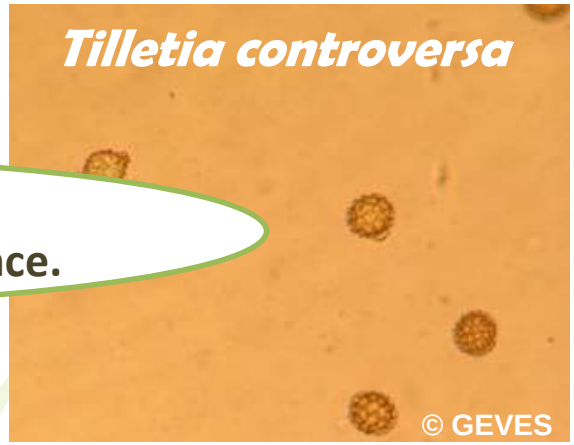
TESTA WP1
Task 1.2:
Protocols for assessing *Tilletia* spp. transmission rates.

GEVES
Expertise & Performance

Testa

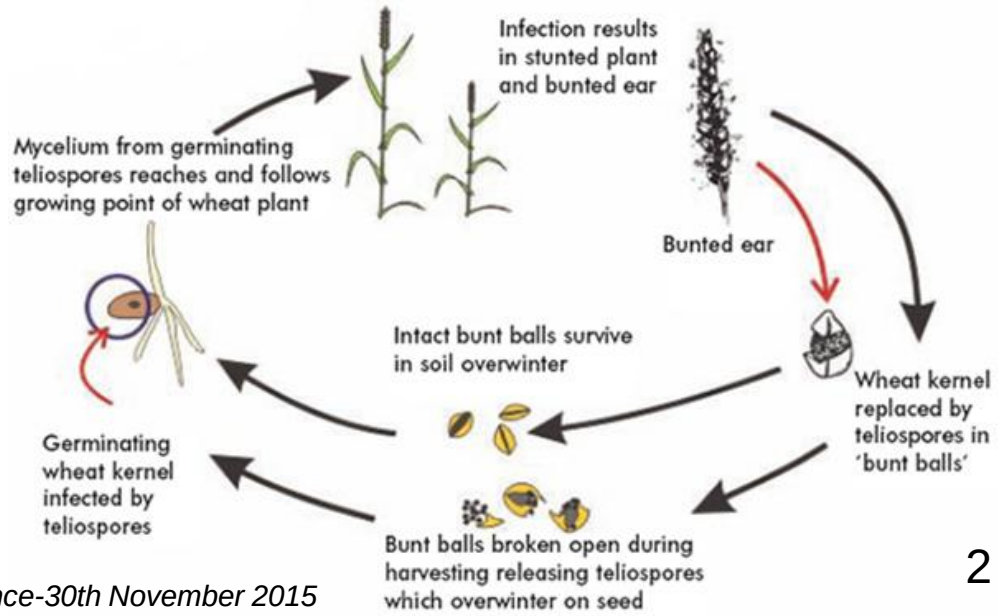
Background : Bunt of Wheat

✓ Pathogens agents of *Tilletia* spp.



FAM project showed that *Tilletia caries* was prevalent in France.

