

# Efficiencies of bacterial transmission from plants to seeds and from seeds to plantlets

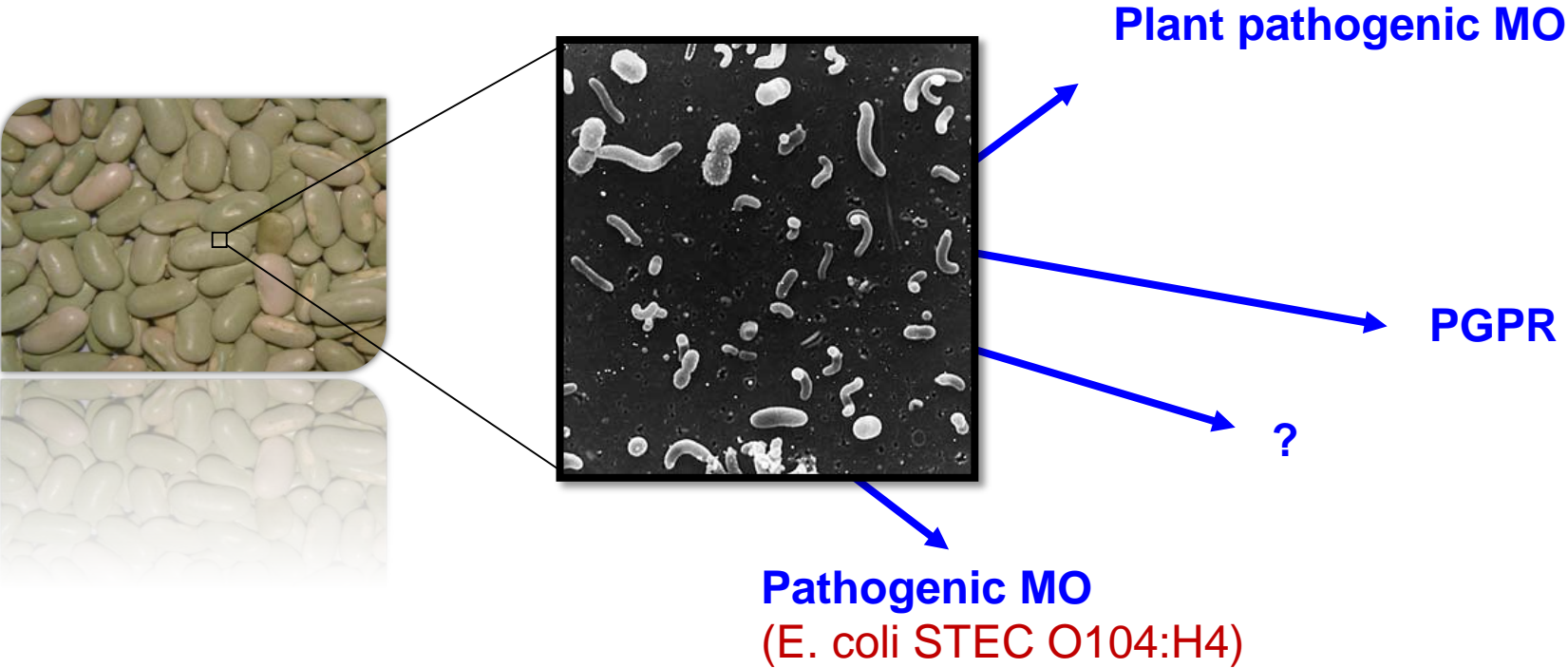
Marie-Agnès Jacques



EMERSYS  
Emergence, systématique  
et écologie des bactéries  
associées aux plantes



# Seeds: vectors of a diversified microbiota



# Seed transmission



Long-term inoculum survival  
Long distance dissemination  
Introduction to new area



