New species and populations in *Fusarium*: examples from the tropics

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Problems and constraints with regard to information on tropical species, populations and plant diseases

1. Data on geographical distribution of species and diversity in the tropics are more related to research activities than to facts;

2. Many regions are absolutely under-investigated. This is true also for the genus *Fusarium*;
Problems and constraints with regard to information on tropical species, populations and plant diseases

3. Causal agents of diseases are not always the same in every country, region or continent;

4. Names used in older reports, like *Fusarium moniliforme* or *Fusarium roseum*, are no longer in use, and stand for a large diversity of different forms and populations.
Fusarium is a typical anamorph form-genus, with species sharing morphological markers.
Telomorphs of *Fusarium* species

**Gibberella**  **Haematonecctria**  **Albonectria**
Why is Fusarium important?

- Biology and ecology
- Economical importance
- Model for species concepts and molecular characterization

Plant pathogen

- Soil fungus → soil and rhizosphere, root rots
- Vascular fungus → wilts, dieback
- Endophyte → plants, seeds, etc.
- Toxin producer → contamination of plant products
- Human pathogen → nosocomial infection
Examples of species with mostly tropical and subtropical distribution:

- *Fusarium lateritium, Fusarium stilboides*
- *Fusarium guttiforme, Fusarium ananatum* - GFC
- *Fusarium mangiferae, F. sterilhyphosum* - GFC
- *Fusarium decemcellulare – Albonectria rigidiuscula*

Form-species, a high diversity of typically tropical populations, many phylogenetic or biological species:

- *Fusarium oxysporum*
- *Fusarium solani – Haematonectria / Neocosmospora*
Species Concepts

- **Morphological Species Concept – MSC**
  Population shares morphological characters

- **Biological Species Concept - BSC**
  Population delimited by reproductive barrier – *mating population*

- **Phylogenetic Species Concept - PSC**
  Population delimited by concordance in groupings generated based on DNA sequences - clades
Morphological Species

Fusarium solani

Morphological markers:
- macro- and microconidia frequent
- chlamydospores frequent
- monophialides in the aerial mycelium are long

Many host plants
- some *formeae speciales*
Also clinical
→ Species Complex
Biological Species

*Biological Species Concept* - BSC

Population of individuals that breed and form fertile descendents

Population is delimited by a reproductive barrier *mating population or breeding population*

Ex. Gibberella fujikuroi Complex
Phylogenetic Species

*Phylogenetic Species Concept* - PSC

Population delimited by concordance in groupings generated based on DNA sequences - clades

Population of individuals that share apomorphmic characters

p.ex. substitution of nucleotides etc.

Geneological Concordance Phylogenetic Species Recognition

Taylor et al. 2000. Fungal Genetics Biology
Fusarium graminearum
Wheat pathogen

PNAS
Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade

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FGB
Examples of tropical crops which develop diseases, caused by *Fusarium* species:

i. **Fruit crops:**
   Mango, pineapple, banana, passionfruit

ii. **Palm trees:**
    oil palm, date palm, canary palm

iii. **Coffee and Cocoa**

iv. **Spices, others:**
    Black pepper, tobacco, vanilla, cotton

v. **Monocots:** Sugarcane, rice, sorghum, maize
Examples of tropical crops which develop diseases, caused by *Fusarium* species:

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Etiology of Mango Malformation in Brazil

Mangifera indica L.

Healthy tree

Symptoms of malformation

Floral - inflorescence
Vegetative - shoot
Objectives

- To identify the causal agents of mango malformation disease in Brazil
  **Methods** AFLP analysis, Gene Sequencing, Morphological Characterization, Pathogenicity Test

- To induce the sexual stage for the causal agents
  **Methods** Identification of mating type (PCR) and crossing

- To develop a PCR-based method for the detection and identification of the causal agents
  **Methods** primer design, tests for specificity and sensitivity
**Disease Distribution in Brazil**

- **Lima 2006, 59 isolates, 8 states**
- **Cunha et al 2000**
Pathogen x Endophyte’s isolation

Malformed tissue
Fusarium only

Asymptomatic tissue
about 15 species
AFLP analysis of *Fusarium* from mango

*Fusarium* sp. nov.

