

Cacao swollen shoot disease is caused by a complex of eight viral species: implications for the development of a polyvalent diagnosis



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Cacao swollen shoot disease

- Characteristic disease symptoms on *Theobroma cacao* :
 - red vein banding in young leaves
 - shoot, stem and root swelling
 - death of the tree



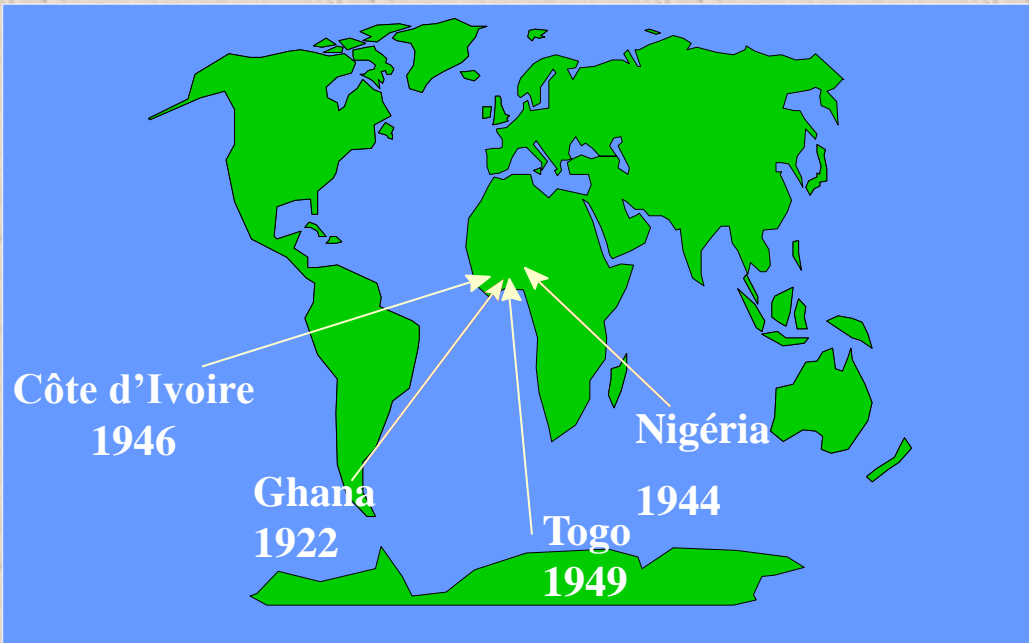
Cacao swollen shoot virus (CSSV)

- Family *Caulimoviridae*
Genus *Badnavirus*
- dsDNA (7-7.3kpb)
- Bacilliform particles
(30-150nm)
- Semi-persistent transmission
by mealybugs
(*Pseudococcidae*)
- No transmission confirmed
by seeds



CSSV epidemics in Ouest Africa: differentiated situations according to the countries