✓ Epidemiology



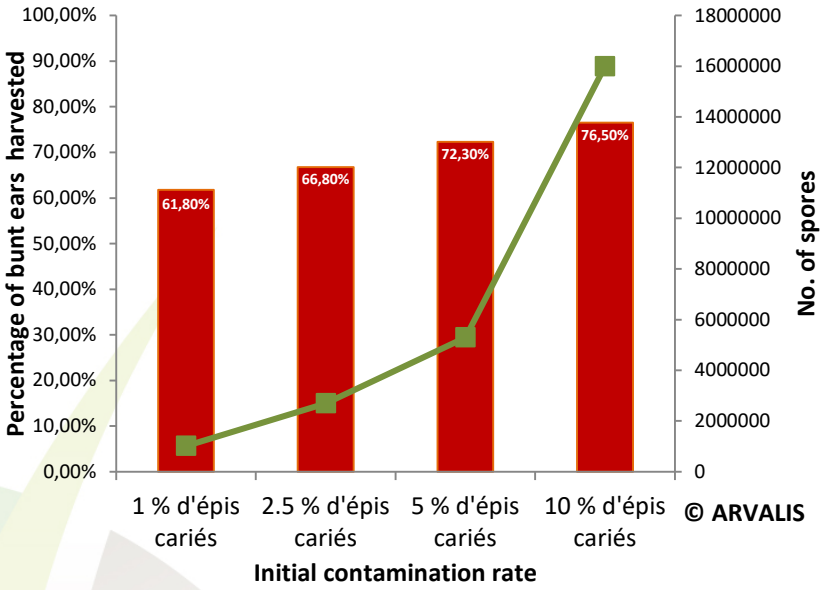
Background : Economic impact

Contamination rate of the wheat seeds:

6000 to 8000 spores / seeds → contaminated seeds

9 billions of spores / seeds→ bunt seeds

Disseminates very easily



Major risk to wheat producers ⇨ **Certification at 0 spores in 2007 in France for seed lots untreated**

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Background : Detection

✓ Method by filtration :

- Agitation of 50 grams(≈1000 seeds) in 200 ml of buffer
- Repeatedly sieved → to eliminate impurities
- Collect (x
  → Malla
- Identification of the spores
- Determination of the concentration (spores/mL)

Method does not estimate the viability of the spores

✓ Alternative method of treatment

→ **Judge the efficiency of treatments**

Aims :

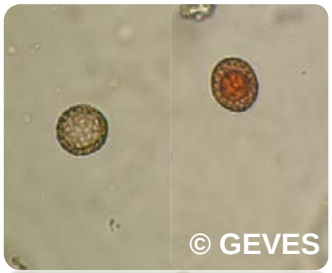
Evaluate viability of *Tilletia* spp. spores.

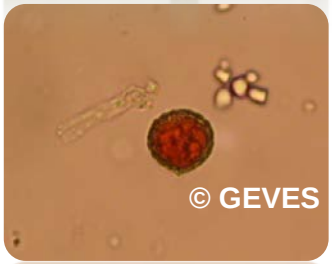
Develop a method to assess pathogen transmission from seeds to plants and from soil to plants

Measure damage threshold → symptoms' expression in field .

Set up protocol for early detection of *Tilletia* spp. by PCR

Estimate viability of spores of *Tilletia* spp.: *in vitro*



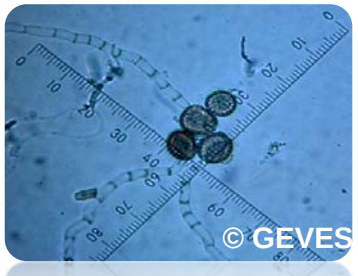


Seed lots naturally or artificially infected.

Staining of spores

Germination of spores

Detection of spores by filtration (ANSES MOA 017)



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Transmission of *T. Caries* in plantlet : *in vivo*

Artificial contamination of seeds and soil

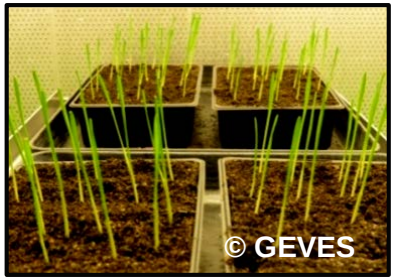
Adapted
(Method CEB N°42)

Controlled
(Detection by filtration)