*F. sterilhyphosum*

*F. mangiferae*
Sequence analysis of genes encoding $\beta$-tubulin and translation elongation factor 1-$\alpha$

Primers tub2 – T1 and T2 - O’Donnell et al., 1998, Mycologia
Primers tef1 – EF1 and EF2 - O’Donnell et al., 1998, PNAS
Sequencing – MegaBACE 500, both directions
Analysis – Maximum Parsimony
Program – PAUP 4 version beta 10
ICCC-12 Session 7B
Species and Populations in Fusarium

MP tree from tef1 + tub2

1252 bp
1 of 2 trees
816 steps
CI 0.659
RI 0.804
HI 0.341

“American”

“Asian”

“African”

Fusarium sp. nov.
F. sterilihyphosum

F. mangiferae

F. pseudoanthophilum
F. brevicatenulatum
F. pseudonygamai
F. verticillioides MP A
F. napiforme
F. ramigenum
F. lactis
F. nygamai MP G
F. pseudocircinatum
F. denticulatum
F. thapsinum MP F
Fusarium sp. NRRL 25221
F. acutatum
F. phyllophilum
F. udum
F. xylarioides
F. dlamini
F. proliferatum MP D
F. globosum
F. fujikuroi MP C
F. concentricum
Fusarium sp. NRRL 25309
MRC 2730
F. sacchari MP B
F. anthophilum
F. succisae
F. bulbicola
F. circinatum MP H
F. bactridioides
F. subglutinans MP E
CML 345
CML 262
CML 389
CML 289
MRC 2802
CML 283
Fusarium sp. NRRL 25346
F. begoniae
F. guttiforme
Maximum Parsimony Tree of Combined tub2 and tef1

F. mangiferae
Ex-paratype MRC 2730

Fusarium sp. nov.

F. sterilihyphosum
Ex-holotype MRC 2802
Crossing

Identification of mating type primers according to Steenkamp et al. (2000)

\[ \text{MAT-2} \quad \text{MAT-1} \]
Fertile crosses only to *Fusarium* sp. nov.
Evidence from morphology, AFLP fingerprint, phylogeny and crossing

_F. mangiferae_ – cosmopolitan, but not found in Brazil

_F. sterilihyphosum_ – South Africa and Brazil

_Gibberella sp. nov._ - Brazil
**Gibberella sp. nov. Lima, Pfenning and Leslie**
**Gibberella** anam. nov. Lima, Pfenning and Leslie
Pathogenicity

Control  Fusarium sp. nov.  F. sterilihyphosum
PCR detection of the causal agents of mango malformation

- Primer Sets designed from the tef1 sequences
  - Fbra – Fusarium sp. nov. and F. sterilihyphosum (~380 bp)
  - Fman – F. mangiferae (~217 bp)

- Specificity – pure DNA from mango pathogens and endophytes

- Sensitivity – DNA dilutions from 20 ng to 1 fg

- Pathogens’ detection in shoot, leaf, bud, flower, fruit peel mango tissue

- Inoculation – according to Freeman et al. (1999)
Specificity

A  Fbra primer set (~380 bp)
B  Fman primer set (~217 bp)

1. Marker
2. Control - water
3. Fusarium sp. nov. (DNA)
4. F. sterilihyphosum (DNA)
5. F. mangiferae (DNA)
6. F. proliferatum
7. F. sacchari
8. F. subglutinans
9. F. pseudocircinatum
10. F. oxysporum
11. F. semitectum
12. F. decemcellulare
13. Neocosmospora sp.
14. Phomopsis mangifera
15. Cladosp. cladosporioides
16. Epiccocum purpurascens
17. Fusiccocum mangiferum
18. Alternaria alternata
19. Coll. gloeosporioides
20. Aspergillus niger
21. Penicillium sp.
22. Pestalotiopsis sp.
23. Chalara fimbriata
24. Lasiodiplodia sp.
25. Phoma sp.
Sensitivity