Geographic and historical distribution of the disease

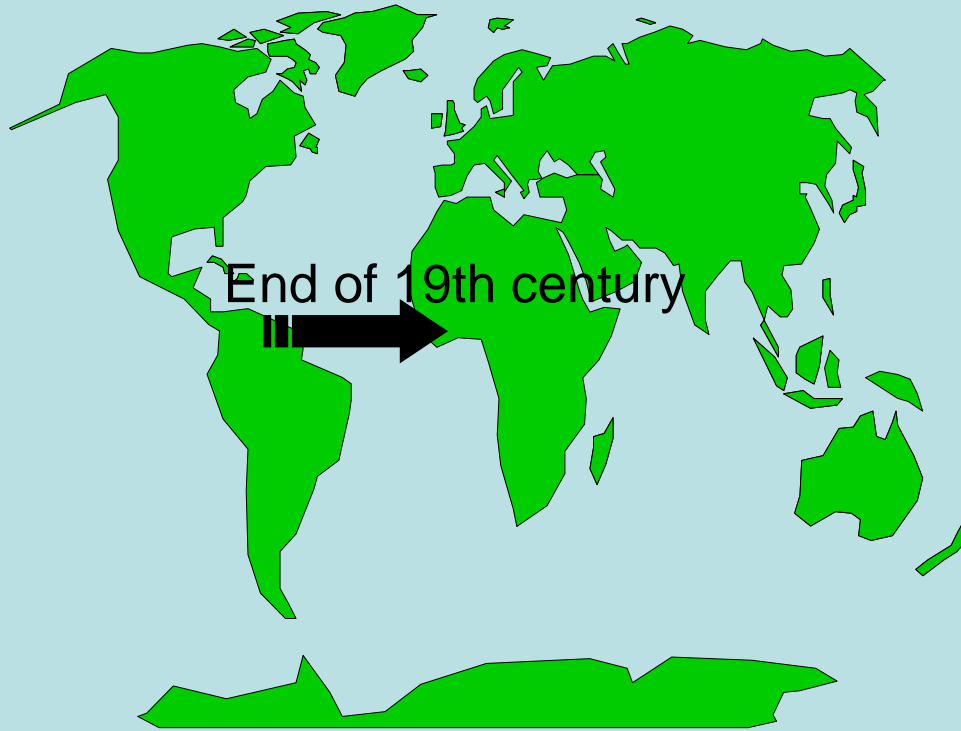


In Ghana: the disease **diffused rather quickly** almost everywhere,
New outbreaks in the West

In Togo: the disease **diffused only** in the south producing area (Kloto) until 2000,
New outbreaks since 2000 in the main producing area (Litimé)

In Côte d'Ivoire: The disease has **not diffused** from outbreaks in the east until 2002
New outbreaks in the Centre West and now everywhere

Introduction of cocoa trees in Owest Africa



Probable origin : host shift (s) from indigenous reservoir plants
(*Sterculiaceae* : *Cola* species, *Bombaceae* : *Adansonia digitata* -baobab-, *Ceiba pentandra* -kapoktree-)

WHAT to do with this disease?

- Indexation with a polyvalent diagnostic taking into account the virus diversity

(more for quarantine than for monitoring in field or only for rootstock and control elite trees)

- Eradication/Replanting varieties with good and sustainable resistance:

Need for an accurate screening of resistance with isolates representative of the virus diversity

And for a more fundamental point of view

- Understanding the epidemiologic aspects of the disease : renewed outbreak in unaffected areas, dispersion of the different isolates

▲ Need to study the virus variability

WHAT about the CSSV variability?

- ✓ Until 2000, only symptom and severity variability described between CSSV isolates
- ✓ a first analysis led to the conclusion that geographical differentiation is more important than differentiation between mild and severe isolates to explain the molecular divergence
- ✓ Alignment of the 6 full length CSSV genomes led to the design of diagnostic primers in the first region of ORF 3

History of Partnerships/Collaborations

**ITRA/ CRA/F
Kpalimé
Togo**

- 1993, first isolate completely sequenced: Agou1 (Kloto area)
- 1998, 2000, 2005, 2010 : 254 (Kloto), 485 (Litimé)

Angèle MISSISSO, Komlan WEGBE



Ghana Cocoa Board
Poised to Maintain Premium Quality Cocoa

- 2000: 30 samples
- 2013 :350 samples (Ashanti, Volta, Western)
- 2014-2015: samples (Eastern, Brong-Ahafo, Central)

CRIG, Ghana

Sammy SACKEY, Henry DZAHINI-OBIATEY, Francis ABROKWAH (PhD student)



- 2009: 355 isolates
- 2010 : 291 isolates

CENTRE NATIONAL DE RECHERCHE AGRONOMIQUE

Koffie KOUAKOU (PhD student and reseacher), Ismael KEBE

Surveys in partnership with the CRA /F, Kpalimé (KLOTO)

