



Exploration of microbial communities associated to Fruitlet Core Rot (FCR) disease in 'Queen' pineapple from Reunion Island

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Ananas comosus

- 'Queen' Variety
- 1st fruit production of Reunion
- Economic losses due to various diseases including FCR disease

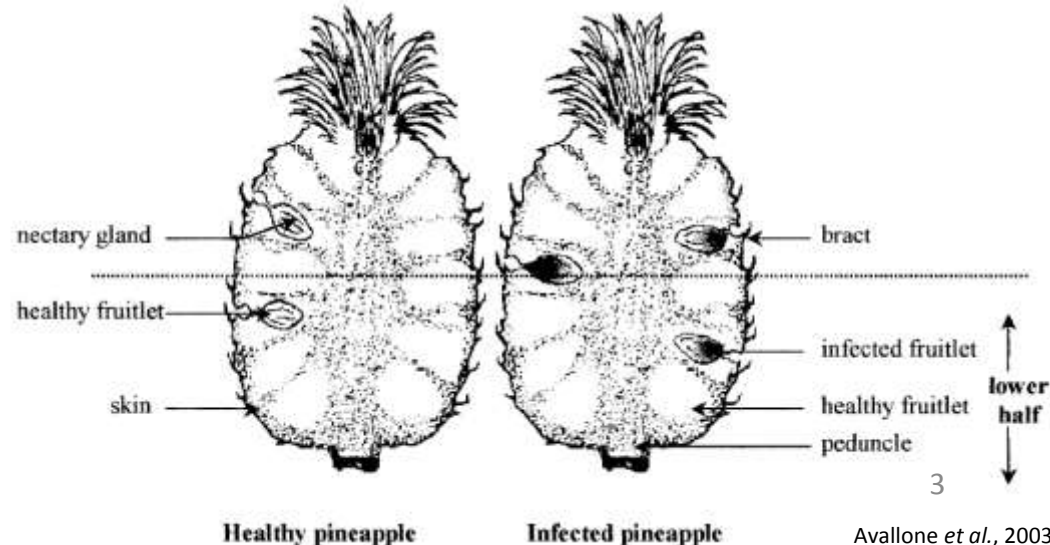


Black Spot disease

- First described in 1896
- originates in the « field »
- Difficult to anticipate
 - Black spots inside the fruit
 - No visible symptoms from the outside



Jean-Christophe Meile , unpublished



Fruitlet Core Rot

- 3 main Phytopathogenic Fungal species

- *Penicillium funiculosum* (*Talaromyces funiculosa*)



<http://www.mycobank.org>

- *Fusarium moniliforme*



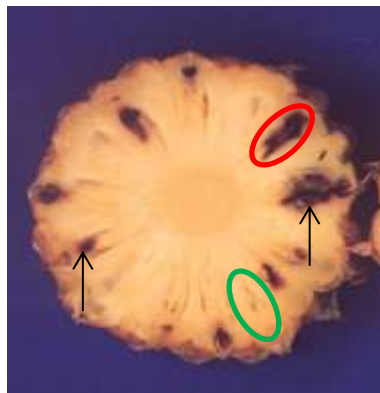
<http://www.mycobank.org>

- *Fusarium ananatum*



Jacobs, 2010

- Fruitlet Browning



Adaptée de Jacobs, 2010

Healthy Fruitlet
Diseased Fruitlet

	Healthy Fruitlets	Infected Fruitlets
Browning	0,33 ± 0,04***	0,58 ± 0,12***
Polyphénol oxydase	0,34 ± 0,35***	3,37 ± 2,26***
Laccase	0,17 ± 0,18***	1,35 ± 0,46***
Saccharose	4,15 ± 0,89***	1,61 ± 0,76***
Glucose	0,91 ± 0,19 ^{ns}	1,38 ± 0,41 ^{ns}
Fructose	0,98 ± 0,17 ^{ns}	1,18 ± 0,28 ^{ns}

Hypotheses

- FCR occurrence linked to various factors :
 - Pedoclimatic conditions
 - Fruit Biochemical composition
 - Agricultural practices
 - **Microbial Communities ?**

Are there specific microbial communities associated to FCR disease ?

Methodology

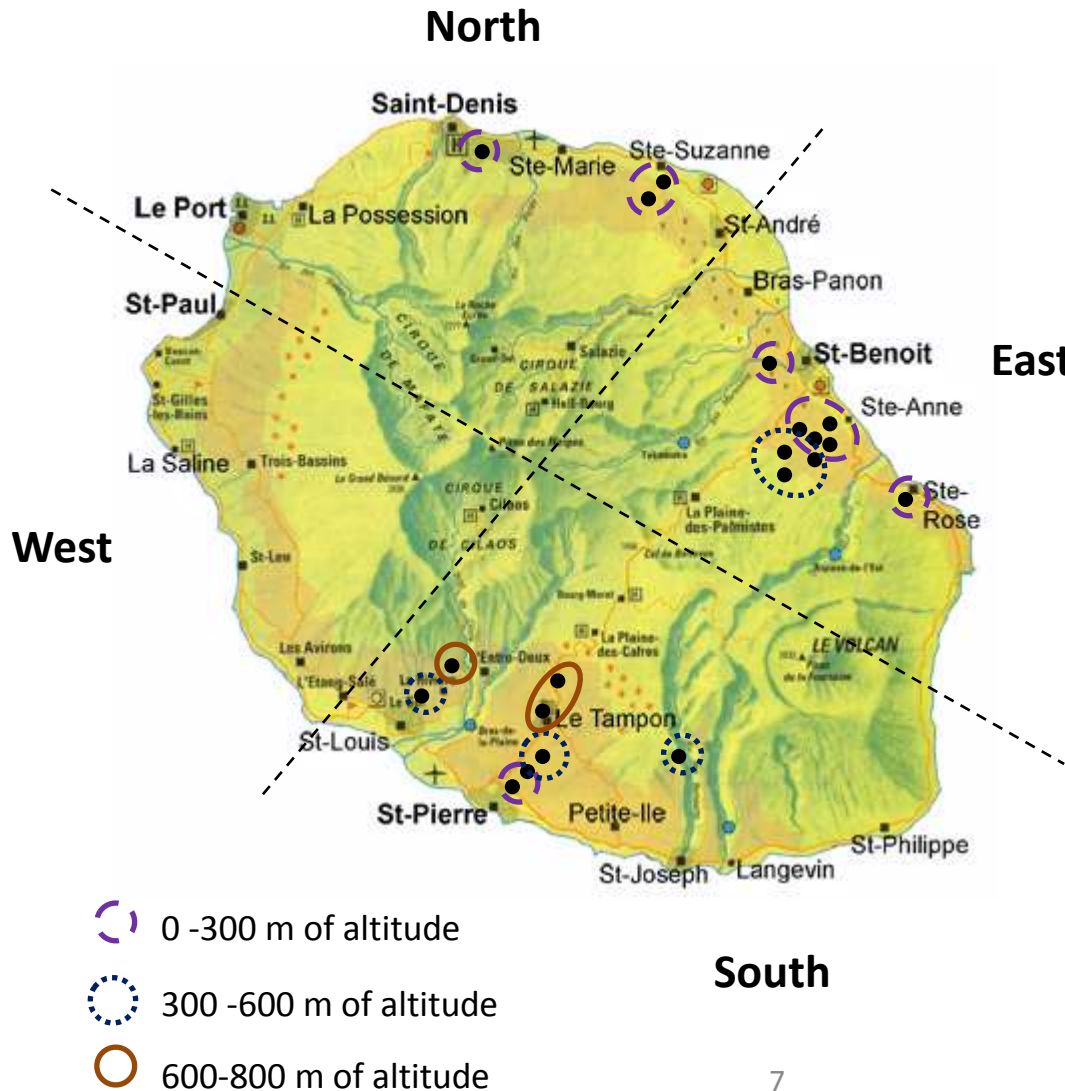
Study of microbial communities associated to :