Sowing in controlled conditions

Transmission of viable pathogen to plantlet

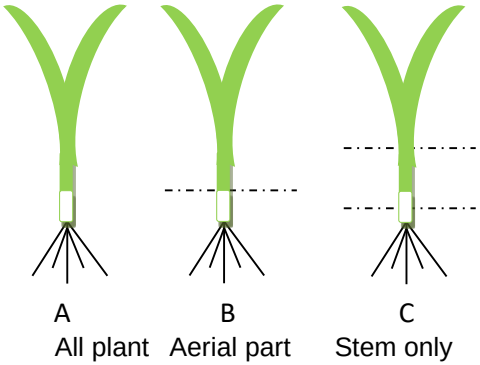
Adapted | **Controlled** | **Validated**



Sampling of plantlets for PCR analyses
Transfert of plantlets in field/greenhouse at 2 leaves stage

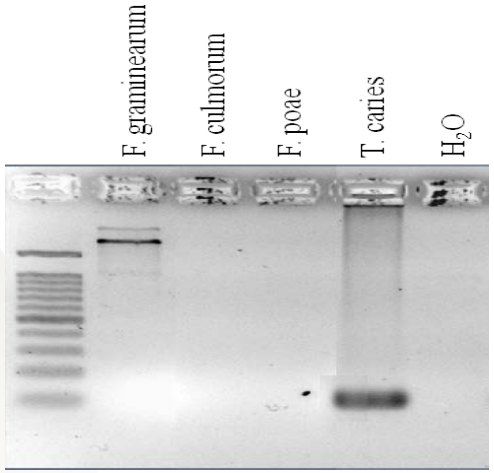
Early detection by PCR

- On plantlets
  - Different stages of development
    - Cotyledons
    - 2-3 leaves → better repeatability
  - Different sampling areas



- On soil
  - Direct sampling
  - Sampling after filtration and centrifugation

Primers provided by Arvalis



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Experimental design

Range of contamination

From 0 to 10 000 spores alive/seed (2013 and 2014).
From 0 to 200 000 spores alives/gram of soil (2015)

Transfert of plantlets

In field (2013 and 2014)
& in greenhouse (2014 and 2015)

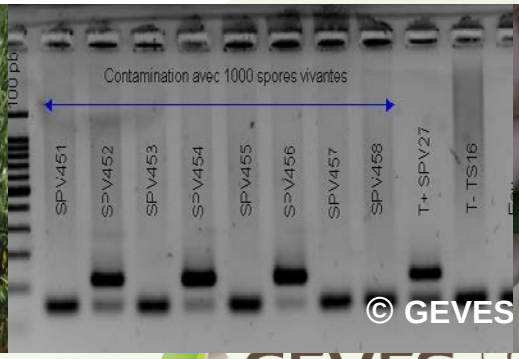
2 experimental fields (FNAMS) :

Brain-sur-l'Authion (49), Bourges (18)

1 greenhouse(GEVES)

Beaucouzé (49)

Observations to evaluate the viability of the spores
% of bunt ears in fields and greenhouse
% of infected plantlets detected by PCR
(3-4 leaf stage)



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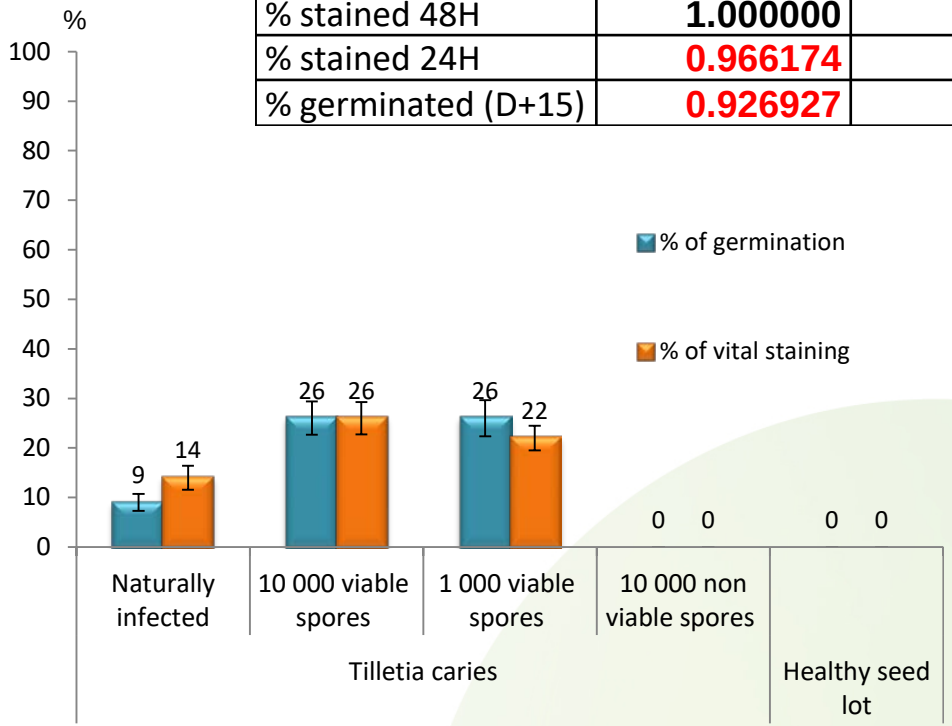
Viability control of spores