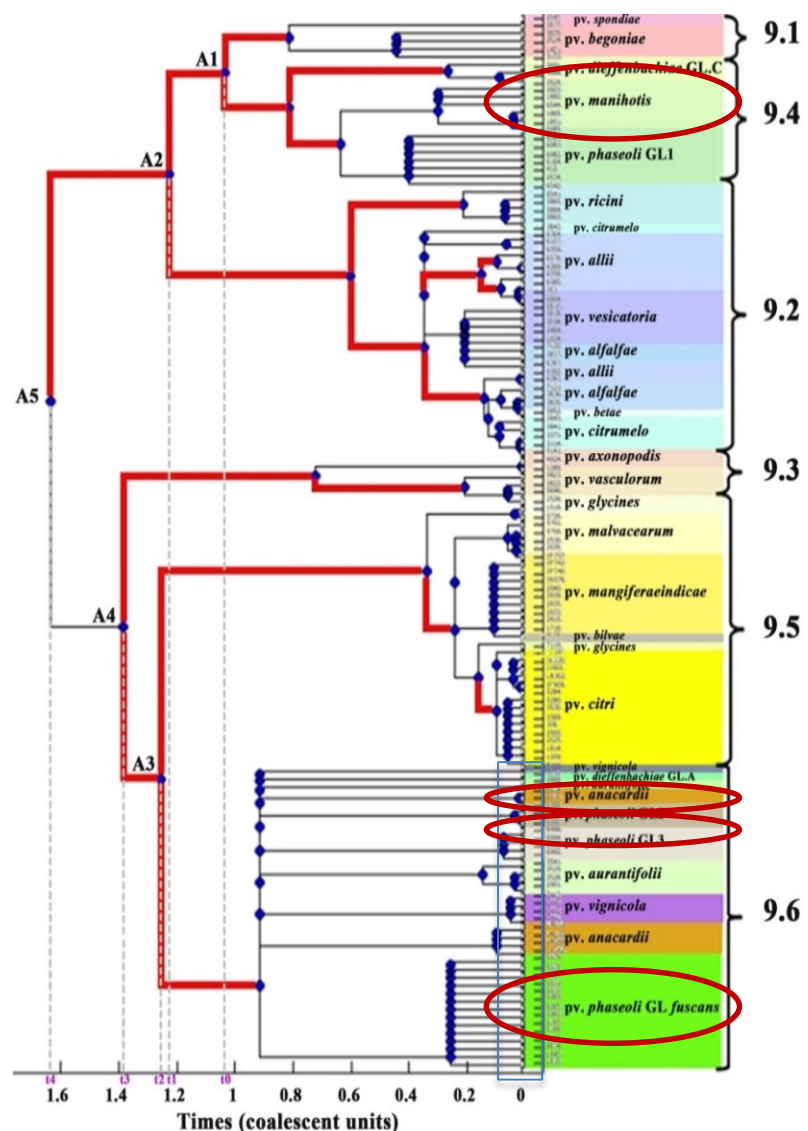
Contaminated seed: source of primary inoculum

Critical step in plant pathogen ecology and  
disease epidemiology

**Strategic step to control diseases**

**Rely on a better understanding of mechanisms  
and pathways used for seed transmission**

# The causal agents of CBB of bean: 4 genetic lineages



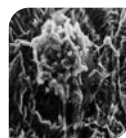
*X. axonopodis* pv. *phaseoli*  
*X. fuscans* subsp. *fuscans*



*Xap 1*



*Phaseolus vulgaris*



*Xap 2*



*Xap 3*

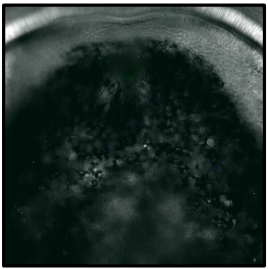


*X. fuscans* subsp. *fuscans*  
*X. citri* pv. *fuscans*  
*X. axonopodis* pv. *phaseoli* var. *fuscans*

*X. axonopodis* sensu Vauterin et al., 2000; Rademaker et al., 2005



**Role of look-alikes in transmission of *Xff* to bean seeds**



Translocation of *gfp*-tagged strain of *Xff* to seeds using confocal scanning-laser microscopy



Efficiencies of transmissions of CBB agents from seeds to plantlets.