- Healthy and diseased fruitlets
- In various climatic contexts
- In various production contexts (agricultural practices)



Comparative study of microbial communities to search for microbial markers of FCR disease

Fruit Sampling



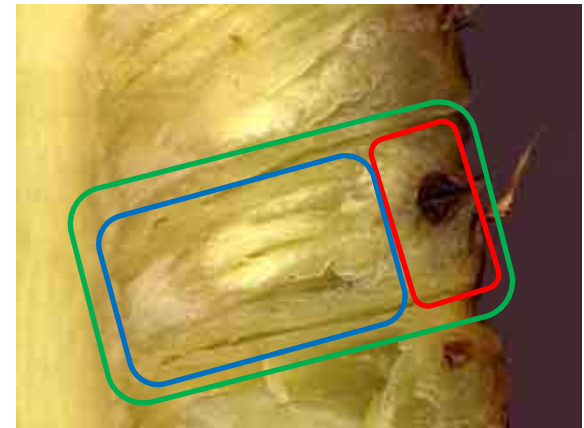
- 20 producers
- 6 fruits/plot
- Jul. to Oct. 2016
- Parameters :
 - Climate
 - Agricultural practices
 - Fruit Biochemical Composition
- Total of 126 samples

Fruitlet Sampling

Healthy and diseased fruitlets



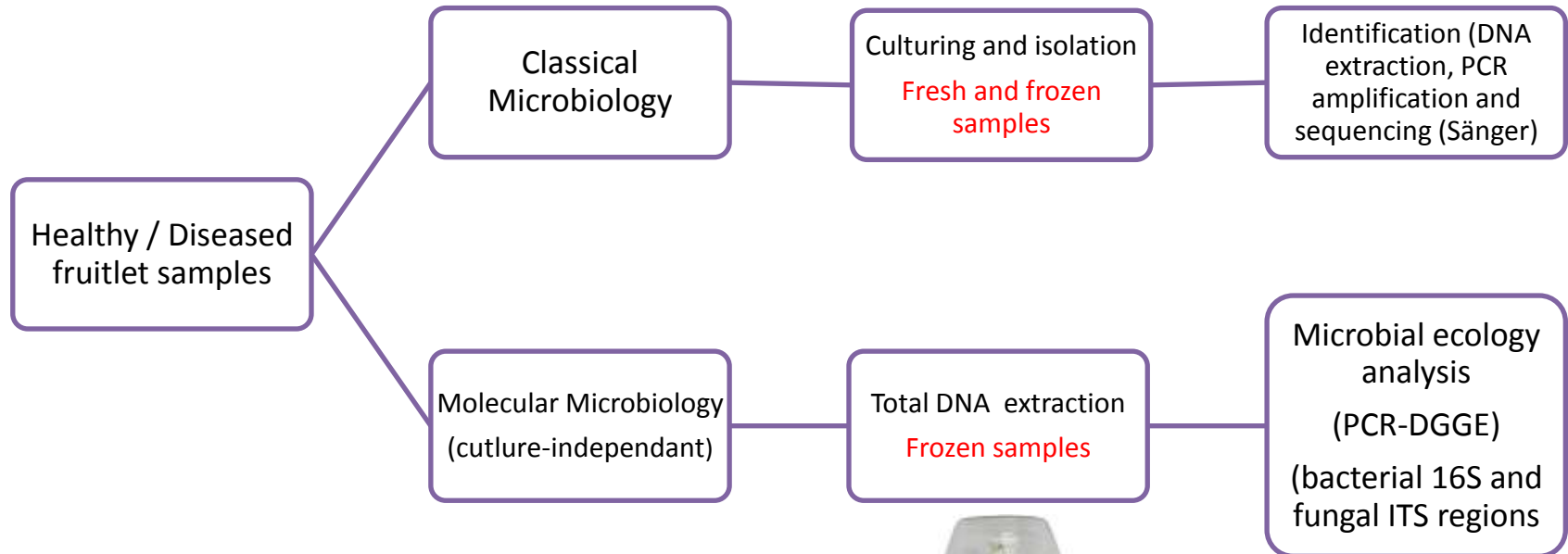
Jean-Christophe Meile , non publiée



Adaptée de : <http://www.snv.jussieu.fr>

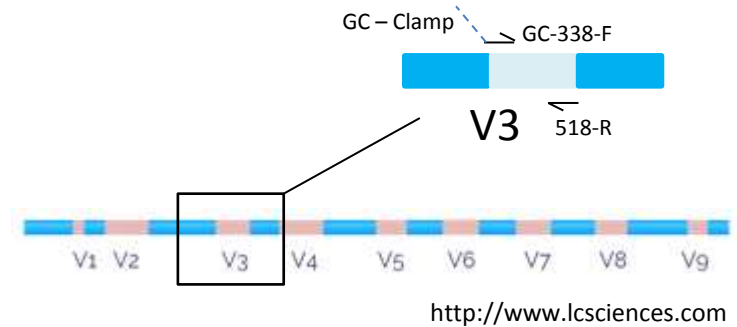


Microbiological analyses of fruitlets

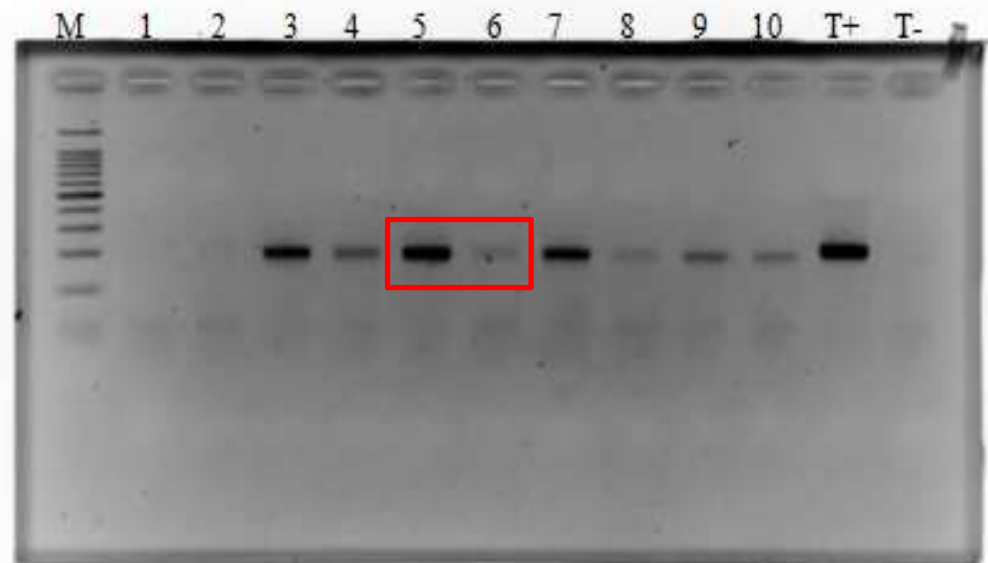


Bacterial Communities (PCR amplification)

