: Distribution and relation to infection by *Fusarium graminearum*. *Phytochemistry* 15, 953–954. doi:10.1016/S0031-9422(00)84377-0.
- Pellan, L., Durand, N., Schorr-Galindo, S., and Strub, C. (2020). Commercial biocontrol agents reveal contrasting compartments against two mycotoxigenic fungi in cereals: *Fusarium graminearum* and *Fusarium verticillioides*. *Toxins* 12, 22. doi:10.3390/toxins12030152.
- Peng, X.-L., Xu, W.-T., Wang, Y., Huang, K.-L., Liang, Z., Zhao, W., et al. (2010). Mycotoxin ochratoxin A-induced cell death and changes in oxidative metabolism of *Arabidopsis thaliana*. *Plant Cell Rep* 29, 153–161. doi:10.1007/s00299-009-0808-x.
- Per, T. S., Fatma, M., Asgher, Mohd., Javied, S., and Khan, N. A. (2017). “Salicylic acid and nutrients interplay in abiotic stress tolerance,” in *Salicylic Acid: A Multifaceted Hormone*, eds. R. Nazar, N. Iqbal, and N. A. Khan (Singapore: Springer Singapore), 221–237. doi:10.1007/978-981-10-6068-7_11.
- Peraza-Reyes, L. (2013). Peroxisomes and sexual development in fungi. *Front. Physiol.* 4, 16. doi:10.3389/fphys.2013.00244.
- Perincherry, L., Lalak-Kańczugowska, J., and Stępień, Ł. (2019). *Fusarium*-produced mycotoxins in plant-pathogen interactions. *Toxins* 11. doi:10.3390/toxins11110664.
- Periyannan, S., Milne, R. J., Figueroa, M., Lagudah, E. S., and Dodds, P. N. (2017). An overview of genetic rust resistance: From broad to specific mechanisms. *PLoS Pathog* 13, e1006380. doi:10.1371/journal.ppat.1006380.
- Perryman, S. A. M., Clark, S. J., and West, J. S. (2014). Splash dispersal of *Phyllosticta citricarpa* conidia from infected citrus fruit. *SCIENTIFIC REPORTS* 4, 8. doi:10.1038/srep06568.

- Picot, A., Hourcade-Marcolla, D., Barreau, C., Pinson-Gadais, L., Caron, D., Richard-Forget, F., et al. (2012). Interactions between *Fusarium verticillioides* and *Fusarium graminearum* in maize ears and consequences for fungal development and mycotoxin accumulation: *Fusarium* spp. interactions in maize ears. *Plant Pathology* 61, 140–151. doi:10.1111/j.1365-3059.2011.02503.x.
- Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., and Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52, 347–375. doi:10.1146/annurev-phyto-082712-102340.
- Ploetz, R. C. (2006). *Fusarium*-induced diseases of tropical, perennial crops. *Phytopathology*® 96, 648–652. doi:10.1094/PHYTO-96-0648.
- Pöggeler, S., Nowrousian, M., Teichert, I., Beier, A., and Kück, U. (2018). “Fruiting-body development in Ascomycetes,” in *The Mycota XV: Physiology and Genetics, 2nd Edition*, eds. T. Anke, and A. Schöffler (Springer, Cham), 56.
- Pogorelko, G., Lionetti, V., Bellincampi, D., and Zobotina, O. (2013). Cell wall integrity: Targeted post-synthetic modifications to reveal its role in plant growth and defense against pathogens. *Plant Signaling & Behavior* 8, e25435. doi:10.4161/psb.25435.
- Proctor, R. H., McCormick, S. P., Kim, H.-S., Cardoza, R. E., Stanley, A. M., Lindo, L., et al. (2018). Evolution of structural diversity of trichothecenes, a family of toxins produced by plant pathogenic and entomopathogenic fungi. *PLoS Pathog* 14, e1006946. doi:10.1371/journal.ppat.1006946.
- Proctor, R. H., Plattner, R. D., Desjardins, A. E., Busman, M., and Butchko, R. A. E. (2006). Fumonisin production in the maize pathogen *Fusarium verticillioides* : Genetic basis of naturally occurring chemical variation. *J. Agric. Food Chem.* 54, 2424–2430. doi:10.1021/jf0527706.
- Qiao, H., Shen, Z., Huang, S. -s. C., Schmitz, R. J., Urich, M. A., Briggs, S. P., et al. (2012). Processing and subcellular trafficking of ER-Tethered EIN2 control response to ethylene gas. *Science* 338, 390–393. doi:10.1126/science.1225974.
- Ramires, F., Masiello, M., Somma, S., Villani, A., Susca, A., Logrieco, A., et al. (2018). Phylogeny and mycotoxin characterization of *Alternaria* species isolated from wheat grown in Tuscany, Italy. *Toxins* 10, 472. doi:10.3390/toxins10110472.
- Ren, Y., Yao, M., Chang, P., Sun, Y., Li, R., Meng, D., et al. (2021). Isolation and characterization of a *Pseudomonas poae* JSU-Y1 with patulin degradation ability and biocontrol potential against *Penicillium expansum*. *Toxicon* 195, 1–6. doi:10.1016/j.toxicon.2021.02.014.
- Reverberi, M., Ricelli, A., Zjalic, S., Fabbri, A. A., and Fanelli, C. (2010). Natural functions of mycotoxins and control of their biosynthesis in fungi. *Appl Microbiol Biotechnol* 87, 899–911. doi:10.1007/s00253-010-2657-5.