A. Fbra - *Fusarium* sp. nov.
B. Fbra - *F. sterilihyphosum*
C. Fman - *F. mangiferae*
**Detection of Fusarium sp. nov. and F. sterilihyphosum**

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Lane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium sp. nov. (DNA)</td>
<td>1</td>
</tr>
<tr>
<td>F. sterilihyphosum (DNA)</td>
<td>2</td>
</tr>
<tr>
<td>F. mangiferae (DNA)</td>
<td>3</td>
</tr>
<tr>
<td>Naturally infected panicle</td>
<td>4</td>
</tr>
<tr>
<td>Asymptomatic inflorescence</td>
<td>5</td>
</tr>
<tr>
<td>Malformed plant inoculated with Fusarium sp. nov.</td>
<td>6</td>
</tr>
<tr>
<td>Malformed plant inoculated with F. sterilihyphosum.</td>
<td>7</td>
</tr>
<tr>
<td>Asymptomatic plant</td>
<td>8-9</td>
</tr>
<tr>
<td>Fruit peel</td>
<td>10-11</td>
</tr>
<tr>
<td>Fruit peduncle</td>
<td>12-13</td>
</tr>
<tr>
<td>Mango leaf</td>
<td>14-15</td>
</tr>
</tbody>
</table>

**Fbra primer set**

[Image of gel electrophoresis with bands for each lane]
Detection of *F. mangiferae*

**Fman primer set**

1. *Fusarium sp. nov.* - DNA
2. *F. sterilihyphosum* - DNA
3. *F. mangiferae* - DNA
4. Naturally infected panicle
5. Asymptomatic inflorescence
6. Malformed plant inoculated with *Fusarium sp. nov.*
7. Malformed plant inoculated with *F. sterilihyphosum*
8. Asymptomatic plant
9. Fruit peel
10. Fruit peduncle
11. Mango leaf
Conclusions

- The main causal agent of mango malformation disease in Brazil corresponds to a new Fusarium species within the Gibberella fujikuroi species complex.

- The teleomorph stage of *Fusarium sp. nov.* can be induced in the laboratory.

- *Fusarium sp. nov.* and *F. sterilhyphosum* are the only known causal agents of the disease in Brazil and can be detected through PCR.
Fusariosis of Pineapple - *Ananas comosus*

Most important disease of this crop, restricted to South America?

*Fusarium subglutinans*

*Fusarium subglutinans f.sp. ananas*

*Fusarium guttiforme*

Morphology, Specificity, tub and tef genes

Limited number of isolates

Nirenberg & O’Donnell 1998

*Gibberella fujikuroi* complex – GFC, but no teleomorph known so far
Fusariosis of Pineapple - *Ananas comosus*

Fusarium associated with pineapple

Symptomatology
Fusariosis of Pineapple - *Ananas comosus*

- Collection of isolates from all over Brazil
- Morphological markers
- Pathogenicity tests
- VCG analysis
- AFLP fingerprint
- Phylogenetic analysis of *tub* and *tef* genes
Fusariosis of Pineapple - *Ananas comosus*

Morphology of *Fusarium guttiforme*
Groups evidenced by VCG and AFLP fingerprint analysis
Fusariosis of Pineapple - *Ananas comosus*

tub2 and tef1

*F. guttiforme* and species in the American clade of GFC

CML - Coleção Micológica de Lavras
NRRL - National Center for Agricultural Utilization Research
Fusariosis of Pineapple - Ananas comosus

Conclusions

- *F. guttiforme* confirmed as the main pathogen of *Fusarium* wilt and resinosis of pineapple in Brazil.
- High genetic diversity observed in the population is consistent with the hypothesis that it is near the center of origin, and the possibility of the occurrence of sexual reproduction in species cannot be excluded.
- The observation of two divergent AFLP groups with similarity below 40% in comparison with the main group suggests that there may be distinct populations causing wilt in pineapple in Brazil.