- PCR amplification :
 - 16S rDNA V3 region
 - DNA primers 338F and 518R



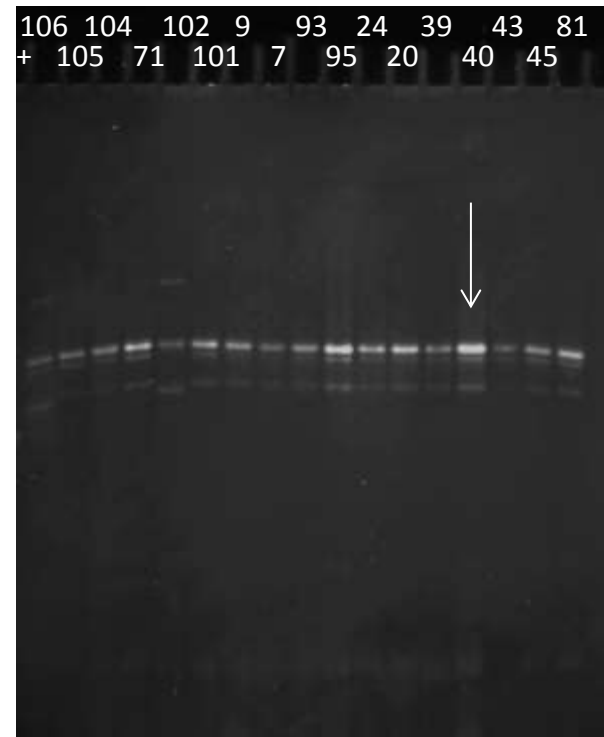
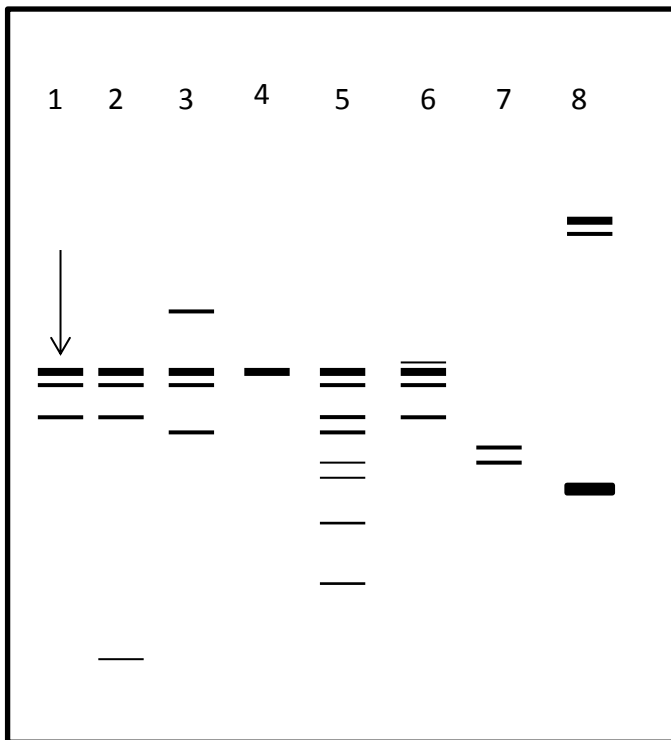
- 200 bp amplicons
- Bacterial DNA amplified in most samples (120/126)



Bacterial Communities Profiling

8 different bacterial profiles

One major DNA band / bacterial species



Bacterial groups

- Principal Component Analysis (PCA)

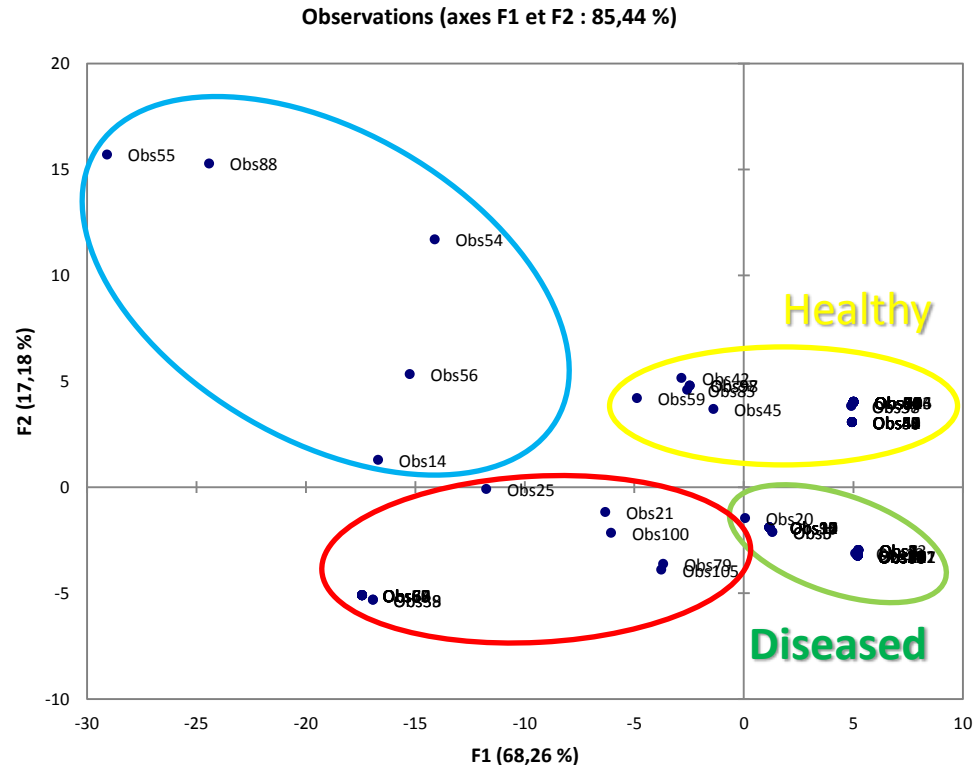
- F1+ F2 represent 85 % of the total variance