# Look-alikes of CBB agents isolated from bean



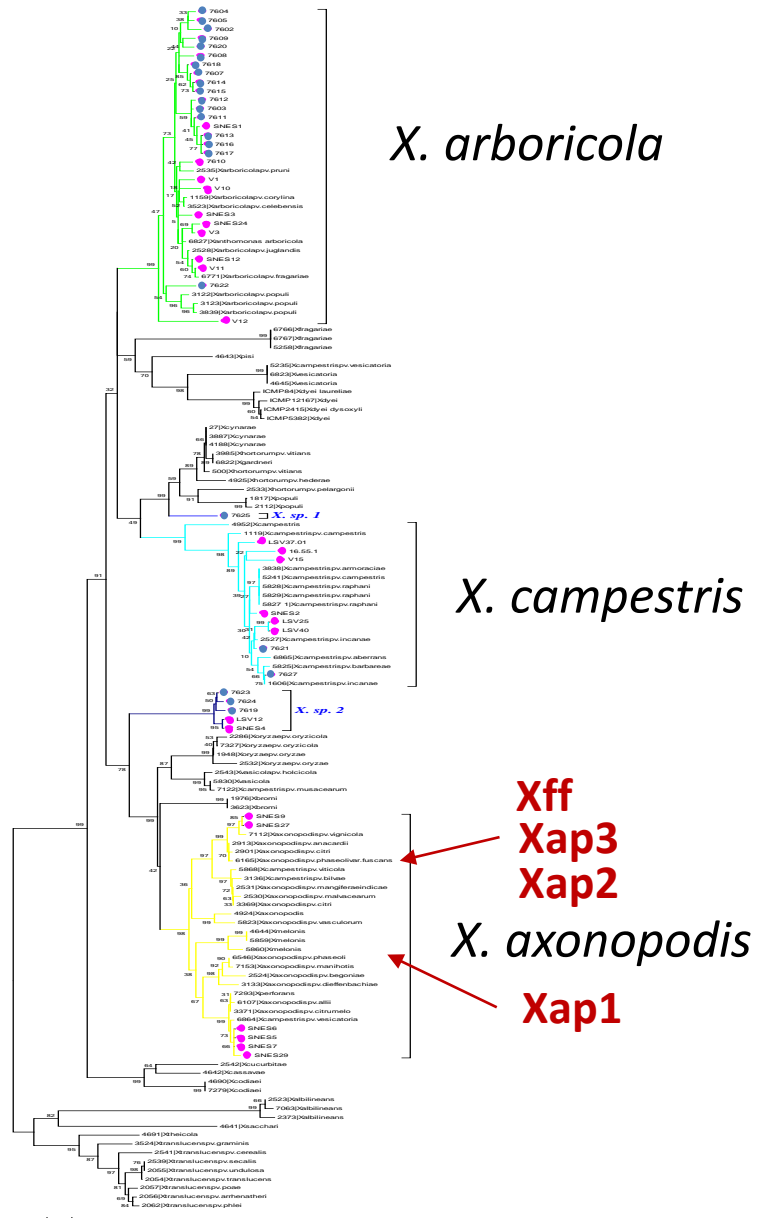
Phylogenetic tree of *Xanthomonas*

Seeds harbor look-alikes of CBB agents

Look-alikes interfere in detection of CBB agents

Bureau et al., 2013; Grimault et al., 2014

- 23 look-alikes of CBB agents

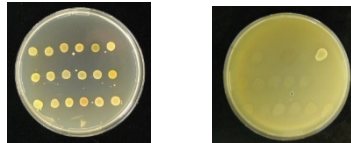


*gyrB*, *rpoD*  
NJ, 1000 bootstraps

# Role of look-alikes in seed transmission of pathogens

*In vitro*

antibiosis



and competition tests



to select strains among the 23 look-alikes that interact with CBB agents

Each look-alike inhibit  
and/or outcompete one  
of the CBB agents

## 4 CBB strains

Xff: 7767-R

Xap GL1: 6546-R

Xap GL2: 6988-R

Xap GL3: 6996-R

## 7 look-alikes

SNES 6

V13

V12

V13

SNES 27

SNES 6

V13



# Role of look-alikes in seed transmission of pathogenic strains

**Pathogen alone**

**Xff: 7767-R**

**SNES 6**

**V13**

**Look-alike alone**

**Xap GL1: 6546-R**

**V12**

**V13**

**Mixtures**

**Xap GL2: 6988-R**

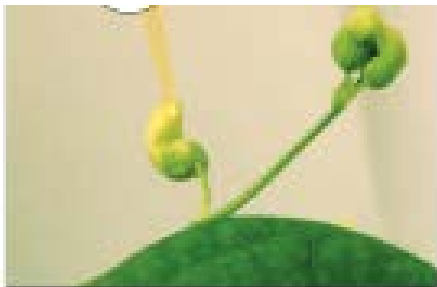
**SNES 27**

**Pathogen:look-alike (1:1)**

**Xap GL3: 6996-R**

**SNES 6**

**V13**



**Flower bud inoculation**

( $2 \times 10 \mu\text{L}$  of a  $1 \times 10^6$  cfu/mL)

