Development of an in-field detection and cost-effective kit for cacao swollen shoot disease (CSSD) in Côte d'Ivoire and Ghana

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Introduction

Cacao Swollen Shoot Disease (CSSD)

CSSD is a vector-born transmitted disease that affects cacao crops in West Africa and causes important economic losses for smallholder cacao farmers. It decreases cacao yield from half in the first year, then cultivars die within 3-4 years following infections. Characteristic symptoms include red vein banding, fern pattern mosaic, small and rounded pods, die-back and, shoot swelling at the origin of the name of this disease. Once established on a farm, there is no chemical to fight against CSSD. The currently recommended control method is based on cutting and replanting infected areas with tolerant planting material and applying good agricultural practices such as agroforestry.

Increased spread and losses

In West Africa, the percentage of plantations affected by CSSD is increasing and economic losses mounting. In Côte d'Ivoire, approximately 12% of the cacao tree plantations are infected, with a reported loss of over 100,000 tons/year. In Ghana, the percentage has soared from 17% to 30% in a 4-year period (2017-2021).

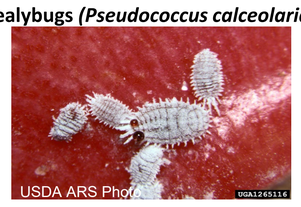
CSSD symptoms on cacao tree branches and leaves

CSSD viruses

Virus characteristics

- The cacao swollen shoot-badnaviruses (CSSD) are naturally transmitted to and between cacao trees by several species of mealybugs during feeding in a semi-persistent manner. The more active in transmission are Formicococcus njalensis (Laing), F. citri (Risso), Ferrisia virgata (Okll).

Mealybugs *(Pseudococcus calceolariae)*



- The CSSD badnaviruses do not replicate in the mealybug vector but are transmitted for several days or longer after they are acquired by the vector.
- Following infection of cacao trees, the asymptomatic phase can last from weeks to a one or more years.
- Several badnaviral species have been associated with CSSD (Ramos-Sobrinho et al., 2020), of which CSSTBV (CSSV), CSSCDV, CSSGMV (CRVBV), and CSSCEV (CRVV) (Chingandu et al., 2017a,b; 2019) infect cacao trees in Côte d'Ivoire and other cacao-producing countries in West Africa, with all but one of them addressed as species considered in the diagnostic tool development.

References: Chingandu, N., Kouakou, K., Aka, R., Gutierrez, O. A., and Brown, J. K. 2017b. J Emerg Virol Dis 3(1): doi http://dx.doi.org/10.16966/2473-1846.128.; Chingandu, N., Kouakou, K., Aka, R., Ameyaw, G., Gutierrez, O.A., and Brown, J.K. 2017b. Virol. J. 14:199; Chingandu, N., Dongo, L., Gutierrez, O.A., and Brown, J.K. 2019.. Plant Dis. 103 (4): 1302-1308; Ramos-Sobrinho, R., Chingandu, N., A. Gutierrez, O., Marelli, J.-P., K. Brown, J. Viruses. 12:443

Current detection methods and challenges

Current detection methods

Laboratory settings and/or central lab facilities are required to perform ELISA, dot blot, and PCR amplification assays. Common drawbacks are the need for transportation of samples to the lab temperature-controlled equipment, long waiting times to obtain results, potential for imprecise labeling of samples, and at times, distrust of results.

The objective of this project was to develop of a new user-friendly technology that can be translated into **a rapid, reliable, and portable detection assay** that can be **carried out on-site by non-scientific staff within the hour.**

DNAFoil® CSSD rapid detection solution

Project kick-off - R&D requirements → **Develop prototype ready for internal testing** → **Prototype finalization and first market activities** → **Final validation by IC taskforce, approval and commercialization**

August 2019

- Project kick-off
- Bioinformatic study to define strategy and feasibility
- Detailed project requirements agreed based on the input from the research partners
- Preparatory project phase

December 2019

- Reaction development and internal evaluation
- External evaluation of the Proof of Concept (PoC) reaction (**The University of Arizona**)
- Prototype adjustment for optimized LoD
- Presentation to the scientific committee

December 2020

- Adjustment of PoC and preparation of minimum viable prototype
- Minimum viable prototype production
- Validation pilot in Côte d'Ivoire
- Customer development and confirmation of customer needs

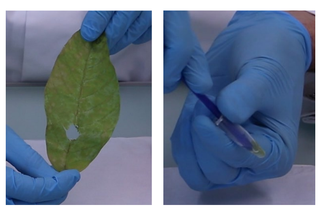
December 2021

- Final validation & expert support by the task force **CNRA, ICRAF, Conseil Café Cacao, FIRCA, Minagri, ANADER**
- Commercialization approval imminent
- Final product preparation for lab application
- Qualification of the best next product
- Customer & research network build-up

November 2022

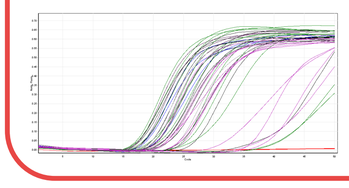
Experimental workflow

1. **Prepare** — Sample preparation — 5 min
2. **Wait** — Incubation — 50 minutes
3. **Know** — Read-out — 1 min



