Complementary approaches to quantify and characterize inocula dynamics and leaf infection at plot level: case of Black Leaf Streak Disease

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Context

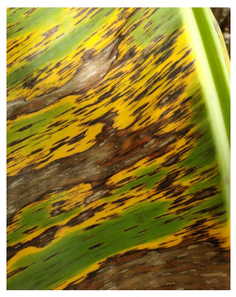
Black Leaf Streak Disease (BLSD) is a major leaf disease of banana, caused by the ascomycete ***Pseudocercospora fijiensis,*** inducing major losses for banana industry and requiring **frequent fungicides applications** worldwide.

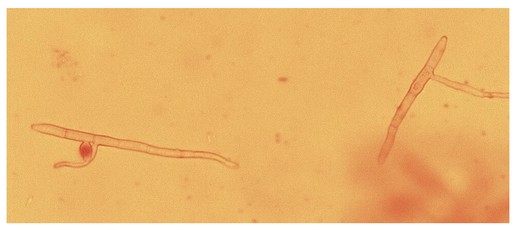
P. fijiensis produces two types of airborne spores, **conidia** and **ascospores**. According to empirical knowledge, plant infection is mainly due to the inoculum from outside the plot (**external inoculum**, supposedly ascospores) whereas the inoculum within the plot (**internal inoculum**, supposedly mainly conidia) plays a minor role.

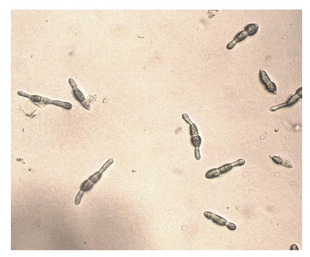
A better understanding of the disease epidemiology, especially on inocula dynamics will allow to **find alternatives** excluding pesticides and optimize current practices.

Main objective

The study aims (i) to **characterize and quantify**, with the proportion of conidia and ascospores, the **external and internal inocula** of a plot, and (ii) to identify which **leaves are infected** by each inoculum, depending on the cultivar and the microclimatic conditions.


Symptoms of BLSD


Conidia of *Pseudocercospora fijiensis*


Ascospores of *Pseudocercospora fijiensis*

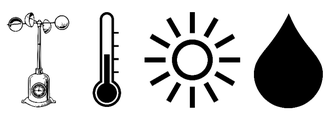
Methodology

The study takes place in Capesterre-Belle-Eau, **Guadeloupe**, FWI in two experimental plots, from **July 2023 to June 2024** : one cultivated with a hybrid **partially resistant** to BLSD (Pointe d'Or), and one cultivated with a cultivar **susceptible** to BLSD (Williams).

(i) Characterization and quantification of the external and internal inocula

(a) Air sampling

Microclimatic conditions




Burkard multi-vial cyclone sampler

Multi-vial volumetric **cyclone samplers** (Burkard) are installed above and under canopy to catch daily respectively the external and internal inocula, sampling 16,5 litres of air per minute, into 1,5 mL Eppendorf vials.

There are two in each cultivar and two in plots without banana as control.

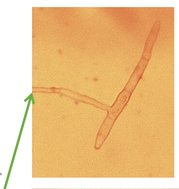
Comparison of the mean number of cells per conidia produced *in vivo* and *in vitro*. The strain GLP 701 has been used to set up the standard curve.

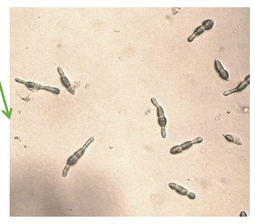
Cultivar/Strain	Type	Number of cells per conidia	
Pointe d'Or	*In vivo*	4,76	b
Williams	*In vivo*	5,22	a
GLP 701	*In vitro*	5,06	ab

(b) Quantifying weekly spores concentration per m^3 of air

2 types of inocula sampled


Inside of the multi-vial cyclone sampler





Microscopy with a hematimeter

Quantification of airborne conidia concentration

Quantitative PCR

ß-tubuline primers

Set up of a standard curve

Concentration of conidia per mL

10^1 10^2 10^3 10^4 10^5 10^6 10^7

30 replicates 10 replicates

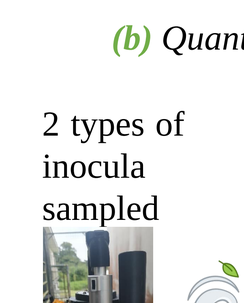
$$Asc = \frac{Cell_{qPCR} - (C_{\mu} \times Cell_{in\ vivo})}{2}$$

With,
Asc, the number of ascospores
$Cell_{qPCR}$, the number of cells found with the qPCR
C_{μ}, the number of conidia counted with the microscope
$Cell_{in\ vivo}$, the number of cells per conidia in vivo

Quantification of airborne ascospores concentration

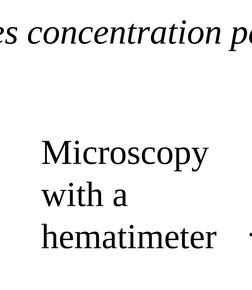
(ii) Highlight leaf infection by external and internal inocula

With sporeproof bags


Unfurled leaf and first leaf protected, avoiding the external inoculum

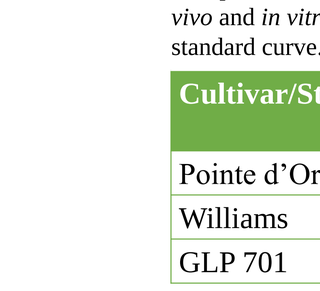
Youngest Leaf Spotted protected, avoiding the internal inoculum

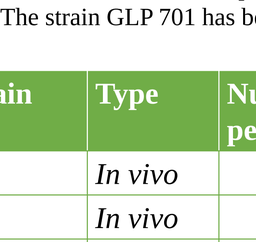
Without sporeproof bags


Control unprotected, exposed to both inocula

Sporeproof bags


First symptoms of BLSD


Photographing the infected leaf



Quantification lesions number between protected and unprotected leaves by image analysis

Quantification of each inoculum within the leaf infection

7 days

37 to 42 days

40 to 45 days

Expected results and perspectives

We expect to quantify the **concentration** of airborne **conidia and ascospores within external and internal inocula**, and to establish the **ratio between the external and the internal inocula** infecting the leaves, both analysed in relation with microclimatic factors. Due to contrasted resistance level of the 2 cultivars, we expect to have a **different ratio** for each one.

This knowledge will allow to **optimize** control methods and to **provide information for epidemiological models** parametrized for BLSD.

Pictures have been taken by Marine SEIDEL, Cirad