The foci of CSSV sampled

LITIME

2000: 30 samples

2004-05: 169 samples

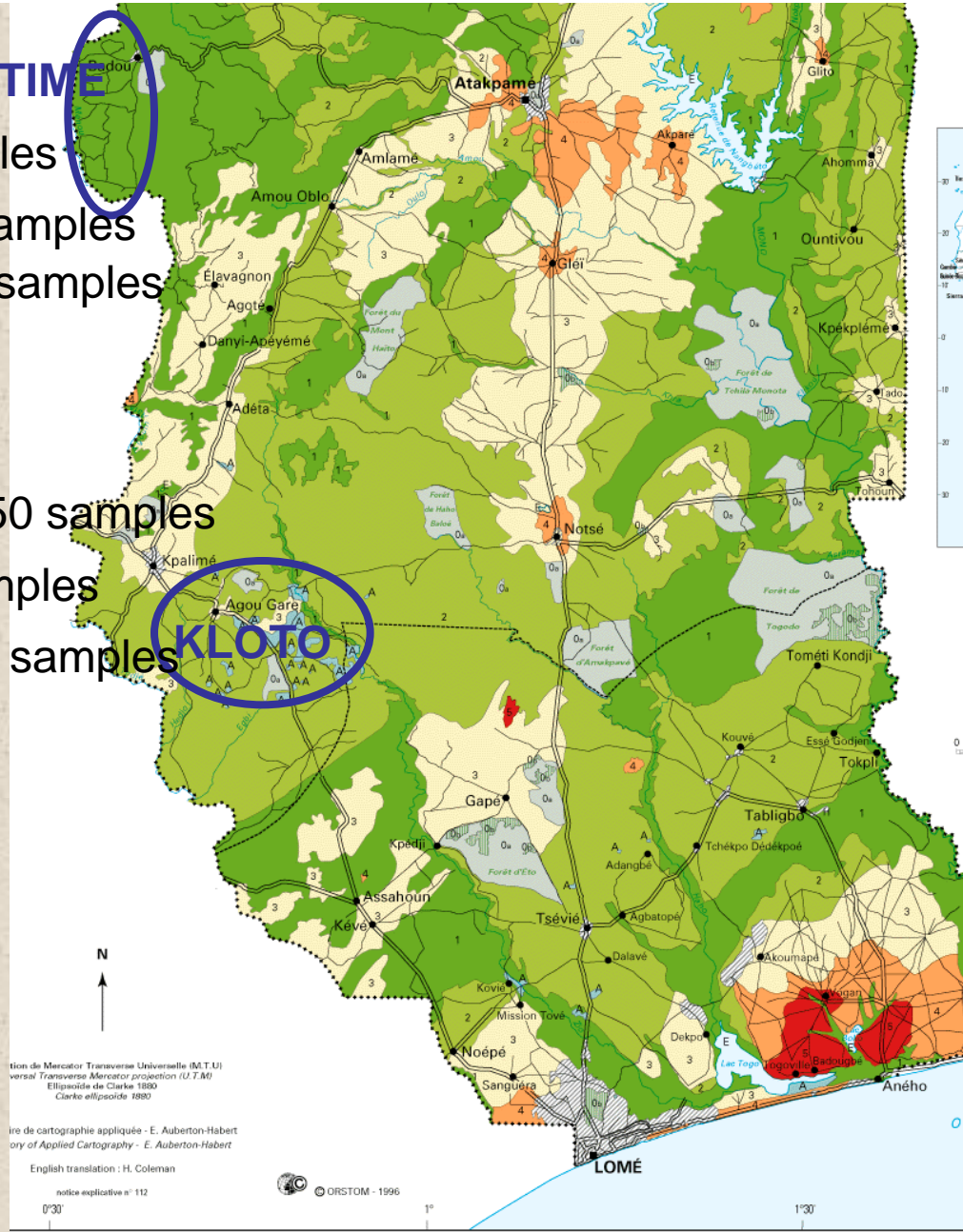
2009-10: 286 samples

1998-2000 : 50 samples

2005 : 62 samples

2009-10: 142 samples

KLOTO



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versus Transverse Mercator projection (U.T.M.)
Ellipsoïde de Clarke 1859
Clarke ellipsoïde 1880

ire de cartographie appliquée - E. Auberton-Habert
ory of Applied Cartography - E. Auberton-Habert

