Banana Black Sigatoka

Mycosphaerella fijiensis



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Presentation:

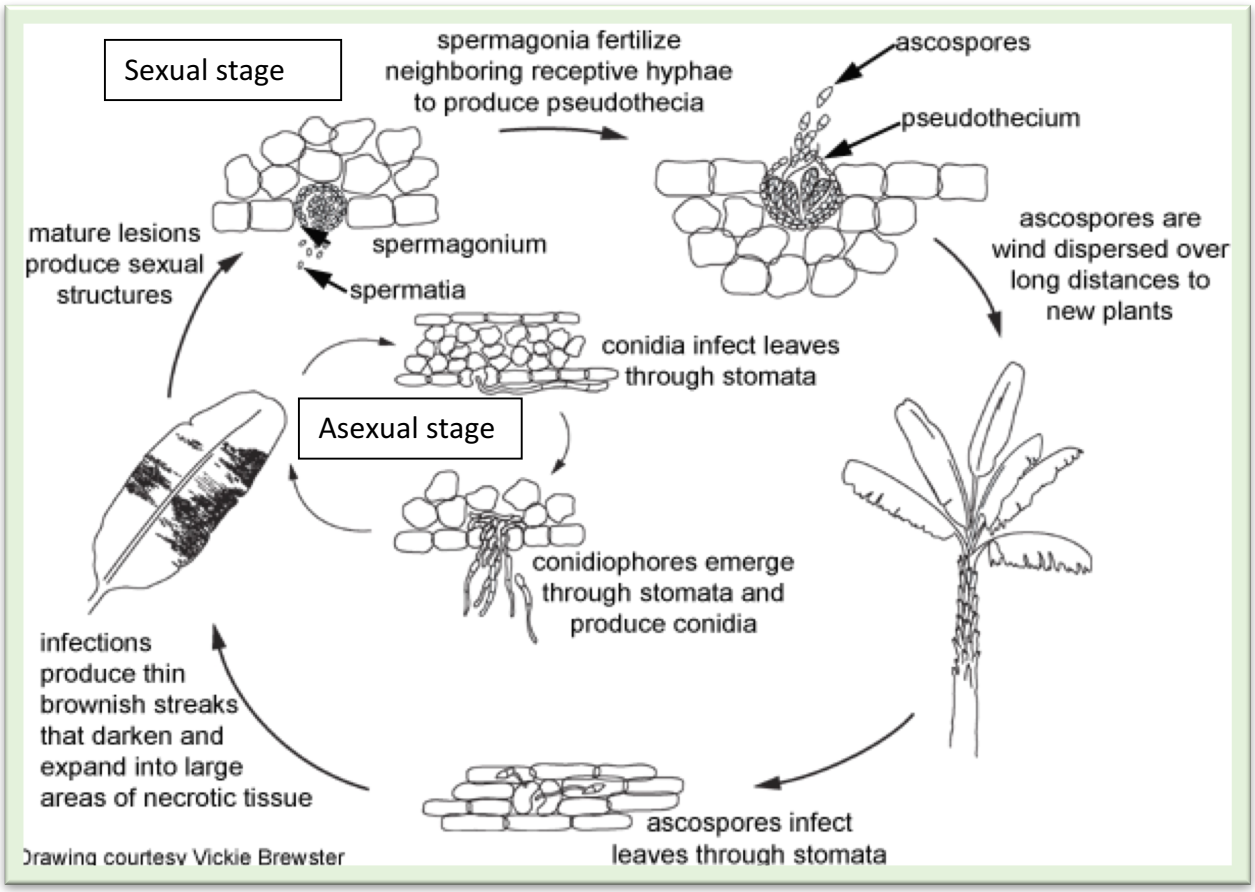
Banana is the fourth most important crop after rice, wheat and maize in the world. The crop is cultivated in the tropical regions of more than 100 countries (Frison et al., 2004).

Black Sigatoka disease is caused by the hemibiotrophic fungus *Mycosphaerella fijiensis* which is the most devastating disease of bananas worldwide (Stover 1980). The fungus attacks the leaves and causes premature ripening of the fruit resulting in significant production losses (Marín et al., 2003).

Actual way to control the pest is based on using fungicides, which leads to environmental and economical concerns especially regarding the emergence of resistant strains of *Mycosphaerella fijiensis (*Brent and Hollomon*, 2007).*

This document deals with pest managements and fungicide alternatives against Black sigatoka actually practicable in the field.