- 4 bacterial groups

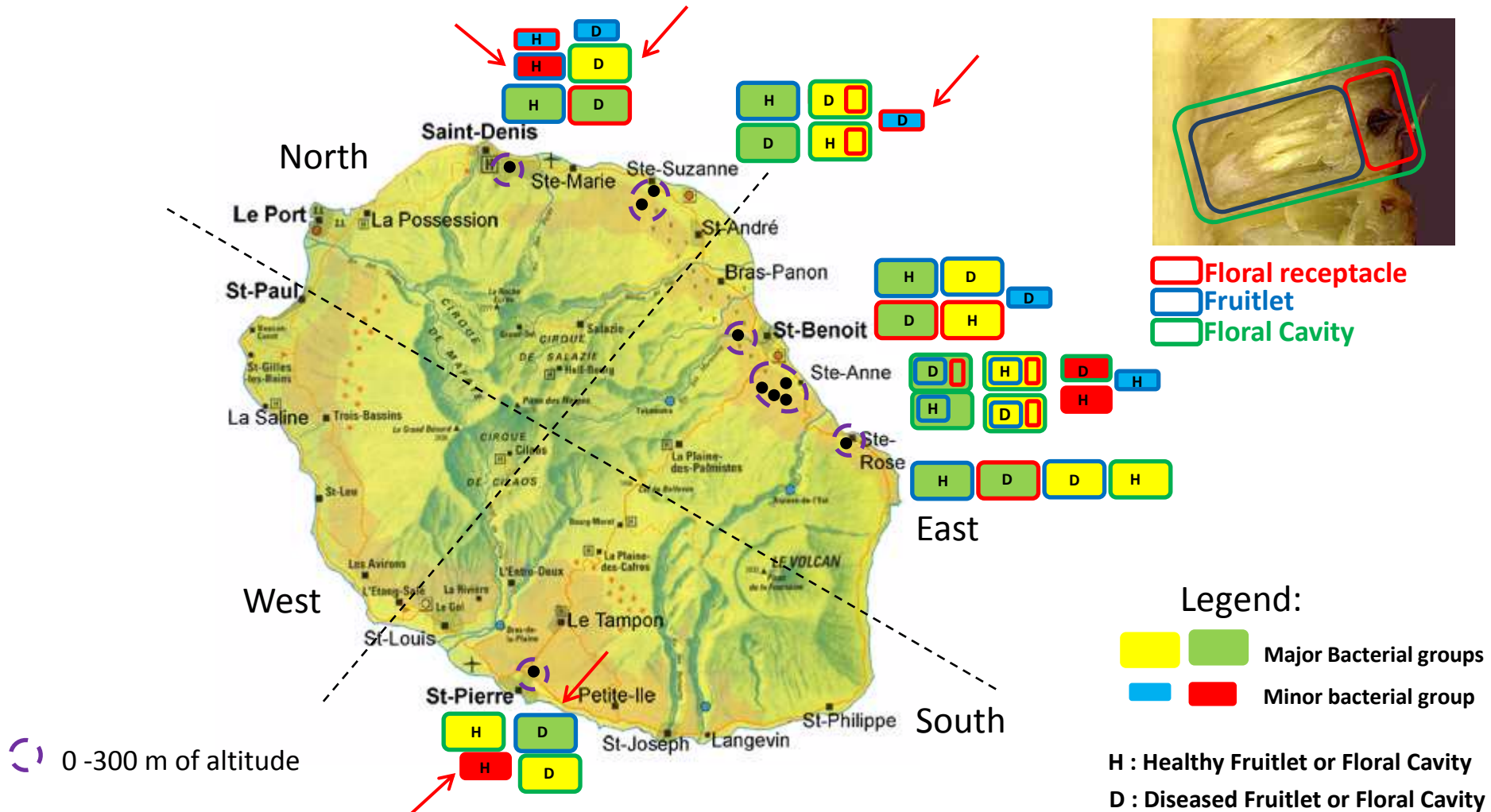
- 2 major groups

- Green : 45 samples (on 107)

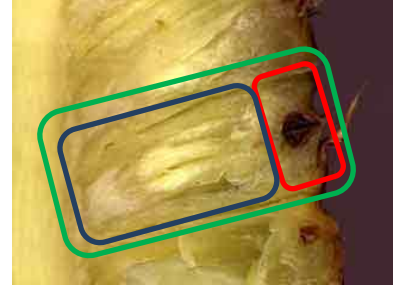
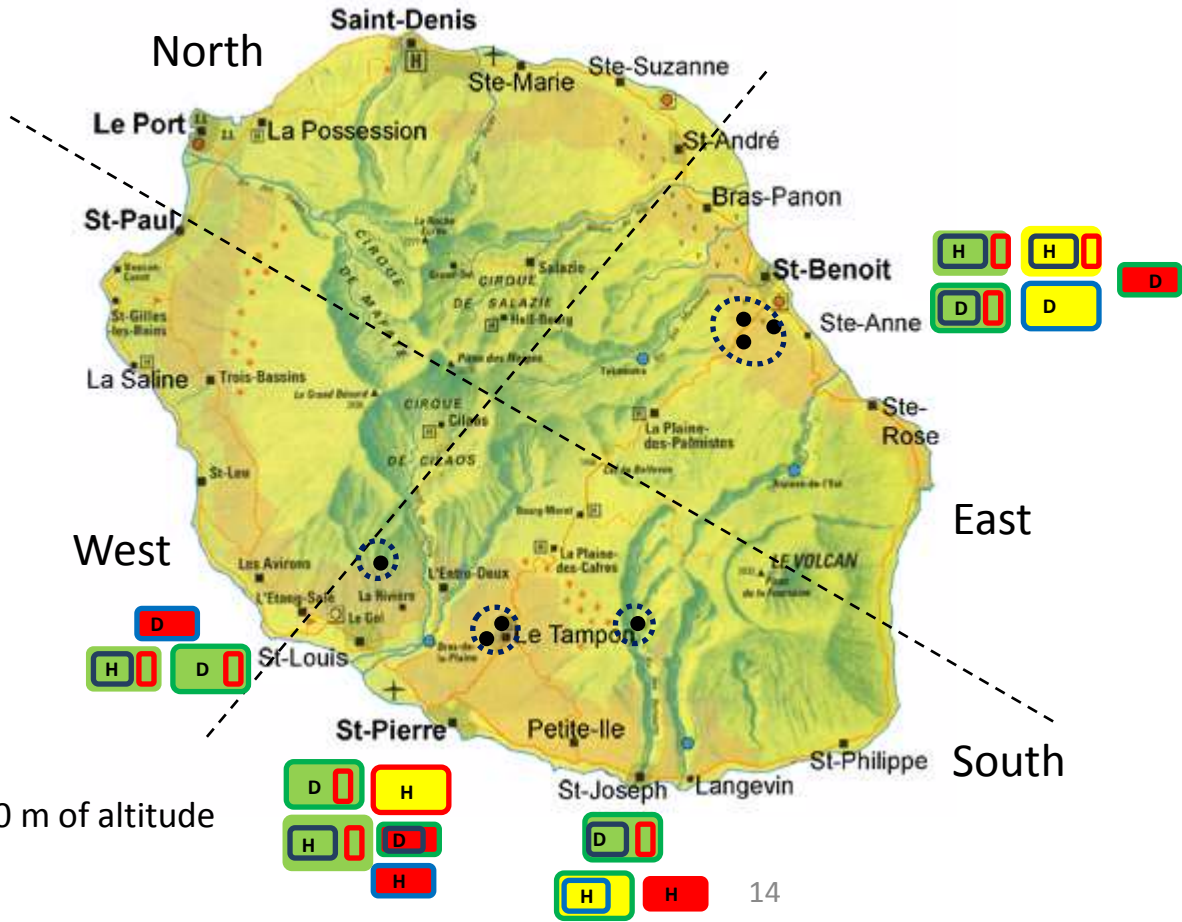
- Yellow : 34 samples