- Ries, L. N. A., Beattie, S. R., Espeso, E. A., Cramer, R. A., and Goldman, G. H. (2016). Diverse regulation of the CreA carbon catabolite repressor in *Aspergillus nidulans*. *Genetics* 203, 335–352. doi:10.1534/genetics.116.187872.
- Rieux, A., Soubeyrand, S., Bonnot, F., Klein, E. K., Ngando, J. E., Mehl, A., et al. (2014). Long-distance wind-dispersal of spores in a fungal plant pathogen: Estimation of anisotropic dispersal kernels from an extensive field experiment. *PLoS ONE* 9, e103225. doi:10.1371/journal.pone.0103225.
- Rohrbach, K. G., and Pfeiffer, J. B. (1976). Susceptibility of pineapple cultivars to fruit diseases incited by *Penicillium funiculosum* and *Fusarium moniliforme*. *Phytopathology*® 66, 1386–1390. doi:10.1094/Phyto-66-1386.
- Rohrbach, K. G., and Taniguchi, G. (1984). Effects of temperature, moisture, and stage of inflorescence development on infection of pineapple by *Penicillium funiculosum* and *Fusarium moniliforme* var. *subglutinans*. *Phytopathology* 74, 995–1000.
- Ruan, J., Zhou, Y., Zhou, M., Yan, J., Khurshid, M., Weng, W., et al. (2019). Jasmonic acid signaling pathway in plants. *IJMS* 20, 2479. doi:10.3390/ijms20102479.
- Ryan, C. A., and Moura, D. S. (2002). Systemic wound signaling in plants: A new perception. *Proceedings of the National Academy of Sciences* 99, 6519–6520. doi:10.1073/pnas.112196499.
- Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J., and Dowling, D. N. (2008). Bacterial endophytes: recent developments and applications. *FEMS Microbiol Lett* 278, 1–9. doi:10.1111/j.1574-6968.2007.00918.x.
- Rzewuski, G., and Sauter, M. (2008). Ethylene biosynthesis and signaling in rice. *Plant Science* 175, 32–42. doi:10.1016/j.plantsci.2008.01.012.
- Saijo, Y., Loo, E. P., and Yasuda, S. (2018). Pattern recognition receptors and signaling in plant-microbe interactions. *Plant J* 93, 592–613. doi:10.1111/tpj.13808.
- Salzman, R. A., Brady, J. A., Finlayson, S. A., Buchanan, C. D., Summer, E. J., Sun, F., et al. (2005). Transcriptional profiling of sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses. *Plant Physiol.* 138, 352–368. doi:10.1104/pp.104.058206.
- Samson, R. A., Yilmaz, N., Houbraeken, J., Spierenburg, H., Seifert, K. A., Peterson, S. W., et al. (2011). Phylogeny and nomenclature of the genus *Talaromyces* and taxa accommodated in *Penicillium* subgenus *Biverticillium*. *Studies in Mycology* 70, 159–183. doi:10.3114/sim.2011.70.04.
- Sánchez-Muñoz, S., Mariano-Silva, G., Leite, M. O., Mura, F. B., Verma, M. L., da Silva, S. S., et al. (2020). “Production of fungal and bacterial pigments and their applications,” in *Biotechnological Production of Bioactive Compounds* (Elsevier), 327–361. doi:10.1016/B978-0-444-64323-0.00011-4.