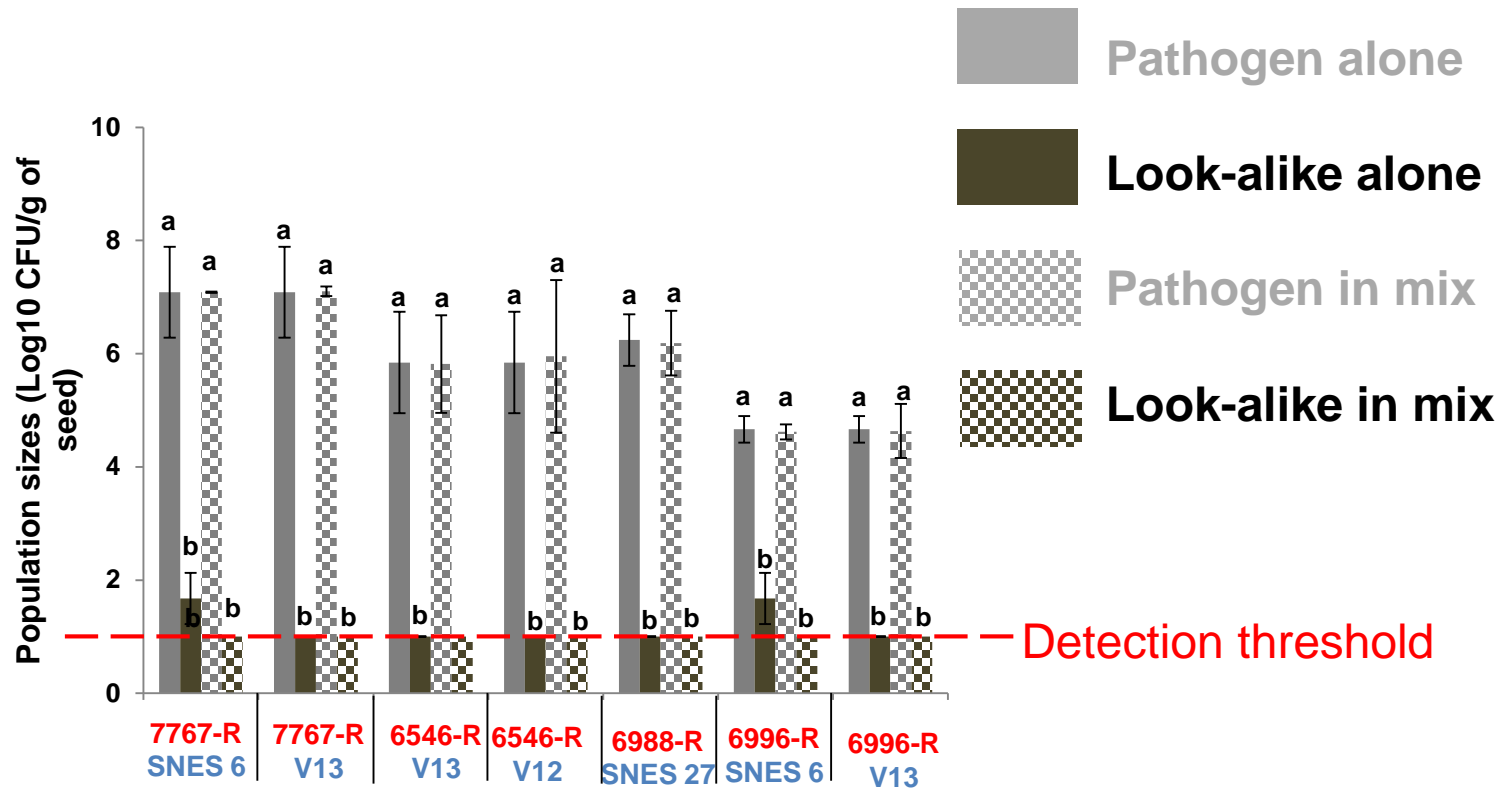
**5 plants / treatment**

**3 flower buds / plant**



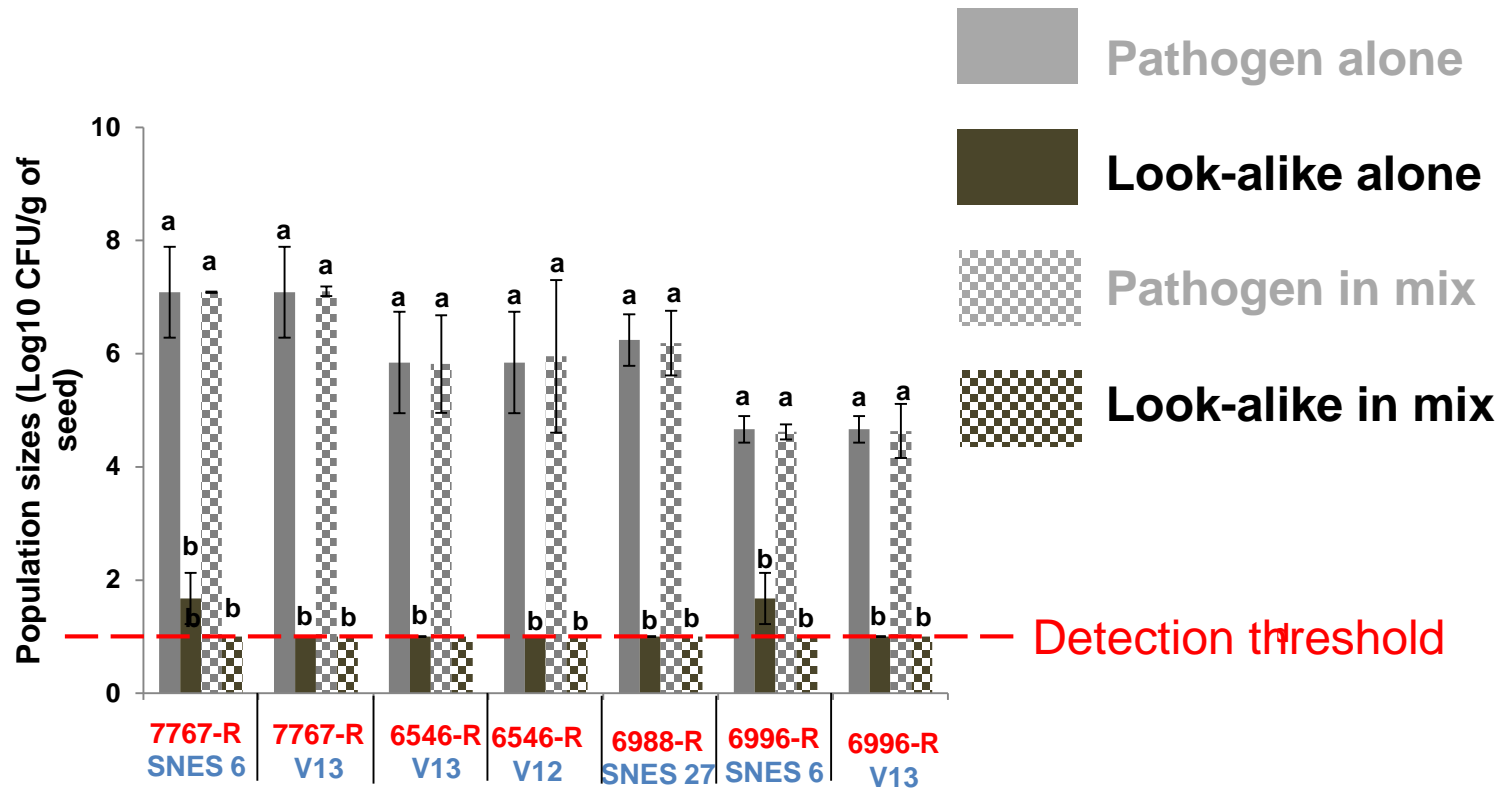


# Role of look-alikes in seed transmission of pathogenic strains





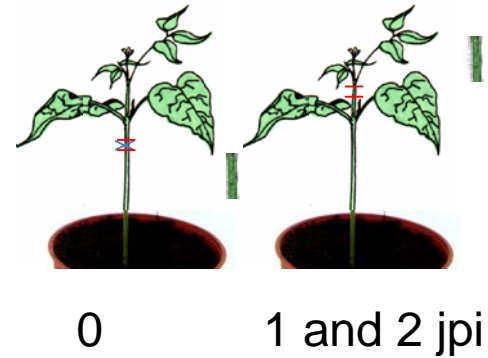