English translation : H. Coleman

notice explicative n° 112

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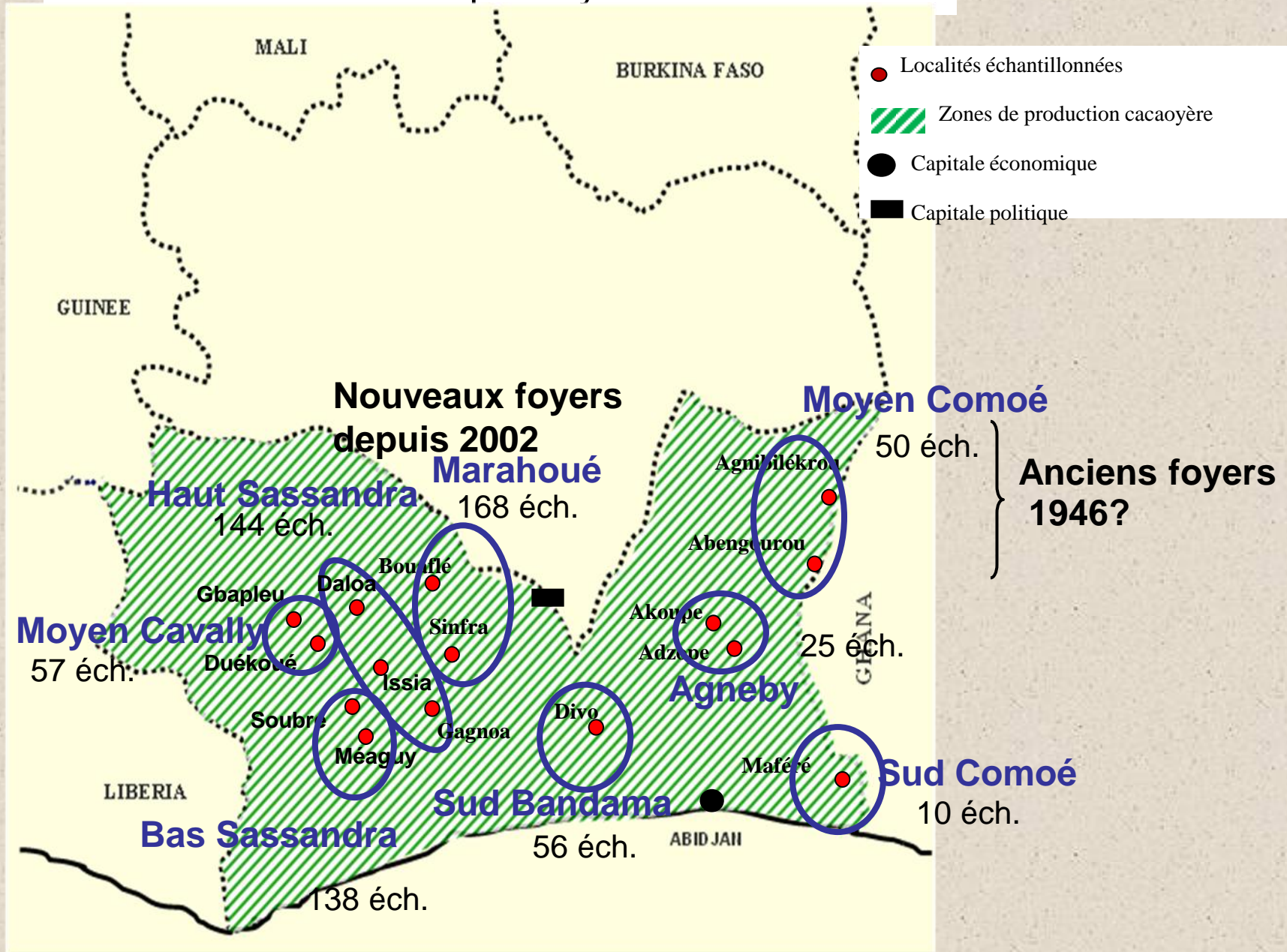