- Sapoukhina, N., Tyutyunov, Y., Sache, I., and Arditi, R. (2010). Spatially mixed crops to control the stratified dispersal of airborne fungal diseases. *Ecological Modelling* 221, 2793–2800. doi:10.1016/j.ecolmodel.2010.08.020.
- Sarrocco, S., Matarese, F., Baroncelli, R., Vannacci, G., Seidl-Seiboth, V., Kubicek, C. P., et al. (2017). The constitutive endopolygalacturonase TvPG2 regulates the induction of plant systemic resistance by *Trichoderma virens*. *Phytopathology*® 107, 537–544. doi:10.1094/PHYTO-03-16-0139-R.
- Seifert, K. A., Aoki, T., Baayen, R. P., Brayford, D., Burgess, L. W., Chulze, S., et al. (2003). The name *Fusarium moniliforme* should no longer be used. *Mycological Research* 107, 643–644. doi:10.1017/S095375620323820X.
- Semeiks, J., Borek, D., Otwinowski, Z., and Grishin, N. V. (2014). Comparative genome sequencing reveals chemotype-specific gene clusters in the toxigenic black mold *Stachybotrys*. *BMC Genomics* 15, 590. doi:10.1186/1471-2164-15-590.
- Shameer, S., and Prasad, T. N. V. K. V. (2018). Plant growth promoting rhizobacteria for sustainable agricultural practices with special reference to biotic and abiotic stresses. *Plant Growth Regul* 84, 603–615. doi:10.1007/s10725-017-0365-1.
- Sheard, L. B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T. R., et al. (2010). Jasmonate perception by inositol-phosphate-potentiated COI1–JAZ co-receptor. *Nature* 468, 400–405. doi:10.1038/nature09430.
- Shearer, C. A. (1995). Fungal competition. *Can. J. Bot.* 73, 1259–1264. doi:10.1139/b95-386.
- Shen, Q., Liu, Y., and Naqvi, N. I. (2018). Fungal effectors at the crossroads of phytohormone signaling. *Current Opinion in Microbiology* 46, 1–6. doi:10.1016/j.mib.2018.01.006.
- Skadsen, R. W., and Hohn, T. M. (2004). Use of *Fusarium graminearum* transformed with gfp to follow infection patterns in barley and *Arabidopsis*. *Physiological and Molecular Plant Pathology* 64, 45–53. doi:10.1016/j.pmpp.2004.04.003.
- Spoel, S. H., and Dong, X. (2008). Making sense of hormone crosstalk during plant immune responses. *Cell Host & Microbe* 3, 348–351. doi:10.1016/j.chom.2008.05.009.
- Spoel, S. H., Johnson, J. S., and Dong, X. (2007). Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proceedings of the National Academy of Sciences* 104, 18842–18847. doi:10.1073/pnas.0708139104.
- Stępień, Ł., Koczyk, G., and Waśkiewicz, A. (2011a). FUM cluster divergence in fumonisins-producing *Fusarium* species. *Fungal Biology* 115, 112–123. doi:10.1016/j.funbio.2010.10.011.
- Stępień, Ł., Koczyk, G., and Waśkiewicz, A. (2011b). Genetic and phenotypic variation of *Fusarium proliferatum* isolates from different host species. *J Appl Genetics* 52, 487–496. doi:10.1007/s13353-011-0059-8.