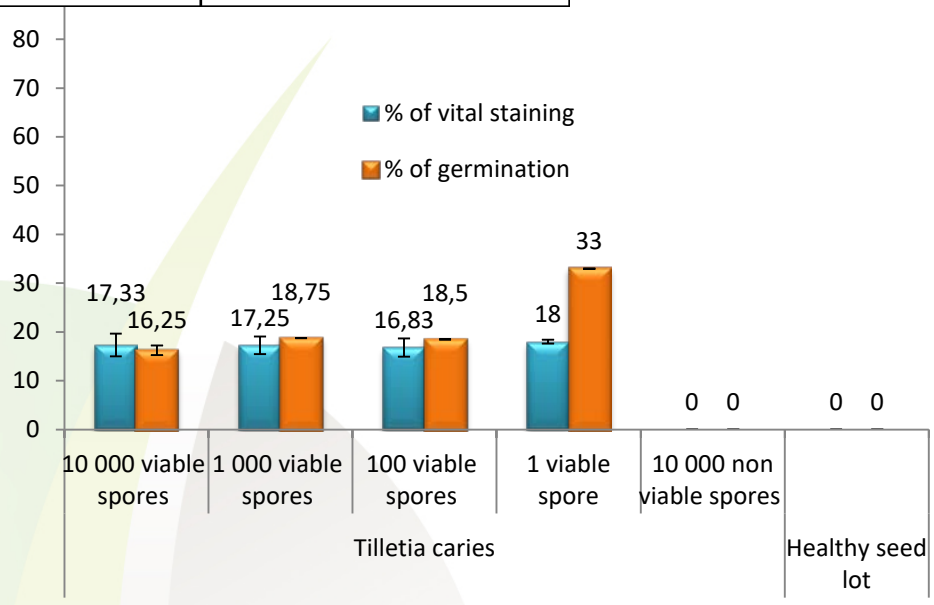
Comparison of the two methods used to estimate viability of spores.

Correlations (Comparison staining-germination)

	% stained 48H	% stained 24H	% germinated (D+15)
% stained 48H	**1.000000**	**0.966174**	**0.926927**
% stained 24H	**0.966174**	**1.000000**	**0.946744**
% germinated (D+15)	**0.926927**	**0.946744**	**1.000000**

Significant correlations at p < .05000 N=72





Very good correlation between staining of the spores of *Tilletia caries* and germination tests