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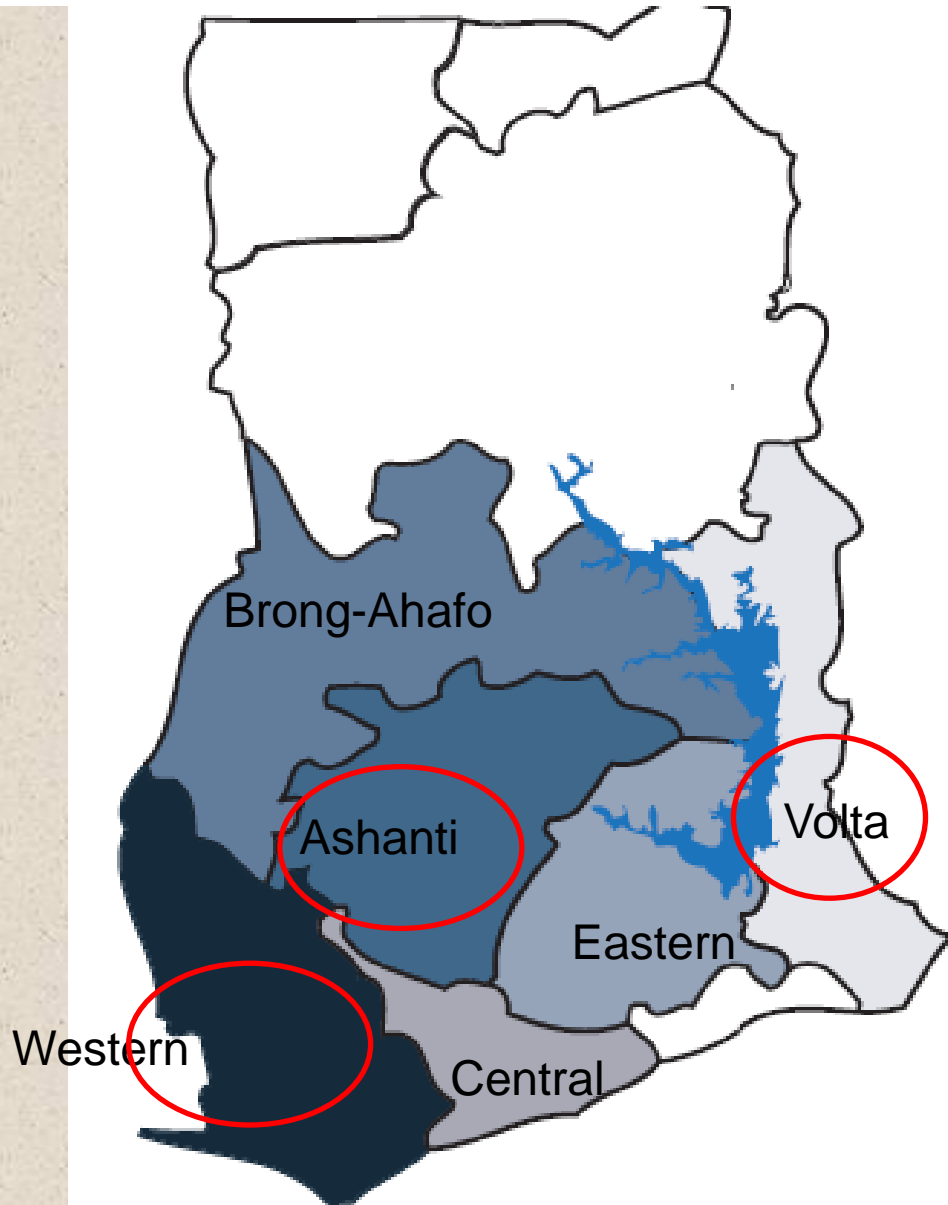
Cocoa growing regions in Ivory Coast