Perspectives and future work

- Further collection, including native species of *Ananas*
- Crossing experiments
- Metabolite profiling
- And …..
Fusarium ananatum sp. nov. in the Gibberella fujikuroi species complex from pineapples with fruit rot in South Africa

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Fusariosis of Pineapple - *Ananas comosus*

Symptoms

Fusarium ananatum

Black spot

Culture morphology
Fusariosis of Pineapple - *Ananas comosus*

Phylogenetic tree of *F. ananatum* and related species produced using parsimony of tef-1a

Informative characters 53
CI = 0.6737
RI = 0.8510
g1 = -0.476216
Panama Disease, Fusariosis, wilt disease

Banana - *Musa* spp.

*Fusarium oxysporum f. sp. cubense*

Teleomorph unknown

Four races ??

- Polyphyletic
- Distinct evolutionary origin
- ... Five species (?), maybe more
Panama disease of banana

O’Donnell et al. 1998
Panama disease of banana

Evolutionary Relationships among the *Fusarium oxysporum* f. sp. *cubense*
Vegetative Compatibility Groups


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Received 16 February 2009/Accepted 21 May 2009

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... clearly showed that ability of *F. oxy. cub* 1 to cause disease on banana has emerged multiple times, independently 2 to cause disease to a specific banana cultivar is also a polyphyletic trait
Panama disease of banana

Disease caused by several pathogens referred to as *Fusarium oxysporum f. sp. cubense*

- *Fusarium oxysporum f. sp. cubense* represents at least five distinct clonal lineages
- Races of *F. oxysporum f. sp. cubense* may belong to different species
- Concept of races is not consistent
- The *formae speciales* concept is not phylogenetically informative

Ploetz 2006
Fusariosis of Passion fruit
*Passiflora* spp. - Disease complex

Causal agents:
1. *Fusarium oxysporum f. sp. passiflorae* - Wilt
2. *Fusarium solani*
   - root rot, stem rot and canker
   - develop to wilting and dieback
   - present also as an endophyte

Nunes & Albuquerque 1995, Pará, Brazil
*Nectria haematococca*

Nirenberg & Brielmeyer-Liebetanz 1996, Germany
*Haematonectria ipomoeae*
Fusariosis of Passion fruit

Wilt syndrom in the field

Photos: Jeferson M. Dariva
Fusariosis of Passion fruit

F. oxysporum f.sp. passiflorae

Fusarium solani

Photos: Jeferson M. Dariva
Morphological markers within the form species *Fusarium solani*

- Growth rate of colony, pigmentation
- Ramification of the conidiophore
- Format, septation and size of micro- and macroconidia
- Specificity, Others?

**Telomorph – Haematonectria**
- Homothallic + heterothallic spp.
- Ascospores
Named species, populations, strains etc.:

Fusarium ambrosium  
Fusarium illudens  
Fusarium martii-phaseoli  
Fusarium striatum (homothallic)  
Fusarium sp.  
Fusarium sp.  
Nectria borneensis  
Nectria plagianthi  
Neocosmospora africana  
N. vasinfected  
N. ornamentata (homothallic)  

India  
Nova Zelandia – tel Nectria illudens  
USA, Phaseolus vulgaris  
Panama – tel Haematonectria ipomoeae  
Guiania  
Venezuela  
Indonesia  
Nova Zelandia  

(O’Donnell 2000)
Named *formae speciales* and *mating populations* in the *Fusarium solani* species complex - FSSC