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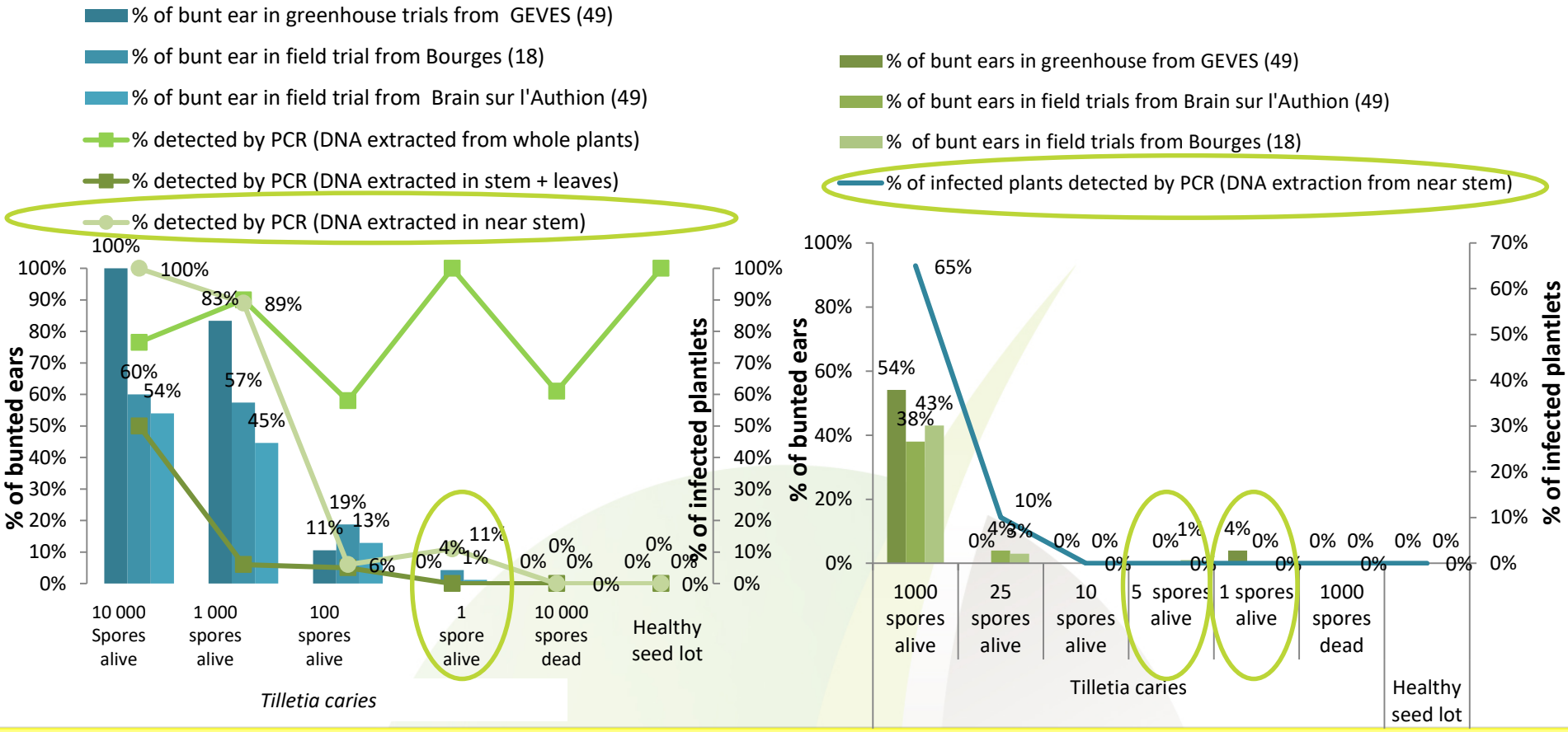
Transmission from seeds to plant