The foci of CSSV sampled by CNRA 2009-2010



Cocoa growing regions in Ghana

The foci of CSSV sampled by CRIG



2000: 30 samples

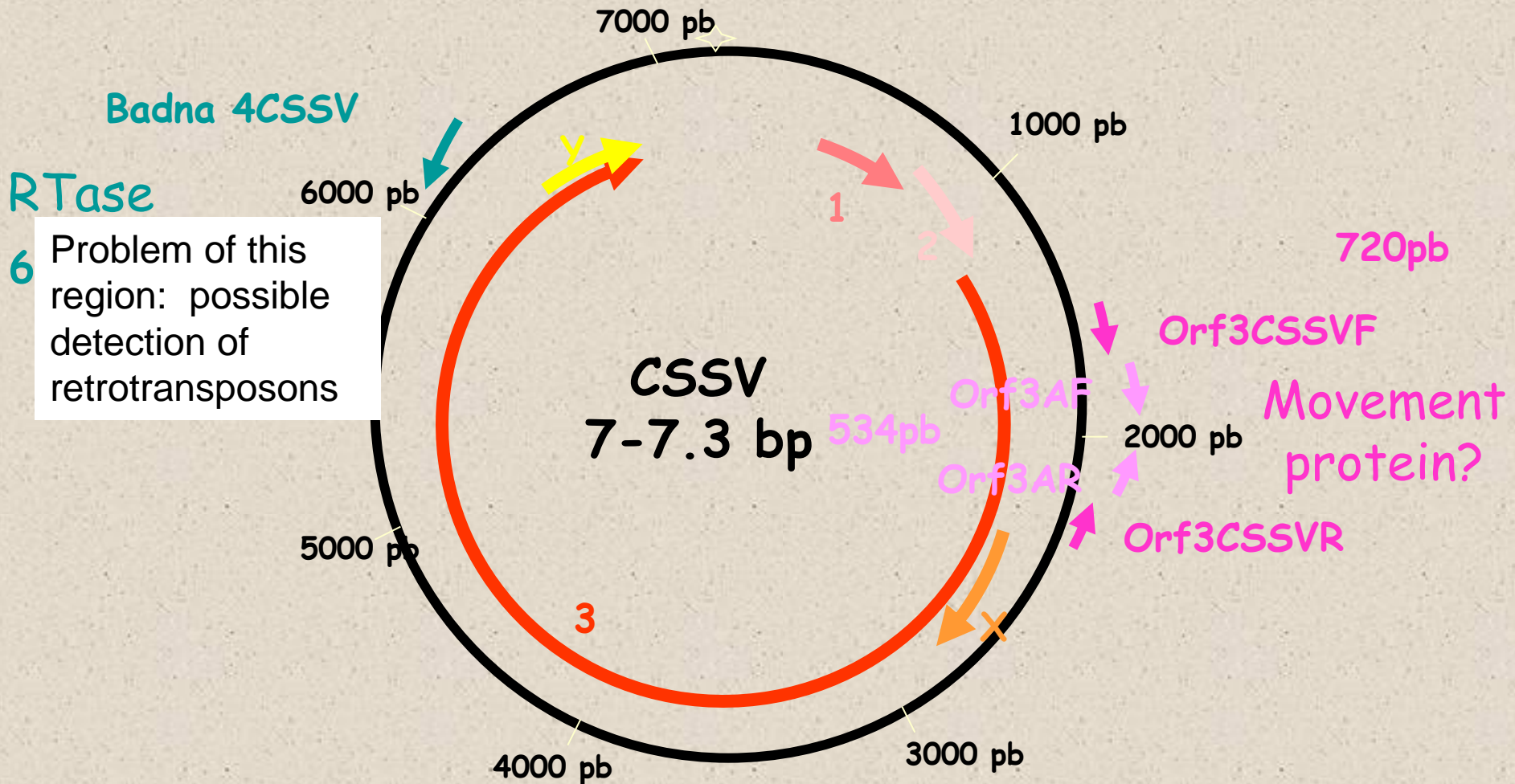
2010: 10 samples

2013: 350 samples
(Volta, Ashanti, Western)

2009/2010

Western	- 173,110 (56.5%)
Ashanti	- 97,310 (15.4%)
Brong Ahafo	- 60,500 (9.6%)
Eastern	- 59,800 (9.5%)
Central	- 56,510 (8.9%)
Volta	- 600 (0.1%)

Variability study by PCR-direct sequencing



Neighbour Joining phylogeny, based on alignment of the first part of ORF3

Group B very ubiquitous

At least 8 CSSV species (more than 20% divergence between sequences): groups B-C, A, D, E, F, G, H and J