1. *F. sol. f. sp. batatas* MP-II *Ipomoea batatas*
2. *F. sol. f. sp. cucurbitae* race 1 MP-I *Cucurbita* spp.
3. *F. sol. f. sp. cucurbitae* race 2 MP-V *Cucurbita* spp.
4. *F. sol. f. sp. glycines* MP-VI *Glycine max*
5. *F. sol. f. sp. mori* MP-III *Morus alba*
6. *F. sol. f. sp. piperis* MP-VII *Piper nigrum*
7. *F. sol. f. sp. pisi* MP-IV *Pisum sativum*
9. *F. sol. f. sp. xanthoxyli* MP-IV *Xanthoxylum sp.*
Clade 3

Asian

Africa

S-American

Clade 2 - SDS pathogens soy beans

S-American

Clade 1

New Zealand

O’Donnell 2000
SDS - Sudden death syndrome of soybean

Causal agent:  *Fusarium solani* f.sp. *glycinis*

DOI 10.1007/s10267-005-0235-y

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Takayuki Aoki · Kerry O’Donnell
María Mercedes Scandiani

*Sudden death syndrome of soybean in South America is caused by four species of Fusarium: Fusarium brasiiliense sp. nov., F. cuneirostrum sp. nov., F. tucumaniae, and F. virguliforme*

*Fusarium solani* on soybean and common bean
Morphology; pathogenicity; ITS, 28S, IGS, EF-1α
5 distinct species, only a few isolates from Brazil were included
SDS - Sudden death syndrome of soybean

Aoki et al. 2005
Etiology of Sudden death syndrome of soybean in Brazil

Objectives

- To identify the causal agents of Sudden death syndrome of soybean in Brazil
  
  **Methods** Gene Sequencing, Morphological Characterization, Pathogenicity Test

- To identify biological species - mating populations
  
  **Methods** Identification of mating type (PCR) and crossing
Clade 2

Clade 3

Brazilian Soybean pathogens
Sexual reproduction in the soybean sudden death syndrome pathogen
Morphological characters

Brazilian isolates

Aoki et al; 2003, 2005
**Piper nigrum** - Black Pepper

*Fusarium solani f. sp. piperis*

Most important disease of black pepper in Brazil

Is supposed to be a distinct species within FSSC

Photos: Lahyre I. Gomes
Cocoa - *Theobroma cacao*

*Fusarium decemcellulare*

*Albonectria rigidiuscula*

Disease: cushion gall
Species and Populations in Fusarium
*Fusarium decemcellulare*

Teleomorph *Albonectria rigidiuscula*
Characterization of populations - approaches

- Morphology \textit{MSC}
- AFLP \textit{fingerprint}
- Sequencing and Phylogenetic analysis \textit{PSC}
- Sexual Compatibility, \textit{mating populations} \textit{BSC}
- Vegetative Compatibility, \textit{vegetative compatibility groups}

Phylogenetic Classification System -

only monophyletic taxa are accepted
Characterization of populations and species

The Ideal World – El Mundo Ideal – O Mundo Ideal

MSC = BSC = PSC
Biological Resource Centers:
Gateway to biodiversity and services to innovation in biotechnology

Our research activities:

- Impact of agriculture on soil fungi diversity
- Diversity and specificity of fungal endophytes in crops and plants of natural vegetation
- Metabolite profiling for chemotaxonomy and bioprospection
- Species concepts and characterization of populations of plant pathogens, for the development of PCR-based protocols for diagnostics and quarantine purposes
Biological Resource Centers:
Gateway to biodiversity and services to innovation in biotechnology
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Acknowledgements

Sarah S. Costa and Virginia G. Elizei, PhD students
Kedma S. Matos and Erica Sfalsin, Master students
Edson L. Rezende, Ana Karla F. Machado, Culture Collection CML
Lucas M. Abreu, Pos-Doc

Cristiano S. Lima - UFRPE, Recife PE, Brazil
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