Results 2013 & 2014



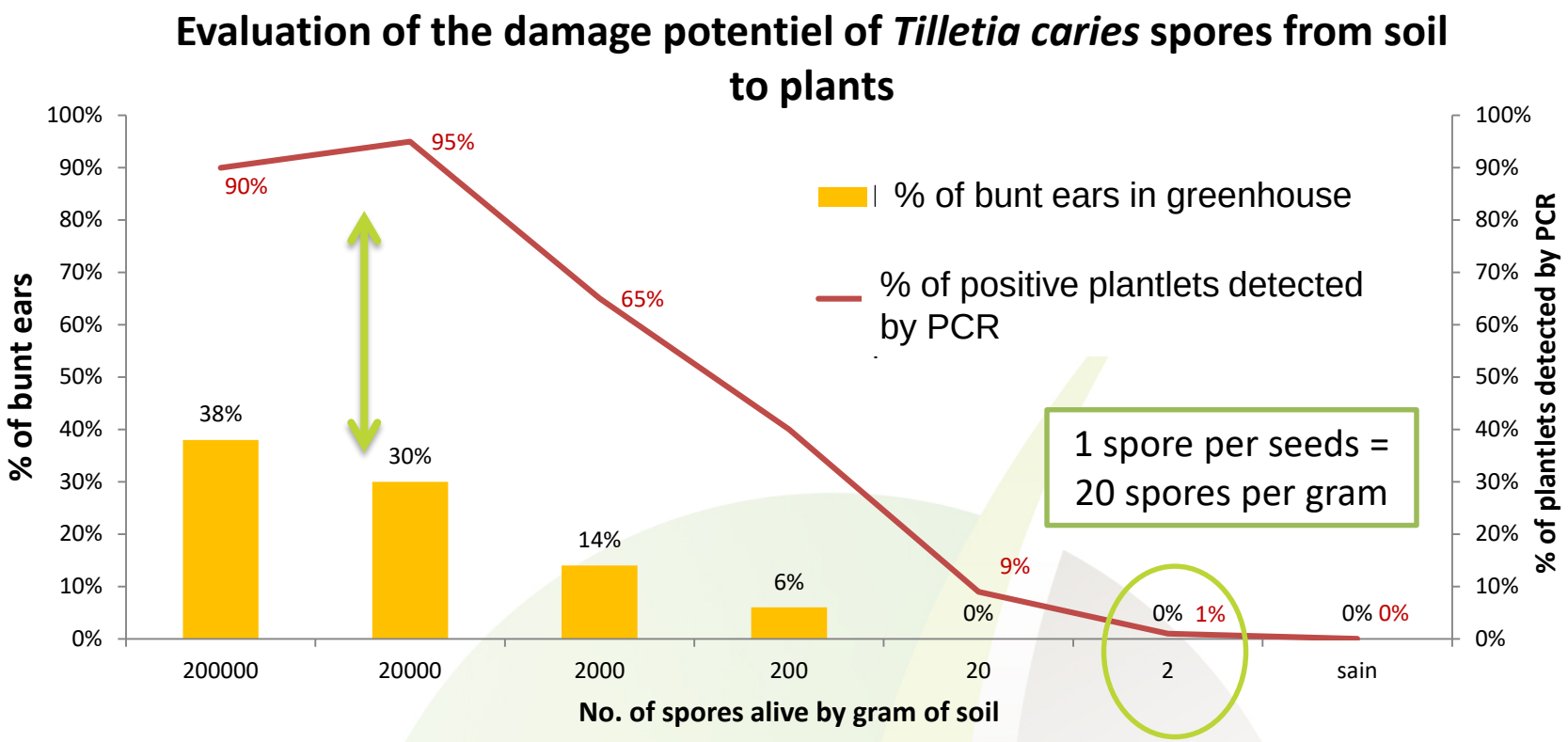
Bunt ears observed only on viable spores conditions

Correlation between % of bunt ears observed in field and infected plants detected by PCR (DNA sampling from the stem)