Easy DNA extraction directly from leaves – no spin columns, no centrifuge

Real-time DNA amplification



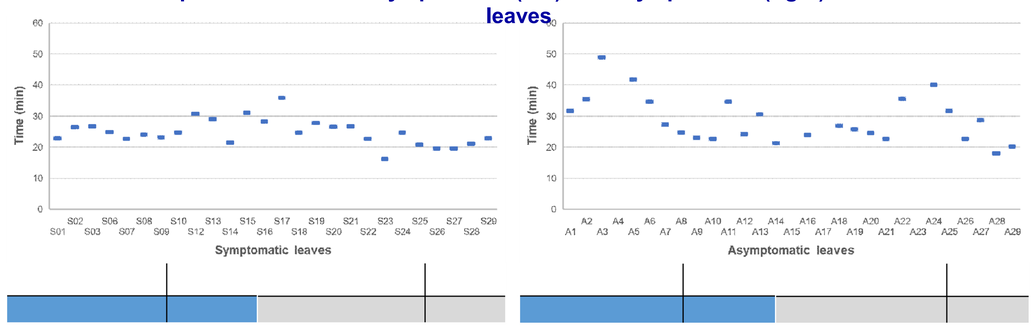
Results within **60 minutes**

Results

Detection of CSSTBV, CSSCDV and CSSGMV in symptomatic and asymptomatic cacao leaf samples

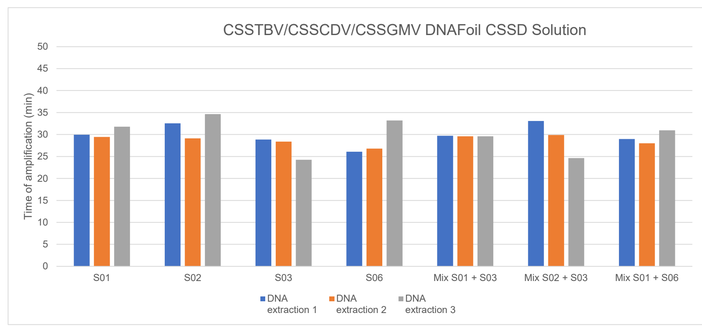
Information from customer		Number positives/Total number		
Sample type	Reported virus type	CSSTBV	CSSCDV	CSSGMV
Symptomatic	CSSTBV	2/2		
Symptomatic	CSSCDV		2/2	
Symptomatic	CSSCDV	4/4		
Symptomatic	CSSGMV	2/2		
Symptomatic	CSSCDV	4/4		
Symptomatic	CSSCDV	4/4		
Symptomatic	CSSCDV		8/8	
Asymptomatic	CSSTBV	2/2		
Asymptomatic	CSSCDV		3/4	
Asymptomatic	CSSCDV	4/4		
Asymptomatic	CSSGMV	3/3		
Asymptomatic	CSSCDV	2/4		
Asymptomatic	CSSCDV	4/4		
Asymptomatic	CSSCDV		7/8	

Amplification times of symptomatic (left) and asymptomatic (right) cacao leaves



Repeatability of DNA markers amplification times when tested on 3 independent extractions

		DNAFoil CSSD Solution for CSSTBV/CSSCDV/CSSGMV Time of amplification (min)		
Leaf sample	Info from customer	DNA extraction 1	DNA extraction 2	DNA extraction 3
S01	CSSTBV	29,95	29,45	31,77
S02	CSSTBV	32,55	29,13	34,65
S03	CSSCDV	28,86	28,39	24,25
S06	CSSCDV	26,09	26,8	33,19
Mix S01 + S03	CSSTBV + CSSCDV	29,7	29,59	29,59
Mix S02 + S03	CSSTBV + CSSCDV	33,08	29,88	24,64
Mix S01 + S06	CSSTBV + CSSCDV	28,97	28	30,95



Conclusions

We developed a technique capable of detecting both symptomatic and asymptomatic viral infections from leaf extracts. The test has an LOD_{95} of **1500 copies**, specificity and sensitivity are >99%

This **portable technology** will allow for an **early detection** of virus infection

Farmers will incur **less losses** by starting with a clean stock and removing infected trees before they infect the whole farm

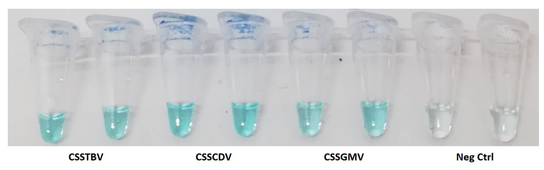
Applications

- CSSD spread mapping
- Certification of Virus-free nursery seedlings
- Surveillance of non-infected areas
- Research and breeding
- Monitoring of seed and clonal gardens for early detection of infection
- Monitoring of remediated farms for early detection of re-infection

Perspectives

- Finalize in-field portable CSSD application for Côte d'Ivoire (up to 8 samples in 1 hour, geo-tagged, aggregated, and analyzed)
- Adapt the test to new mutations and specific strains (new regions and **Ghana**)
- Develop pooled samples extraction for rapid testing in **nurseries**
- In the mid-term **onshore the manufacturing** in West Africa (PPP or private partnership)

Next Versions



- Colorimetric readout
- Simple, robust and highly portable
- Smartphone-based application