Bacterial groups on the map I



Bacterial groups on the map II



 Floral receptacle
 Fruitlet
 Floral Cavity

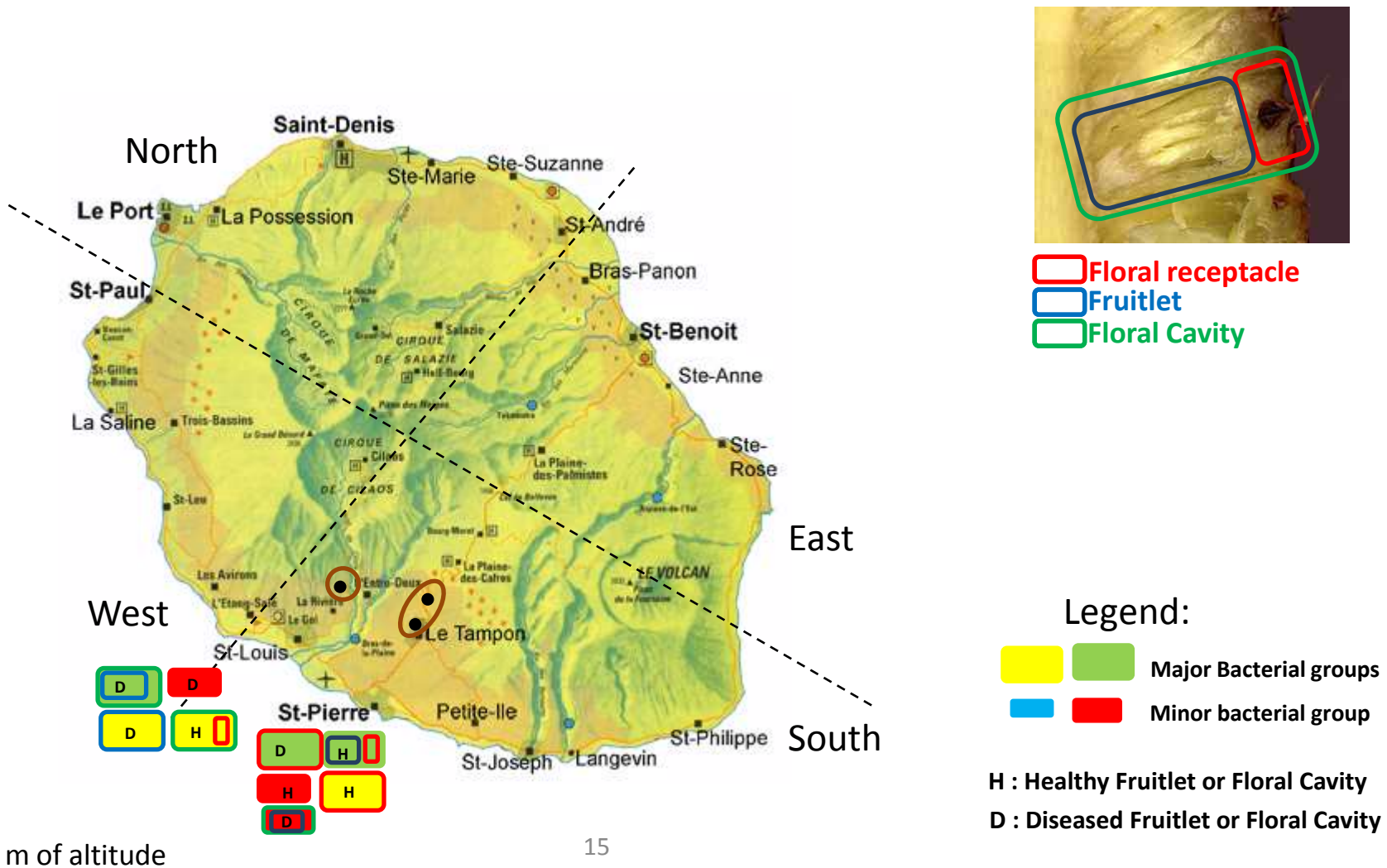
Legend:

Major Bacterial groups

 Minor bacterial group

H : Healthy Fruitlet or Floral Cavity
D : Diseased Fruitlet or Floral Cavity

Bacterial groups on the map III

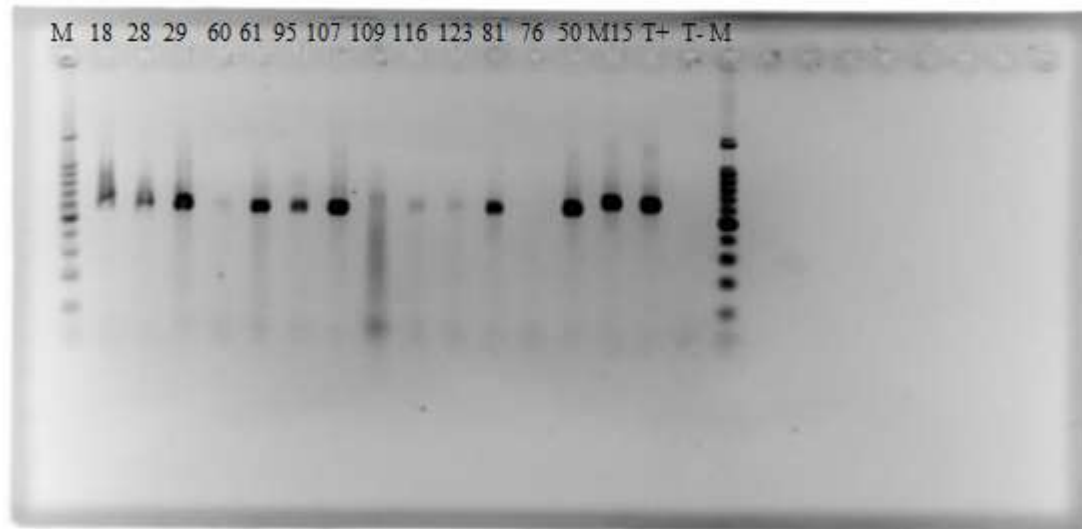
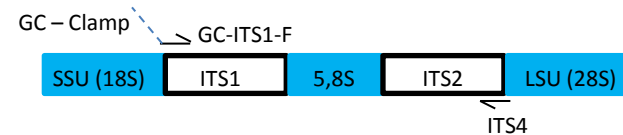


Bacterial Communities : Conclusions

- Most bacterial groups are present at all altitudes and type of fruitlet (Healthy and Diseased)
- Blue bacterial group
 - Low altitude
 - North and East sides of the Island
- Yellow bacterial group not found in St Louis samples (Soil management ? Climate ?)