I. Pathogen cycle and plant defence mechanisms



The utilization of fungicides is the important and more compelling techniques for diseases control yet but chemical control of Black sigatoka is not the solution in the long run for sustainable environment (Ganry, Foure, de Bellaire, and Lescot 2012). One of them would be the identification of potential **biological control agents** for banana diseases.

The defence mechanisms involved in host plants are PR protein antimicrobial metabolites (phytoalexins), proteins and thickening of cell wall by producing callose and lignin (Cavero et al., 2015). There are research going on to finding out new resistant banana cultivar; example: research on mutants resistant to juglone (a toxin produced in black Sigatoka disease) by using gamma rays (Reyes-Borja et al., 2005).

However, inherent plant defences are easily overcome by the fungus. Moreover, this tropical/sub-tropical climate disease is complicated to fight against as it is foliar fungi having both sexual and asexual phase, so the cycle lasts all year round (Marin et al., 2003).

II. Potential biological control against *Mycosphaerella fijiensis*

Fungus *Trichoderma Atroviride* (on the market)

T. Atroviride can be used in a sprayed spore suspension on the leaves**.**
The production of conidia can be done in autoclaved rice amended with $CaCO_3$ and with 50% humidity kept in bags opened at 3 days after inoculation of *T. Atroviride*. Therefore, it does not require a significant investment. **A combined application of the biocontrol agent with fungicides** (Chlorotalonil, Flutriafol, Copper Oxychloride, and Azoxystrobin) may be performed when required for the integrated management of the disease (Poholl et al., 2015). Mycoparasitism is expected to be involved in the activity of this antagonist.

Biological fungicide (on the market)

The use of **NECO**, an extra oil extra leaf from ***Ocimum gratissimum*** (easy to obtain, only a small amount is necessary). It can be spread on the leaves with an optimum effect observed at dose of 20 ml / l in the field.
NECO is dominated by thymol, a phenolic terpene that seems to exert a direct action on reducing mycelia growth (Kassi et al., 2014).

Use of botanical extracts in small extract amount

1. The use of botanical extracts like, ***Azadirachta indica, Cinnamomum zeylanicum*** have shown the potential to reduce Black Sigatoka in banana plantlets (30% concentration), it directly reduces mycelia growth
2. ***Capsicum annum*** also can be applied as foliar sprays to 1-2 months-old plants. (Journal of Agricultural Science; Vol. 9, No. 4; 2017).
3. Moreover, the use of isolate of a plant growth-promoting rhizobacterium ***Pseudomonas fluorescens*** and an entophytic fungus ***Fusarium oxysporum*** can be applied on the soil (Mortensen 2013).

Microbial fungicide based on Bacillus subtilis EA-CB0015 (on the market)

The microbial fungicide is greatly interesting in controlling Black Sigatoka disease. Applied in the field, it displays reductions comparable to fungicide chlorothalonil and mancozeb (Gutierrez-Monsalve and al., 2015). At the moment, **only two promising commercial products** have been evaluated in the field (*B. subtilis* QST 713 from Serenade® and *B. pumilus* QST 2808 from Sonata®) (Serrano et al., 2013). **Lipopeptides fengycin C and iturin A in B** are produced by ***B. subtilis EA-CB0015*** during fermentations and it have been shown that these lipopeptides could inhibit fungi growth by disrupting the cell membrane (Tao et al., 2011 ; Romero et al., 2007).

Bacterium Burkholderia spinose

Cell suspensions of ***B. spinosa*** can be applied to banana plants as a foliage spray in weekly intervals.
Several modes of action have been suggested to explain the biocontrol activity, namely competition for nutrients and space between pathogen and the antagonist, direct parasitism, antibiosis and induced resistance of the host plan (Silva and De Costa 2014).

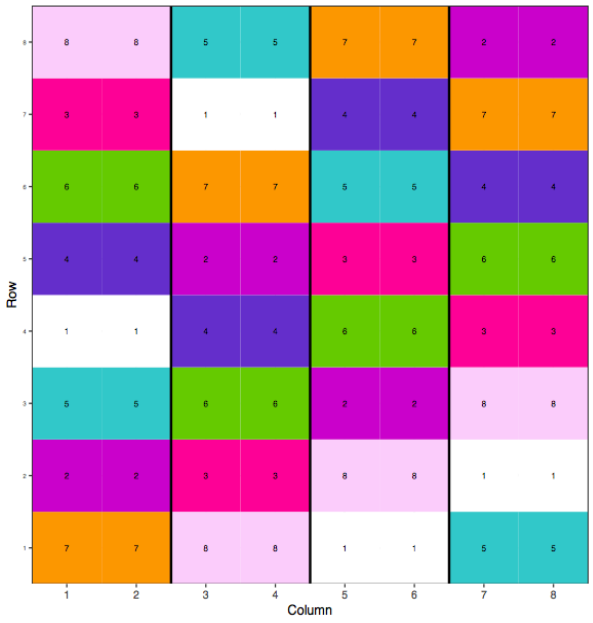
III. Limits and innovative experiment for the future

Trichoderma atroviride is a filamentous soil fungus (Chet 1987) and *Bacillus Subtilis* is mostly found in soils too (Kilian and al., 2000). The use of those products on leaves could maybe be improved by applying them on the soil as they can induce plant resistance. This approach needs more study and field trial in this case of foliar pathogen.