Ubiquist groups or groups specific to certain areas

Group C South of Togo+ Ghana

Group A center of Togo + Ghana

Group D center of Côte d'Ivoire

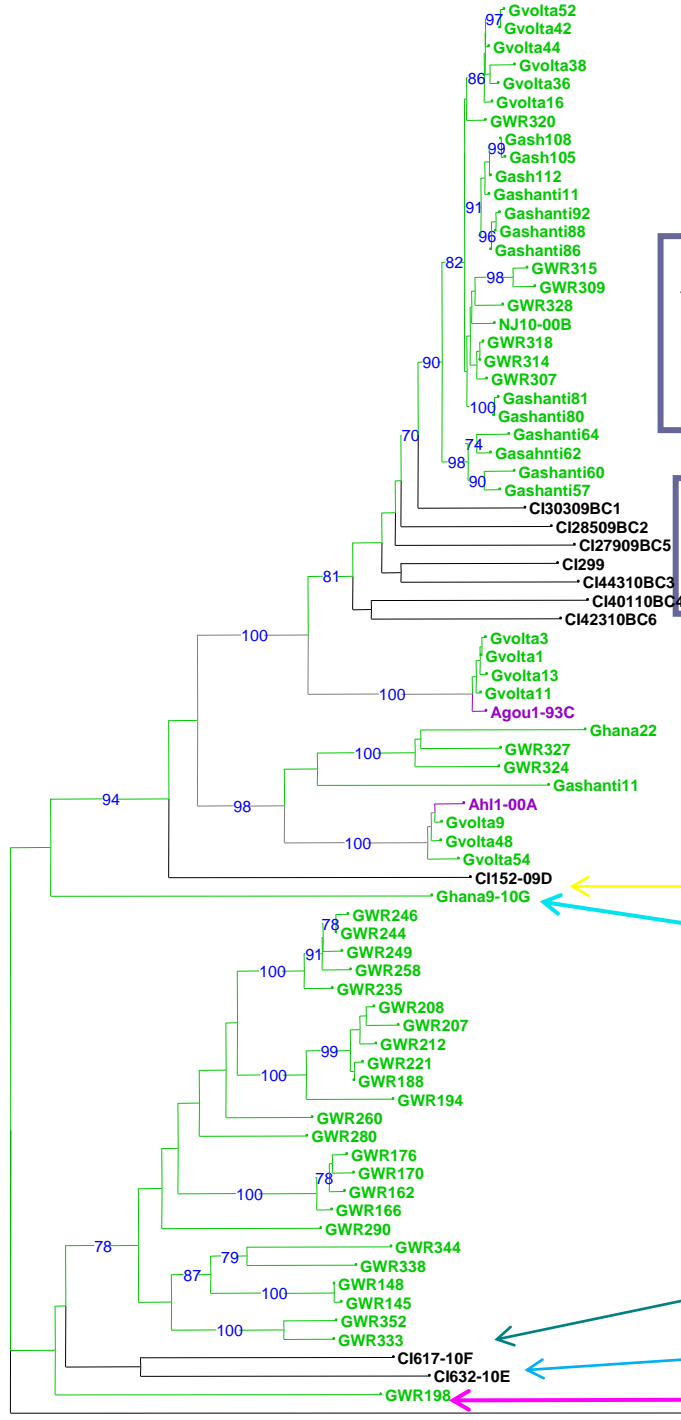
Group G Ghana

Group J Ghana western region

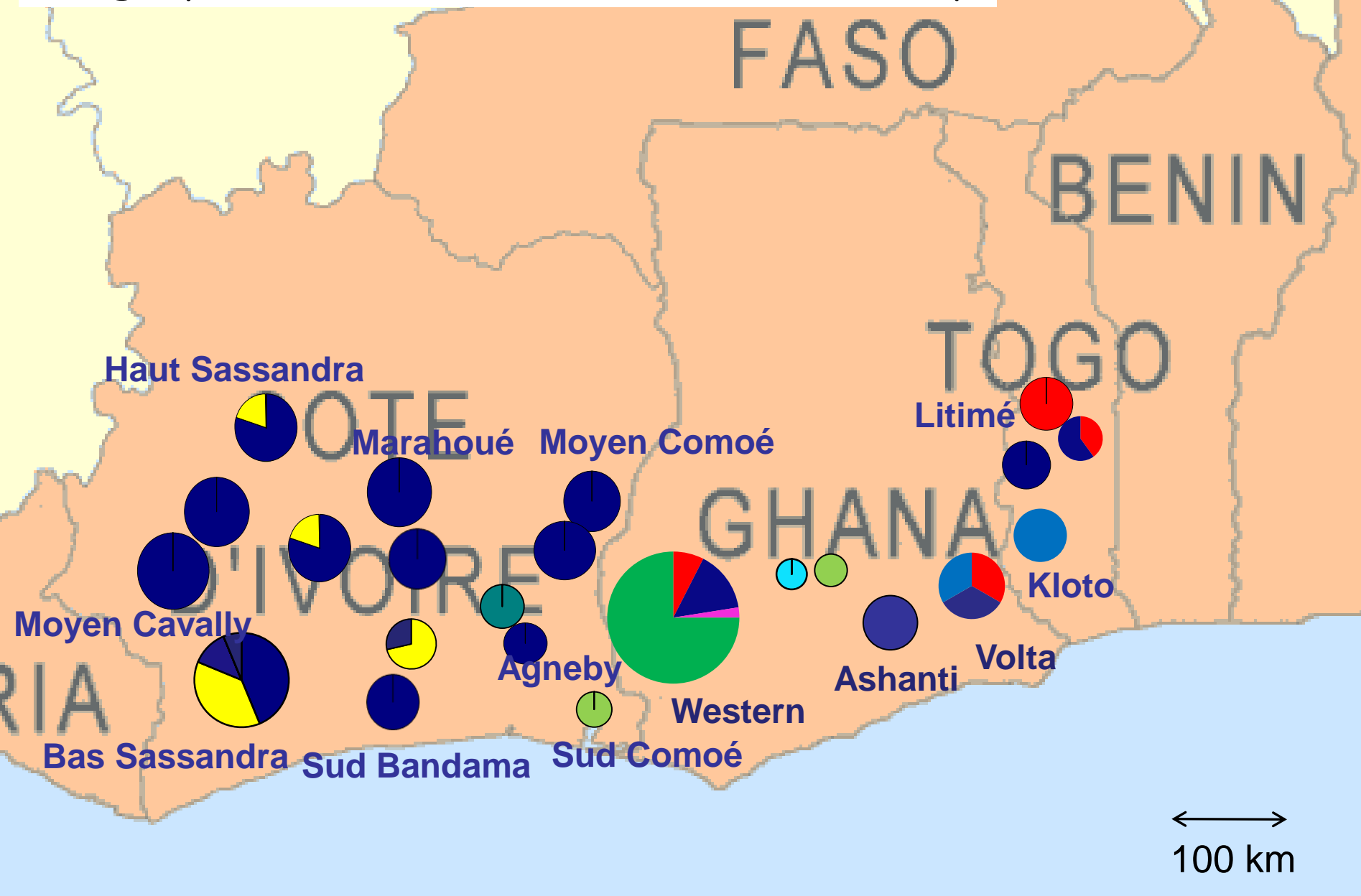
Group F: East of Côte d'Ivoire

Group E East of Côte d'Ivoire + Ghana

Group H Ghana western region




Geographical distribution of CSSV diversity



Conclusions


- The Cocoa swollen shoot disease is actually caused by a complex of different species as some other disease caused by badnaviruses (BSV, DBV)
 - ..with probably for each species, mild and severe isolates
 - ...and with recombinant virus between these 8 species, which complicate the picture of diversity
- The amplification of the first part of ORF3 could be a good tool for a polyvalent detection of CSSV but still need to be improved for a detection of all species

Perspectives




Finish to establish the geographical distribution (Ghana and Nigeria) of viral species responsible for the disease to get an effective molecular diagnostic

or set up a polyvalent serum for an IC-PCR?



Cocoa varieties screening should use at least one severe isolate of each phylogenetic group (species)

What type of screening? Open field trials in area infected, experimental transmission by mealybugs, or artificial transmission by biolistic?



Need more sampling from other foci, other country (Nigeria) and indigenous hosts to understand the origin of the variability observed