Damage threshold : 1 spores/seeds in greenhouse & field

Transmission from soil to plant

Results 2015



Damage threshold : 2 spores/ gram of soil

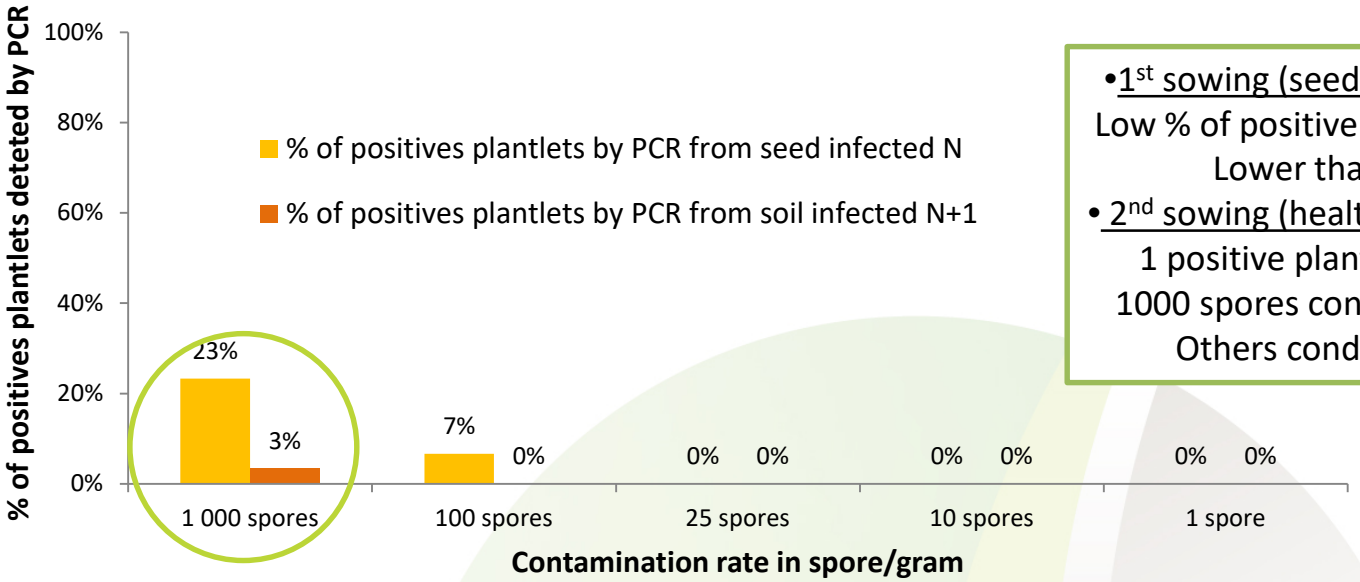
Gap between % of positive plantlets detected by PCR compared to % of bunt ears

→ Deferred sowing in time

Transmission from seed to soil and from soil to plant
Results 2015

Objective : Evaluate the dissemination between too sowing

1) Seeds artificially infected are sown in healthy soil → plantlets are analyzed by PCR after 5 weeks
2) Healthy seeds are sown in the same soil after the first sampling
→ plantlets are analyzed by PCR after 5 weeks



- 1st sowing (seeds infected in healthy soil) :
  Low % of positive plantlets detected by PCR.
  Lower than previews results.
- 2nd sowing (healthy seeds in the same soil) :
  1 positive plantlet detected by PCR for 1000 spores condition (1/30 whether 3%)
  Others conditions werenot tested

Seeds artificially infected provided from contamination of 2014 (with spores from 2013) :
Decreasing of the viability → lower transmission from seeds to plants
Nevertheless transmission of spore from seeds to soil to plant at high contamination rate
A new test is ongoing with seeds artificially infected with new spores from 2014.

Summary

2012 → Validation of a staining method to evaluate viability of *Tilletia caries* spores