- Stępień, Ł., Koczyk, G., and Waśkiewicz, A. (2013). Diversity of *Fusarium* species and mycotoxins contaminating pineapple. *J Appl Genetics* 54, 367–380. doi:10.1007/s13353-013-0146-0.
- Stępień, Ł., and Waśkiewicz, A. (2013). Sequence divergence of the enniatin synthase gene in relation to production of beauvericin and enniatins in *Fusarium* species. *Toxins* 5, 19. doi:10.3390/toxins5030537.
- Stotz, H. U., Thomson, J., and Wang, Y. (2009). Plant defensins: Defense, development and application. *Plant Signaling & Behavior* 4, 1010–1012. doi:10.4161/psb.4.11.9755.
- Strange, R. N., Majer, J. R., and Smith, H. (1974). The isolation and identification of choline and betaine as the two major components in anthers and wheat germ that stimulate *Fusarium graminearum* in vitro. *Physiological Plant Pathology* 4, 277–290. doi:10.1016/0048-4059(74)90015-0.
- Studt, L., Wiemann, P., Kleigrewe, K., Humpf, H.-U., and Tudzynski, B. (2012). Biosynthesis of fusarubins accounts for pigmentation of *Fusarium fujikuroi* perithecia. *Appl. Environ. Microbiol.* 78, 4468–4480. doi:10.1128/AEM.00823-12.
- Summerell, B. A., and Leslie, J. F. (2011). Fifty years of *Fusarium*: How could nine species have ever been enough? *Fungal Diversity* 50, 135–144. doi:10.1007/s13225-011-0132-y.
- Tamás, L., Čiamporová, M., and Luxová, M. (1998). Accumulation of pathogenesis-related proteins in barley leaf intercellular spaces during leaf senescence. *Biologia Plantarum* 41, 451–460.
- Tao, Y., Yuan, F., Leister, R. T., Ausubel, F. M., and Katagiri, F. (2000). Mutational analysis of the Arabidopsis nucleotide binding site–leucine-rich repeat resistance gene RPS2. *The Plant Cell* 12, 2541–2554.
- Tao, Z., Ying, Z., XiaoPeng, J., JinTao, L., Hong, G., FuHang, S., et al. (2013). Cloning and characterization of the gene cluster required for beauvericin biosynthesis in *Fusarium proliferatum*. *Sci. China Life Sci.* 56, 10. doi:10.1007/s11427-013-4505-1.
- Tarr, D. E. K., and Alexander, H. M. (2009). TIR-NBS-LRR genes are rare in monocots: evidence from diverse monocot orders. *BMC Res Notes* 2, 197. doi:10.1186/1756-0500-2-197.
- Teisson, C. (2002). Examen des nuisances sur ananas en Afrique de l’Ouest. Montpellier: CIRAD-FLHOR.
- Torres, M. A., Jones, J. D. G., and Dangl, J. L. (2006). Reactive oxygen species signaling in response to pathogens. *Plant Physiol.* 141, 373–378. doi:10.1104/pp.106.079467.
- Tudzynski, B. (2014). Nitrogen regulation of fungal secondary metabolism in fungi. *Front. Microbiol.* 5, 15. doi:10.3389/fmicb.2014.00656.
- Turzhanova, A., Khapilina, O. N., Tumenbayeva, A., Shevtsov, V., Raiser, O., and Kalendar, R. (2020). Genetic diversity of *Alternaria* species associated with black point in wheat grains. *PeerJ* 8, e9097. doi:10.7717/peerj.9097.