Fungal Communities (PCR amplification)

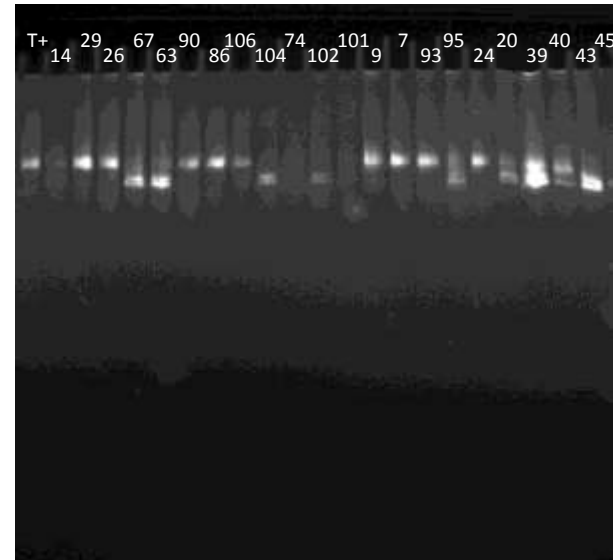
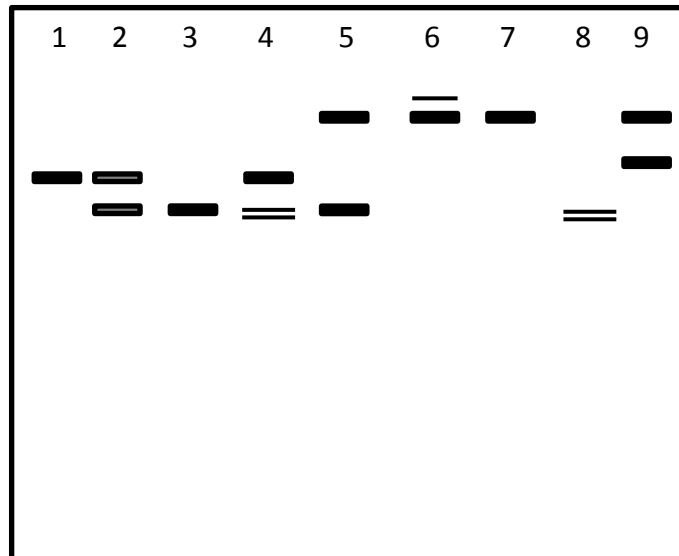
- PCR amplification :
 - Genomic region ITS1-5,8S-ITS2
 - DNA primers ITS1F and ITS4



- 500-700 bp amplicons
- Fungal DNA amplified for 120 samples on 126

Fungal Communities Profiling

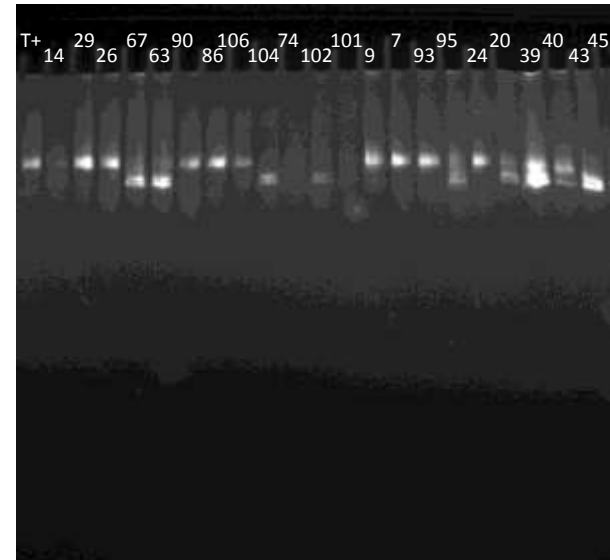
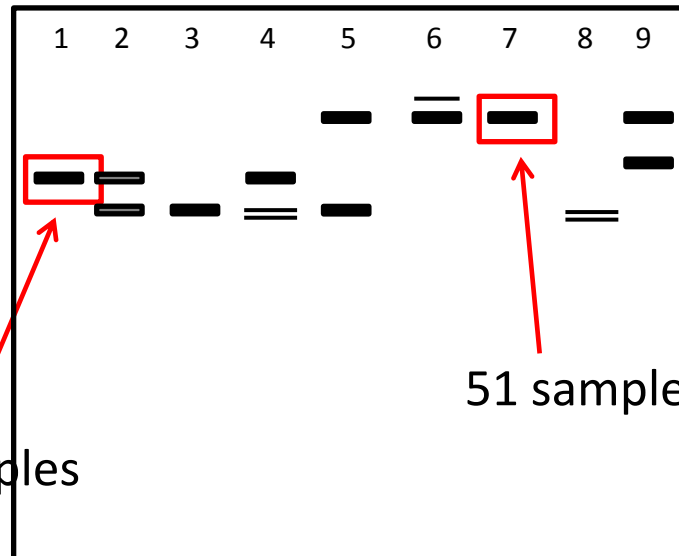
- 9 different fungal profiles



Fungal Communities Profiling

- 9 different fungal profiles

Two Major DNA bands / Fungal species



Fungal Groups

- PCA

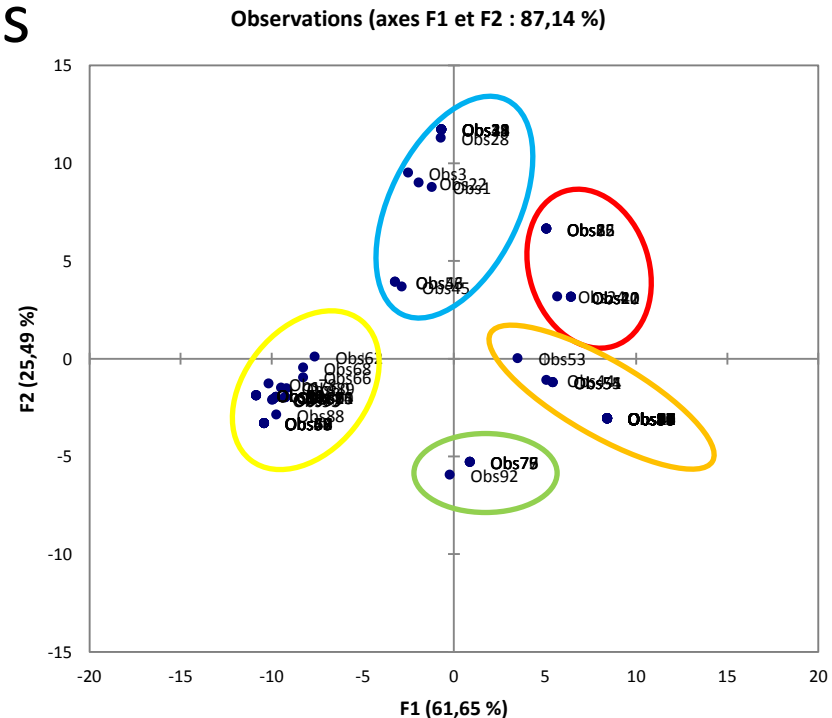
- F1 + F2 represent 87% of the total variance

