

**INTERNATIONAL WORKSHOP ON THE COCOA SWOLLEN SHOOT DISEASE**

**26 - 28 NOVEMBER 2013 – N'SA HOTEL, GRAND BASSAM, CÔTE D'IVOIRE**

# **Identification of vectors of cocoa swollen shoot virus and control methods : A perspective from Côte d'Ivoire**

**Presented by Pierre Walet N'GUESSAN**

# Outline

## **1. BACKGROUND**

## **2. CURRENT STATUS OF KNOWLEDGE**

- **Morphological identification of mealybugs**
- **Molecular identification of mealybugs**
- **Detection on virus into mealybugs**
- **Control of CSSV vectors**

## **3. DIFFICULTIES**

## **4. CONCLUSION**

## **5. PERSPECTIVES**

# 1. BACKGROUND

# Background

- ❑ It is known that CSSV is transmitted by mealybugs (Family : Pseudococcidae)
- ❑ At least 14 species of mealybugs are known as vectors of CSSV;
- ❑ Previous works on mealybugs control were difficult :
  - ✓ Some chemicals non effective or those which are effective are also toxic
  - ✓ Works on biological control are done in some countries like Ghana but there is not yet application in fields



## **2. CURRENT STATUS OF KNOWLEDGE**

➤ **MORPHOLOGICAL  
IDENTIFICATION  
OF MEALYBUGS**

# Morphological identification of mealybugs

- ❑ **Survey in cocoa orchards and identification :**
  - ✓ Trees random selected per field ;
  - ✓ Looking for mealybugs on pods, trunk, leaves, flowers, chupons ;
  - ✓ Identification based on external body of female adults
  - ✓ Counting all colonies found ;
  - ✓ Identification in laboratory of non identified specimens in fields using binocular magnifying.

# Morphological identification of mealybugs

## 3 Species which do not belong to Pseudococcidae family



*Stictococcus gowdeyi*  
(Stictococcidae)



*Ceroplastes spp.*  
(Coccidae)



*Stictococcus sjöstedti*  
(Stictococcidae)