- Umemura, K., Satou, J., Iwata, M., Uozumi, N., Koga, J., Kawano, T., et al. (2009). Contribution of salicylic acid glucosyltransferase, OsSGT1, to chemically induced disease resistance in rice plants. *The Plant Journal* 57, 463–472. doi:10.1111/j.1365-3113X.2008.03697.x.
- Uroz, S., Courty, P. E., and Oger, P. (2019). Plant symbionts are engineers of the plant-associated microbiome. *Trends in Plant Science* 24, 905–916. doi:10.1016/j.tplants.2019.06.008.
- van Damme, M., Bozkurt, T. O., Cakir, C., Schornack, S., Sklenar, J., Jones, A. M. E., et al. (2012). The Irish potato famine pathogen *Phytophthora infestans* translocates the CRN8 kinase into host plant cells. *PLoS Pathog* 8, e1002875. doi:10.1371/journal.ppat.1002875.
- van Loon, L. C., Rep, M., and Pieterse, C. M. J. (2006). Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* 44, 135–162. doi:10.1146/annurev.phyto.44.070505.143425.
- van Loon, L. C., and van Kammen, A. (1970). Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. ‘Samsun’ and ‘Samsun NN’: II. Changes in protein constitution after infection with tobacco mosaic virus. *Virology* 40, 199–211. doi:https://doi.org/10.1016/0042-6822(70)90395-8.
- van Loon, L. C., and van Strien, E. A. (1999). The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiological and Molecular Plant Pathology* 55, 85–97. doi:10.1006/pmpp.1999.0213.
- van Schie, C. C. N., and Takken, F. L. W. (2014). Susceptibility genes 101: How to be a good host. *Annu. Rev. Phytopathol.* 52, 551–581. doi:10.1146/annurev-phyto-102313-045854.
- Vanholme, R., Demedts, B., Morreel, K., Ralph, J., and Boerjan, W. (2010). Lignin biosynthesis and structure. *Plant Physiol.* 153, 895–905. doi:10.1104/pp.110.155119.
- Vanhoutte, I., Audenaert, K., and De Gelder, L. (2016). Biodegradation of mycotoxins: Tales from known and unexplored worlds. *Front. Microbiol.* 7, 20. doi:10.3389/fmicb.2016.00561.
- Varbanova, M., Porter, K., Lu, F., Ralph, J., Hammerschmidt, R., Jones, A. D., et al. (2011). Molecular and biochemical basis for stress-induced accumulation of free and bound p-coumaraldehyde in cucumber. *Plant Physiol.* 157, 1056–1066. doi:10.1104/pp.111.184358.
- Venkatesh, N., and Keller, N. P. (2019). Mycotoxins in conversation with bacteria and fungi. *Front. Microbiol.* 10, 10. doi:10.3389/fmicb.2019.00403.
- Ventura, J. A., Zambolim, L., and Chaves, G. M. (1993). Integrated management system for pineapple *Fusarium* disease control. *Acta Horticulturae* 334, 439–454. doi:10.17660/ActaHortic.1993.334.48.