Some of mechanism, the one involved for *Capsicum annum* for instance are not yet know, or not detailed enough and **need more research**. Knowing the mechanisms is essential to make better biocontrol products (Mortensen 2013 ; Silva and De Costa 2014).

Formulating and producing antifungal metabolites that control *Mycosphaerella fijiensis* like lipopeptides fengycin C and iturin A in B needs **further studies** (Tao et al., 2011 ; Romero et al., 2007).

Major problematic in biological controls against Black sigatoka is the lack of knowledge concerning **comparison between different method** efficiencies directly in cropping systems. Here, we present a **field trial** to assess and contrast methods previously presented.



Field experiment

5 trial stations in 5 different locations in Queensland, Australia from June to November /Thailand from November to April limiting inherent field effects

Field layout of the banana experiment showing the layout of replicate blocks, and random allocation of biological controls within each replicate block

1: Control without **any fungicide**

2: **Chemical fungicides :** azoxystrobin/ carbendazim: (23% suspension concentration) once a month

3: Foliage spray spore suspension of Fungus ***Trichoderma Atroviride*** (10^8 conidia per milliliter suspension)

4: Foliage spray suspension of Bacterium ***Burkholderia spinose*** (100 µg mL^{-1}).

5: Foliage sprayed suspension of ***Bacillus subtilis EA-CB0015*** (100 µg mL^{-1}).

6: Foliage spray spore suspension of Fungus **Ocimum gratissimum** (NECO) (30% concentration).

7: Foliage botanical extract spray of ***Azadirachta indica, Cinnamomum zeylanicum and Capsicum annuum*** (30% concentration).

8: Isolate of rhizobacterium ***Pseudomonas fluorescens*** and entophytic fungus ***Fusarium oxysporum*** applied on the soil (30% concentration).

Every extract is applied once a week.

Statistical analysis

- **U**, 1
- **Block**, $b = 4$, $df_b = 3$
- **Mainplot**, $m = 32$, $df_m = 28$
- **Subplot**, $p = 64$, $df_p = 32$
- **Plant**, $pl = 640$, $df_{pl} = 576$
- **Leaf**, $lf = 1280$, $df_{lf} = 640$

Full structural model for the foliar disease experiment considering that 2 leaves per plant and 10 plants per subplot are sampled. Quantitative leaf disease impacts are assessed with disease assessment scales.

The model for this data could be written as linear model:

Leaf disease = mean + **Biological control** + *Block* + *Block:Mainplo*t + *Block:Mainplot:Subplot*

A mathematical model for this data could be written as :

$$LD_{ijk} = \mu + bc_{r(j)} + b_i + m_{ij} + e_{ijk}$$

Where LD_{ijk} is the quantitative response (Leaf disease) for the observation in the ith block of the jth mainplot of the kth subplot; i = 1...4, j = 1...32 and k = 1...64. The explanatory component contains μ, the overall population mean, and $bc_{r(j)}$, the effects of biological control r applied to mainplot j, for r = 1...8. The structural component contains the block effects, b_i, i = 1 . . . 4, and the mainplot effects m_{ij}, i = 1...4, j = 1...32. Finally, the error term (Rep.Mainplot.Subplot) is associated with the deviations, e_{ijk} for i = 1...4, j = 1...32 and k = 1...64. Assuming the deviations as IID with a normal distribution with common variance.

The linear model could be computed with anova functions of R-software and others functions limiting field heterogeneity effects.

Conclusion:

Inherent plant resistance disease mechanisms are easily overcome by *Mycosphaerella fijiensis*. At the moment, biological controls constitute the major alternative to fungicides.

Various biological control products are developed and based on reliable and consistent scientific results. However, more research work in field condition has to be done to know the precise use (Pajot 2001).

The randomized field trial presented previously, takes in account different biological products and shows good future perspectives for Black sigatoka disease management. Others products might be added to the field layout depending on funds raised during the project.

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