# Morphological identification of mealybugs

## 7 Species of Pseudococcidae family



*Formicococcus*  
(*Planococcoides*) *njalensis*



*Planococcus kenyae*



*Ferrisia virgata*



*Dysmicoccus brevipes*



*Phenacoccus hargreavesi*



*Planococcus citri*



*Pseudococcus longispinus*<sup>9</sup>



# **MOLECULAR IDENTIFICATION OF MEALYBUGS**

# Molecular identification of mealybugs

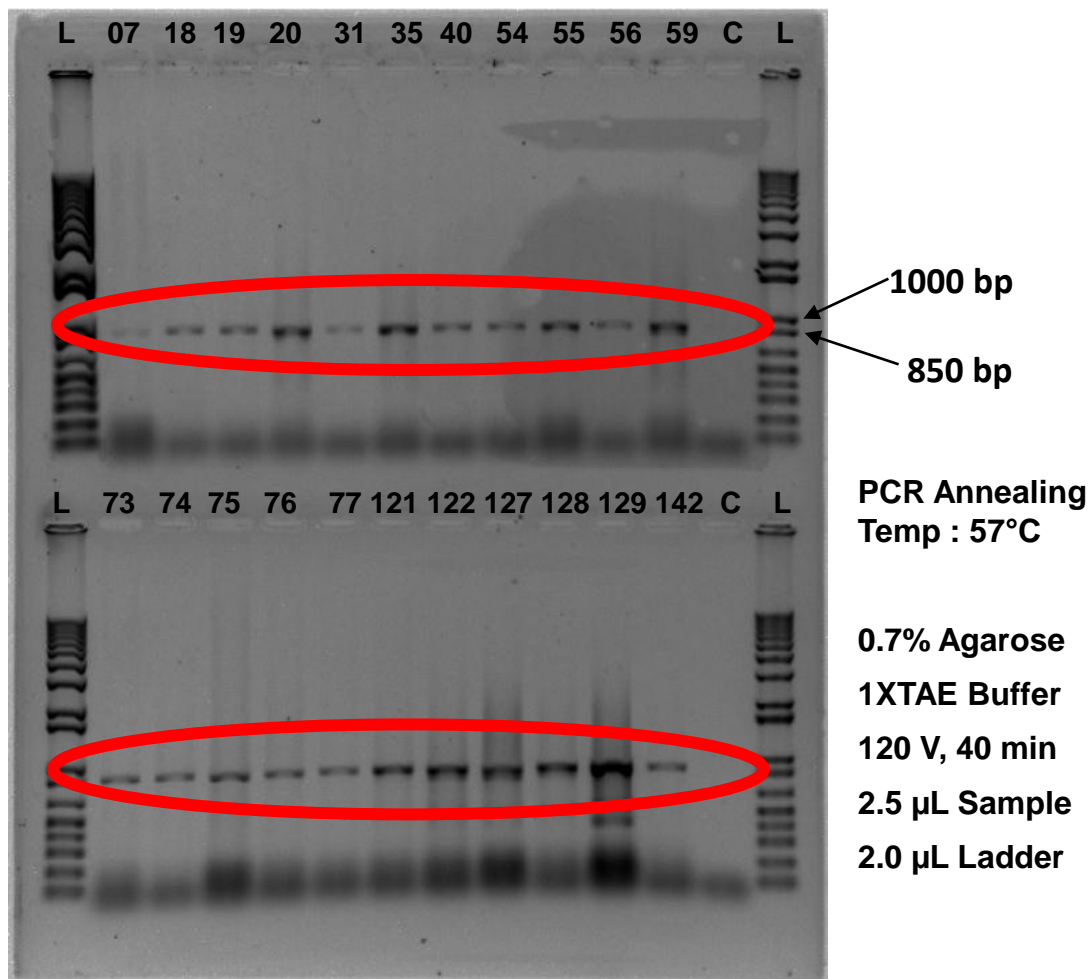
- ❑ **Sampling in fields**
  
- ❑ **Molecular analysis :**
  - ✓ Extraction of mealybugs' DNA ;
  - ✓ PCR with MTD 10/12 primers to amplify mtCOI gene ;
  - ✓ Agarose gel electrophoresis
  - ✓ Sequencing and analyze of sequences ;
  - ✓ Building the phylogenetic tree.