- Verma, V. C., Singh, S. K., and Prakash, S. (2011). Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. *J. Basic Microbiol.* 51, 550–556. doi:10.1002/jobm.201000155.
- Vleesschauwer, D. D., Sei, H. S., Demeestere, K., and Höfte, M. (2016). The DELLA protein SLR1 integrates and amplifies salicylic acid- and jasmonic acid-dependent innate immunity in rice. *Plant Physiol.* 170, 1831–1847. doi:10.1104/pp.15.01515.
- Wang, J., and Chai, J. (2020). Structural insights into the plant immune receptors PRRs and NLRs. *Plant Physiol.* 182, 1566–1581. doi:10.1104/pp.19.01252.
- Weller, D. M. (2007). *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology*® 97, 250–256. doi:10.1094/PHYTO-97-2-0250.
- Wicklow, D. (1992). “Interference competition,” in *The fungal community its organization and role in the ecosystem - 2nd edition* Mycology series., eds. G. Carroll and D. Wicklow (New York), 11.
- Wildermuth, M. C., Dewdney, J., Wu, G., and Ausubel, F. M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature* 414, 562–565. doi:10.1038/35107108.
- Willige, B. C., Isono, E., Richter, R., Zourelidou, M., and Schwechheimer, C. (2011). Gibberellin regulates PIN-FORMED abundance and is required for auxin transport-dependent growth and development in *Arabidopsis thaliana*. *The Plant Cell* 23, 2184–2195. doi:10.1105/tpc.111.086355.
- Wollenberg, R. D., Taft, M. H., Giese, S., Thiel, C., Balázs, Z., Giese, H., et al. (2018). Phenamacril is a reversible and noncompetitive inhibitor of *Fusarium* class I myosin. *J. Biol. Chem.* 10. doi:10.1074/jbc.RA118.005408.
- Wyatt, T. T., Wösten, H. A. B., and Dijksterhuis, J. Fungal spores for dispersion in space and time. *Adv Appl Microbiol* 85, 49. doi:10.1016/b978-0-12-407672-3.00002-2.
- Yang, D.-L., Yao, J., Mei, C.-S., Tong, X.-H., Zeng, L.-J., Li, Q., et al. (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *PNAS* 109, E1192–E1200. doi:10.1073/pnas.1201616109.
- Yang, M., Zhang, H., Kong, X., van der Lee, T., Waalwijk, C., van Diepeningen, A., et al. (2018). Host and cropping system shape the *Fusarium* population: 3ADON-producers are ubiquitous in wheat whereas NIV-producers are more prevalent in rice. *Toxins* 10, 115. doi:10.3390/toxins10030115.
- Yang, Y., Qi, M., and Mei, C. (2004). Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress: Anti-oxidative role of salicylic acid in rice. *The Plant Journal* 40, 909–919. doi:10.1111/j.1365-313X.2004.02267.x.

- Yilmaz, N., Hagen, F., Meis, J. F., Houbraeken, J., and Samson, R. A. (2016). Discovery of a sexual cycle in *Talaromyces amestolkiae*. *Mycologia* 108, 70–79. doi:10.3852/15-014.
- Yilmaz, N., Houbraeken, J., Hoekstra, E. S., Frisvad, J. C., Visagie, C. M., and Samson, R. A. (2012). Delimitation and characterisation of *Talaromyces purpurogenus* and related species. *Pers - Int Mycol J* 29, 39–54. doi:10.3767/003158512X659500.
- Yilmaz, N., Visagie, C. M., Houbraeken, J., Frisvad, J. C., and Samson, R. A. (2014). Polyphasic taxonomy of the genus *Talaromyces*. *Studies in Mycology* 78, 175–341. doi:10.1016/j.simyco.2014.08.001.
- Yu, J.-H., and Keller, N. (2005). Regulation of secondary metabolism in filamentous fungi. *Annu. Rev. Phytopathol.* 43, 437–458. doi:10.1146/annurev.phyto.43.040204.140214.
- Yuan, Y., Wang, X., Hatab, S., Wang, Z., Wang, Y., Luo, Y., et al. (2014). Patulin reduction in apple juice by inactivated *Alicyclobacillus* spp. *Lett Appl Microbiol* 59, 604–609. doi:10.1111/lam.12315.
- Zhan, R.-L., Yang, S.-J., Ho, H.-H., Liu, F., Zhao, Y.-L., Chang, J.-M., et al. (2010). Mango malformation disease in south China caused by *Fusarium proliferatum*. *Journal of Phytopathology* 158, 721–725. doi:10.1111/j.1439-0434.2010.01688.x.
- Zhang, H., Van der Lee, T., Waalwijk, C., Chen, W., Xu, J., Xu, J., et al. (2012). Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. *PLoS ONE* 7, e31722. doi:10.1371/journal.pone.0031722.
- Zhang, J., Liu, J., and Ming, R. (2014). Genomic analyses of the CAM plant pineapple. *Journal of Experimental Botany* 65, 3395–3404. doi:10.1093/jxb/eru101.
- Zheng, H., Kim, J., Liew, M., Yan, J. K., Herrera, O., Bok, J. W., et al. (2015). Redox metabolites signal polymicrobial biofilm development via the NapA oxidative stress cascade in *Aspergillus*. *Current Biology* 25, 29–37. doi:10.1016/j.cub.2014.11.018.
- Zheng, Z., Appiano, M., Pavan, S., Bracuto, V., Ricciardi, L., Visser, R. G. F., et al. (2016). Genome-wide study of the tomato SIMLO gene family and its functional characterization in response to the powdery Mildew fungus *Oidium neolycopersici*. *Front. Plant Sci.* 7. doi:10.3389/fpls.2016.00380.
- Zipfel, C. (2009). Early molecular events in PAMP-triggered immunity. *Current Opinion in Plant Biology* 12, 414–420. doi:10.1016/j.pbi.2009.06.003.
- Ziv, C., Zhao, Z., Gao, Y. G., and Xia, Y. (2018). Multifunctional roles of plant cuticle during plant-pathogen interactions. *Front. Plant Sci.* 9, 1088. doi:10.3389/fpls.2018.01088.