- 5 fungal groups

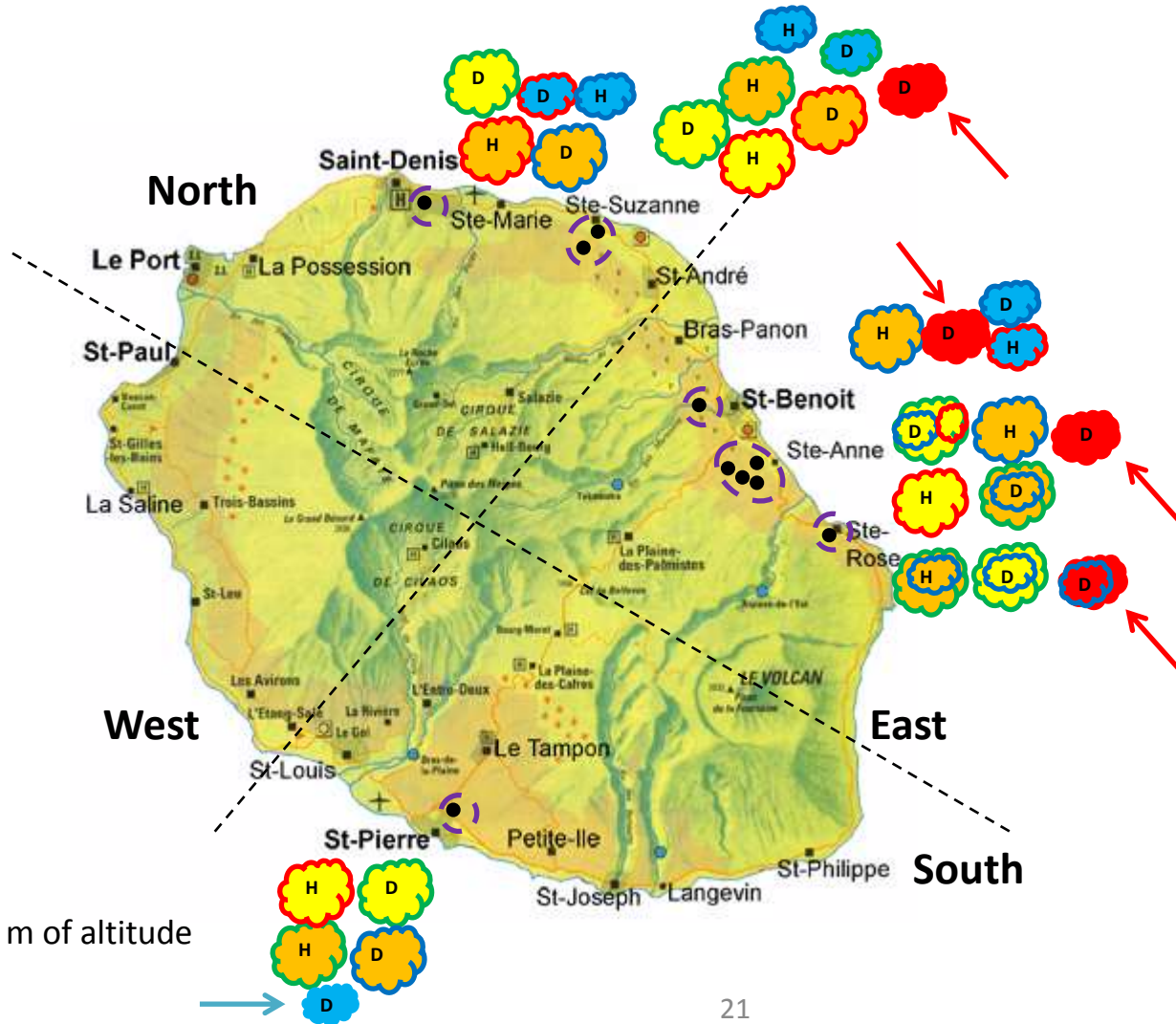
- Yellow & Orange major groups

- Yellow : 32 samples on 97,
Mostly Diseased fruitlets

- Orange : 35 samples
Healthy and Diseased fruitlets



Map of fungal communities I



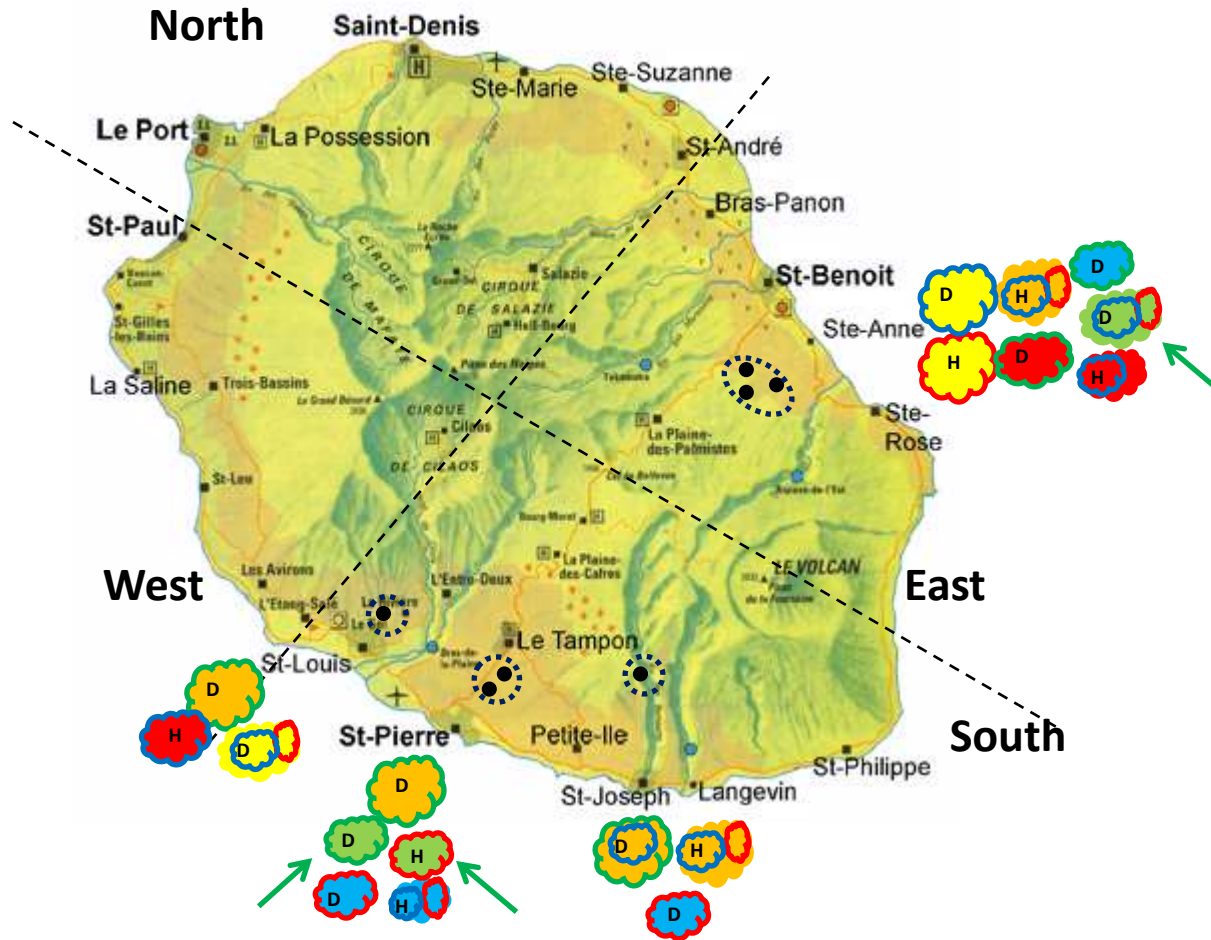
Floral receptacle
 Fruitlet
 Floral Cavity