# Molecular identification of mealybugs

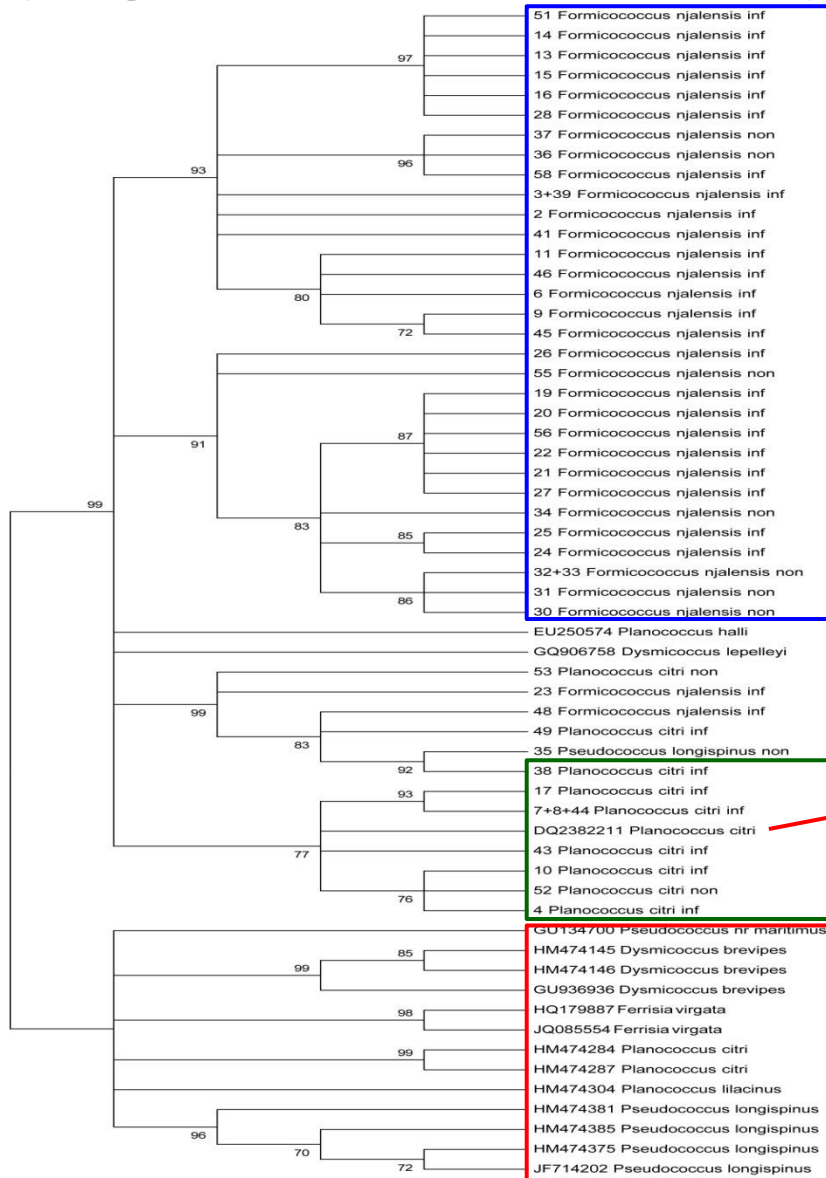
## Migration of DNA after Agarose gel electrophoresis

- ✓ Good reaction : migration of DNA at 800 ~ 850 pb.