CONTRIBUTION SCIENTIFIQUE

Articles acceptés dans des revues internationales à comité de lecture :

- 1| **Vignassa, M.**, Meile, J.-C., Chiroleu, F., Soria, C., Leneveu-Jenvrin, C., Schorr-Galindo, S., and Chillet, M. (2021). Pineapple Mycobiome Related to Fruitlet Core Rot Occurrence and the Influence of Fungal Species Dispersion Patterns. *J. Fungi* 7, 175. doi:10.3390/jof7030175.

- 2| Fabre, F., **Vignassa, M.**, Urbach, S., Langin, T., and Bonhomme, L. (2019). Time-resolved dissection of the molecular crosstalk driving *Fusarium* head blight in wheat provides new insights into host susceptibility determinism. *Plant Cell Environ* 42, 2291–2308. doi:10.1111/pce.13549.

Communications orales lors de congrès :

Certains de ces travaux de thèse devaient être présentés lors des congrès indiqués ci-dessous. Ces derniers ont toutefois été annulés suite à la pandémie du COVID-19.

- 1| Xth International Pineapple Symposium, (Uvero Alto, Dominican Republic), 18 – 22 Août 2020.

- 2| 8^{ème} édition des Rencontres de l'Agroalimentaire en Océan Indien, (Antananarivo, Madagascar), 23 – 24 novembre 2020.

ABSTRACT

In Reunion Island, pineapple crops are exposed to high parasitic pressure promoted by the subtropical climate of the island. The Fruitlet Core Rot (FCR) disease is caused by a set of fungal pathogenic species in which *Fusarium ananatum* has been the most described so far. The development of brown discoloration in mature fruits represents a major issue affecting notably the quality of the 'Queen Victoria' pineapple cultivar due to its high susceptibility to FCR. Until now, the management of epidemics lies on the combination of suitable agricultural practices and the use of fungicide treatments. Nevertheless, these strategies are unsuccessful in the presence of climatic conditions that favor the development and dispersion of causal agents. Mycotoxins accumulation in the flesh of infected fruits is also of concern in the preservation of sanitary quality of fruit productions. In order to develop novel alternatives for sustainable sources of FCR resistance, my research work focused on the determinants of 'Queen Victoria' pineapple susceptibility.