2013 → 1% to 4% of bunt ears in field at 1 spore alive/ seed

2014 → 4 % of bunt ears in greenhouse at 1 spore alive/ seed condition

2015 → 1% of positive plantlet detected by PCR at 2 spores alive/ gram of soil

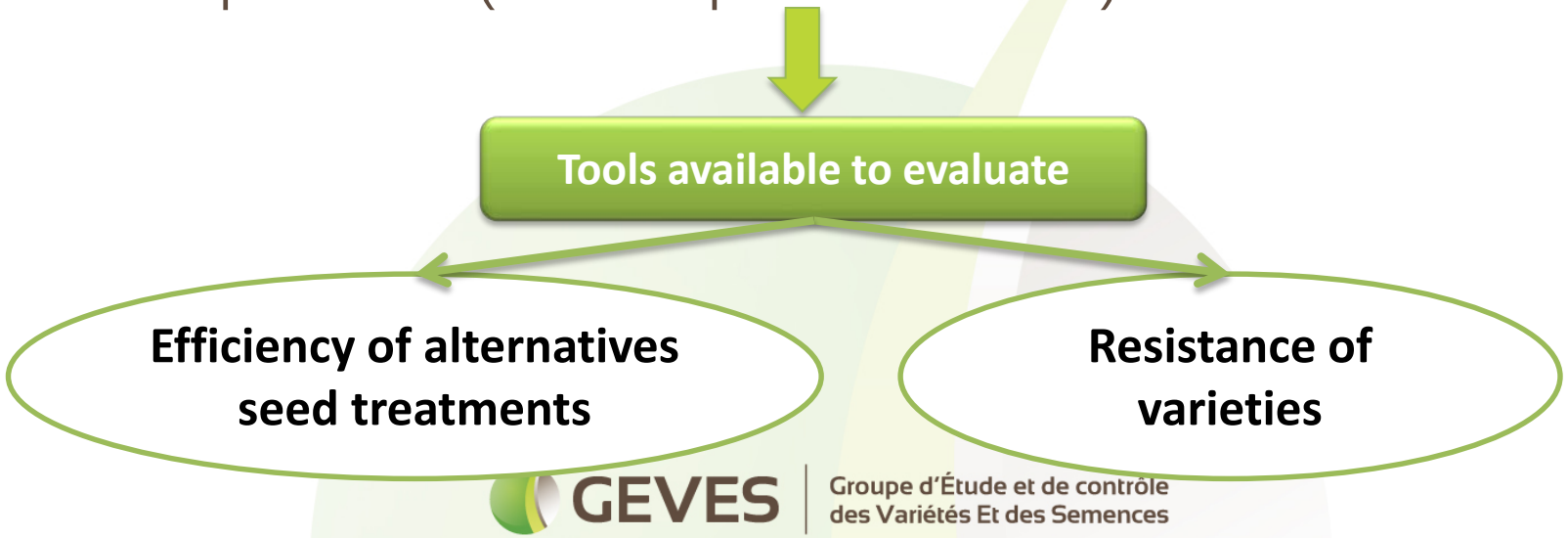
Results presented & used by French ministary

Derogation of norm at 0 spore/seed lot

Conclusion

Protocols available

- Detection and identification of *Tilletia* species in seed lot
- Evaluation of viability of spores of *Tilletia caries*
- Grow out and early detection by PCR
  - ⟹ On soil after filtration and centrifugation
  - ⟹ On plantlets at 2-3 leaves stages
- Inoculum production (seeds or plantlets infected)



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Acknowledgment

- Matthieu Rolland and Aurélie DUPUY from **BioGEVES**, France
- Romain Valade and Clément Compagnon from **ARVALIS**, France
- Julie Gombert and Fabien Colombel from **FNAMS**, France.
- Berta Killerman and Robert Bauer from **LfL Pflanzenbau**, Germany.
- Laure Weisskopf from **Agrocope**, Switzerland.
- Eckhard Koch from **JKI**, Germany.
- Valérie Cockerell and Marian McNeil from **SASA**, Scotland.

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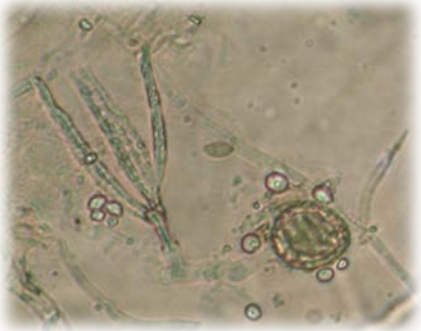
Thanks for your attention





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