# Molecular identification of mealybugs

## Phylogenetic tree of 47 mealybugs CI and 16 ref. genebank



*Formicococcus njalensis* CI

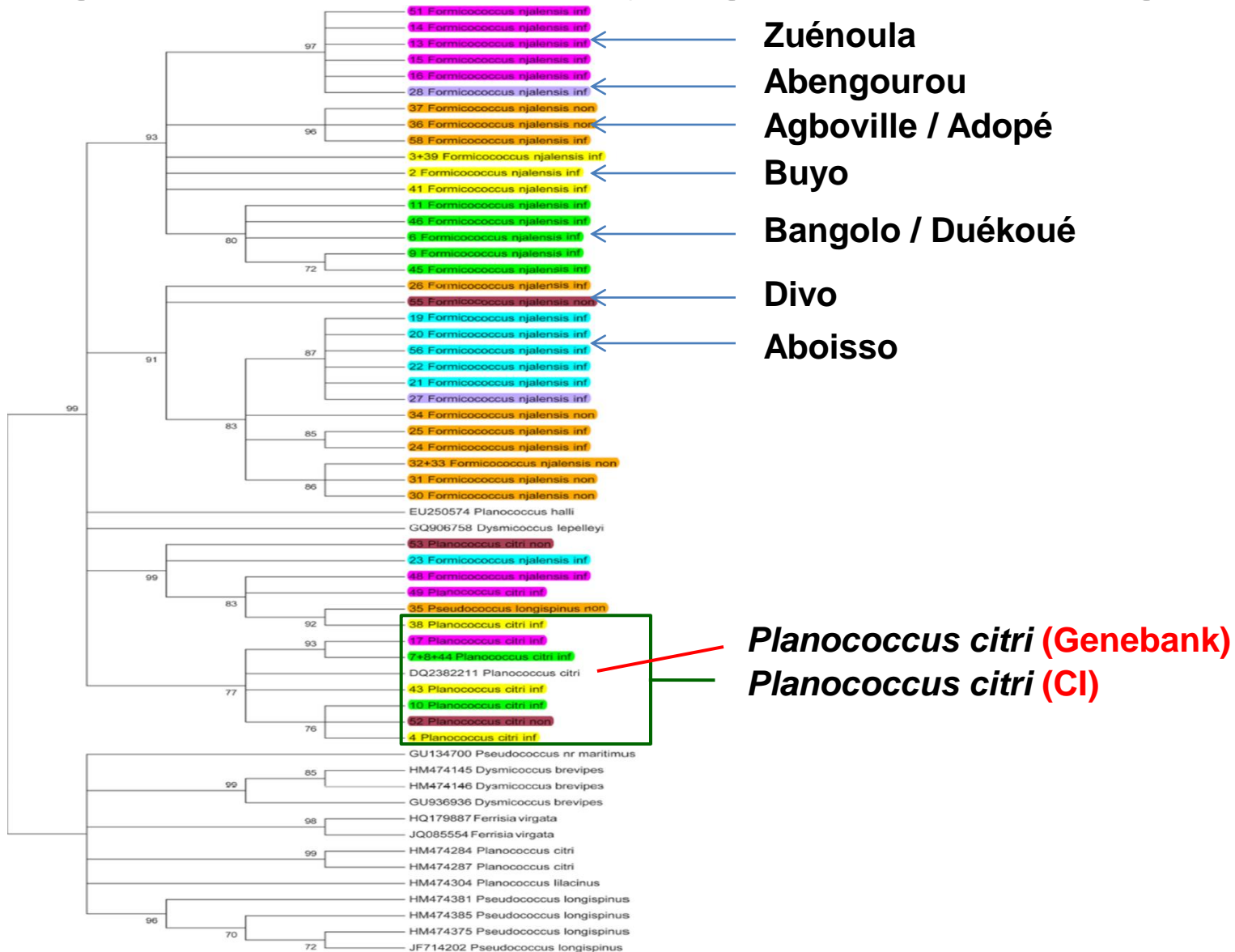
*Planococcus citri* (Genebank)

*Planococcus citri* (CI)

Others mealybugs species (Genebank)

# Molecular identification of mealybugs

## Phylogenetic tree of 47 mealybugs CI and 16 ref. genebank





# **DETECTION OF CSSV INTO MEALYBUGS**

# Detection of CSSV into mealybugs

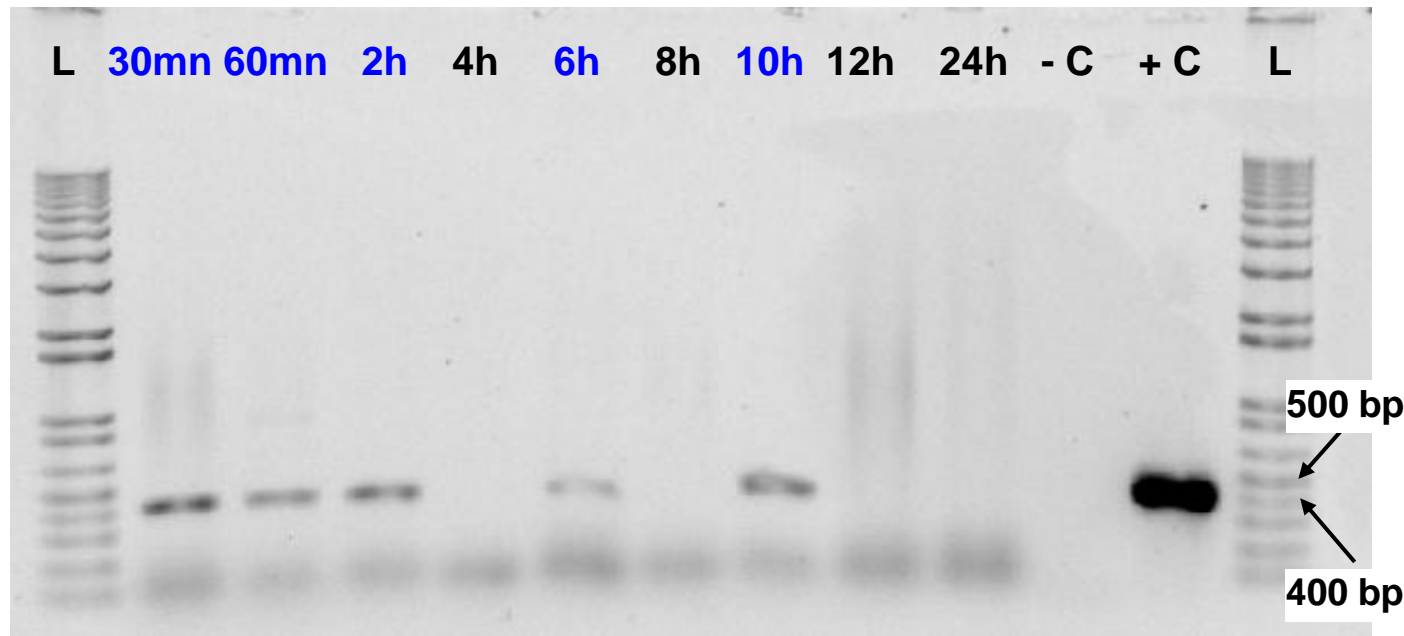
## How long mealybugs are viruliferous ?