An epidemiological approach permitted to establish that Fruitlet Core Rot occurrence is positively correlated to contamination patterns resulting from aerial dispersion of the pathogen spores. Moreover, the prevalence of fungal species belonging to the complexes *Fusarium fujikuroi* and *Talaromyces purpureogenus* within the fruit mycobiome have demonstrated the role of a pathogenic fungal set composed of *Fusarium proliferatum*, *Fusarium ananatum*, *Fusarium oxysporum*, *Fusarium sacchari*, *Talaromyces stollii* and *Talaromyces amestolkiae* in the disease expression. The *in vitro* study of interaction profiles between four of those species have evidenced the growth antagonism of *T. stollii* on the pathogenic *Fusarium* species. Significant variations of mycotoxin contents (fumonisins B₁, B₂ and beauvericin) were also measured during dual culture of pathogens. Finally, the analysis based on varietal comparison of the molecular signal promoting early defense responses show that susceptibility of 'Queen Victoria' cultivar is partly supported by a low constitutive expression of genes involved in the synthesis of PR proteins. The results suggest a fungal strategy based on the repression of defense signal transduction in pineapple during the first 72 hours of the host - pathogen interaction leading to the disease establishment.

Keywords : *Ananas comosus*, Fruitlet Core Rot, *Fusarium*, Host – pathogen interaction, *Talaromyces*

RÉSUMÉ

Les cultures d'ananas de la Réunion sont soumises à une forte pression parasitaire favorisée par le climat subtropical de l'île. La maladie de la tache noire est causée par un cortège de champignons filamenteux dont *Fusarium ananatum* est l'espèce la plus décrite à ce jour. Le développement de taches noires dans les fruits matures constitue une problématique majeure de par son impact sur la qualité de l'ananas 'Queen Victoria' présentant une forte sensibilité à cette pathologie. La gestion des épisodes épidémiques repose actuellement sur des méthodes associant des pratiques culturales adaptées et l'utilisation de fongicides. Toutefois, ces stratégies s'avèrent infructueuses pour des conditions climatiques fortement favorables au développement et à la dispersion des agents pathogènes. L'accumulation de mycotoxines au sein des tissus infectés représente également une préoccupation d'envergure dans la préservation de la sécurité sanitaire des productions d'ananas. Dans l'objectif de développer de nouveaux leviers de résistance plus durables, des recherches visant à caractériser les déterminants de la sensibilité de l'ananas 'Queen Victoria' ont été menées sur chaque composante du pathosystème.

Une approche épidémiologique a permis d'établir que l'occurrence de la tache noire est positivement corrélée à une contamination résultant d'une dispersion aérienne des spores d'espèces pathogènes. De plus, la prédominance d'espèces fongiques appartenant aux complexes *Fusarium fujikuroi* et *Talaromyces purpureogenus* au sein du mycobiome du fruit a démontré l'implication d'un cortège pathogène constitué de *Fusarium proliferatum*, *Fusarium ananatum*, *Fusarium oxysporum*, *Fusarium sacchari*, *Talaromyces stollii* et *Talaromyces amestolkiae* dans l'expression de cette pathologie. L'étude *in vitro* des profils d'interaction entre quatre de ces espèces a mis en évidence le pouvoir antagoniste de *T. stollii* sur la croissance des espèces pathogènes de *Fusarium*. D'importantes variations dans les concentrations en mycotoxines (fumonisines B₁, B₂ et beauvericine) ont également été mesurées au cours de confrontations entre les pathogènes. Enfin, l'analyse par comparaison variétale des voies de signalisation moléculaire conditionnant les réponses de défense précoces montre que la sensibilité du cultivar 'Queen Victoria' est en partie imputable à une faible expression constitutive des gènes impliqués dans la synthèse de protéines PR. Les résultats obtenus suggèrent la mise en œuvre d'une stratégie fongique basée sur la répression des signaux de défenses transduits chez l'ananas au cours des 72 premières heures de l'interaction hôte – pathogène menant à l'établissement de la maladie.

Mots clés : *Ananas comosus*, *Fusarium*, interaction hôte-pathogène, maladie de la tache noire, *Talaromyces*