Legend :

● ● Major Fungal groups
● ● ● Minor Fungal groups

H : Healthy Fruitlet or Floral Cavity
D : Diseased Fruitlet or Floral Cavity

Map of fungal communities II



 Floral receptacle
 Fruitlet
 Floral Cavity

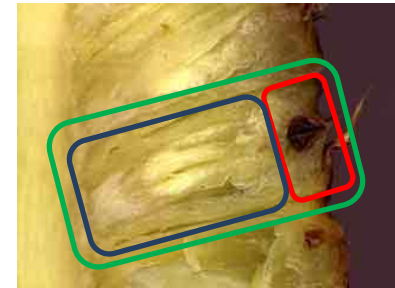
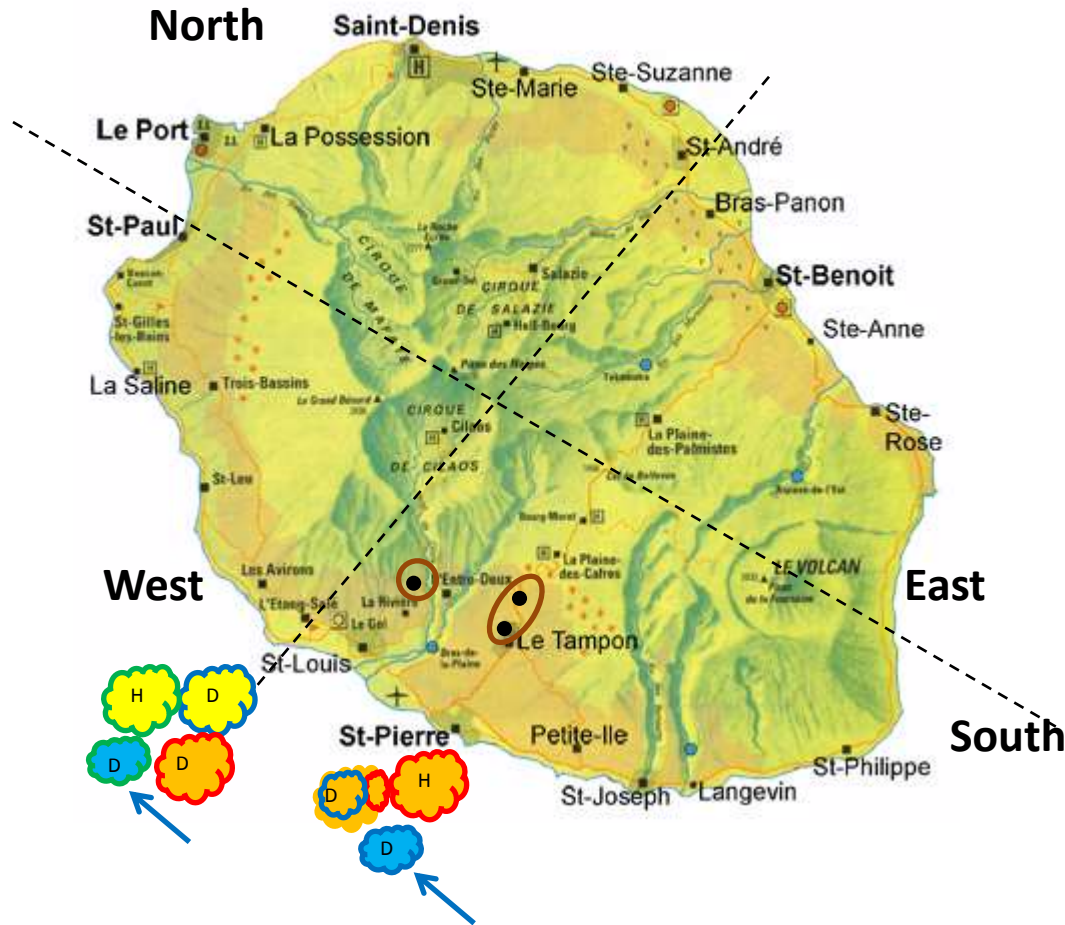
Legend :

● ● Major Fungal groups
● ● ● Minor Fungal groups

H : Healthy Fruitlet or Floral Cavity
D : Diseased Fruitlet or Floral Cavity

300 -600 m of altitude

Map of fungal communities III



- Floral receptacle
- Fruitlet
- Floral Cavity

Legend :

- ● Major Fungal groups
- ● ● Minor Fungal groups

- H : Healthy Fruitlet or Floral Cavity**
- D : Diseased Fruitlet or Floral Cavity**

 600-800 m of altitude

Fungal Communities : Conclusions

- Red Fungal group :
 - Detected in diseased fruitlets at low altitude (<300m)
- Green fungal group
 - Only detected at medium altitude (300-600m)
- Blue Fungal group
 - Detected in diseased fruitlets at high altitude (600-800m)

Conclusions

- Microbial (bacterial and fungal) DNA detected in almost all (>95%) sampled fruitlets
- Potential microbial markers of FCR disease :
 - At low altitude (<300m)
 - Yellow fungal & bacterial groups (North)
 - Red fungal group (East)
 - Green bacterial group (South)
 - At medium altitude (300-600m)
 - Green & Blue fungal & red bacterial groups (East)
 - Orange Fungal group (South-West)
 - Blue Fungal group (South)
 - At high Altitude (600-800m)
 - Blue Fungal group

Conclusions - Perspectives

- Potential microbial markers of FCR vary according to:
 - Altitude
 - Region of production (climate, practices)
- Perspectives
 - Identification of microbial DNA sequences composing the different microbial groups/profiles (current)
 - Get complementary data with Next Generation Sequencing approaches
 - Functional approaches (RNA) to identify which microbial species are metabolically active (RT-PCR-DGGE, RNAseq)



Aknowledgments

- **Mathilde Hoarau (Cirad)**
- **Marc Chillet (Cirad)**
- **Ananabio Project & Alain Soler (Cirad)**
- **Julie De Stefano, Master student (Toulouse)**
- **Lola Filippi, Ing. school student (Toulouse)**
- **Bastien Barral, PhD student (Cirad)**





**Thank you
for your attention !
Merci pour votre
attention !**