- Feeding non viruliferous mealybugs on infected seedlings for 3 days in greenhouse
- Killing mealybugs with 75° ethanol
- DNA extraction, PCR and agarose gel electrophoresis



# Detection of CSSV into mealybugs

## Agarose gel electrophoresis



0.7% Agarose,  
1XTAE Buffer,  
120 V, 40 min,  
2.5  $\mu$ L Samples,  
2.0  $\mu$ L Ladder.

- ❑ Positive reaction of some samples : Y30mn, Y60mn, Y2h, Y6h, Y10 h
- ❑ Migration of the positive control at the same size (~400 bp)
- ❑ Presence of CSSV into mealybugs .



# **CONTROL OF CSSV VECTORS**

# Control of CSSV vectors

## □ Chemical control

- ✓ New insecticides available and already used in mirids control (imidacloprid, acetamiprid, thiacloprid)
- ✓ Test the effectiveness of these insecticides on mealybugs
- ✓ Study the effect of these news insecticides on cocoa beans quality

# 3. DIFFICULTIES

# DIFFICULTIES

- ❑ Morphological identification is difficult :
  - ✓ Adapted key of identification ;
  - ✓ Slide preparation ;
  - ✓ Need taxonomist to confirm.
  
- ❑ DNA extraction not easy for all development stages of mealybugs :
  
- ❑ No sequence of *Formicococcus* (*Planococcoides*) *njalensis* in genebank;

# 4. CONCLUSION

# CONCLUSION

- ❑ Morphological identification is difficult ;
- ❑ Techniques are available for molecular identification of mealybugs;
- ❑ CSSV can be detected into mealybugs ;
- ❑ New systemic insecticides are available to be tested for the control of mealybugs.

# 5. PERSPECTIVES



# PERSPECTIVES

- ❑ Improve the molecular technique for mealybugs identification ;
- ❑ Determine the genetic diversity of mealybugs in Côte d'Ivoire ;
- ❑ Continue detection of CSSV in different species of mealybugs in greenhouse and fields;
- ❑ Identify and control mealybugs hosts plants in cocoa orchards



# ACKNOWLEDGMENTS



Le Conseil de Régulation, de Stabilisation et de Développement de la Filière Café-Cacao



FONDS INTERPROFESSIONNEL POUR LA RECHERCHE ET LE CONSEIL AGRICOLES



World Cocoa  
Foundation



**THANK YOU FOR**



**YOUR ATTENTION**