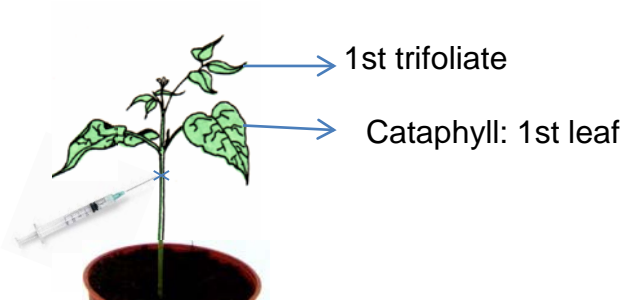
# Role of look-alikes in seed transmission of pathogenic strains



**No transmission of look-alikes**

**No effect of look-alikes on pathogen transmission**

# Xylem colonization by look-alikes and pathogenic strains



**7767-R**

**SNES 6**

V13

**6546-R**

V12

V13

**6988-R**

**SNES 27**

**6996-R**

**SNES 6**

V13

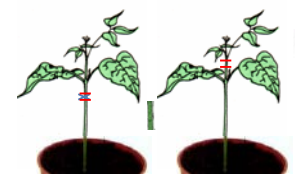
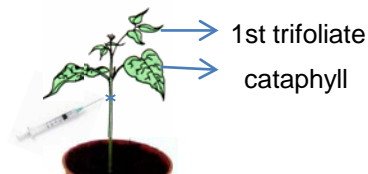
Pathogen alone

**Look-alike alone**

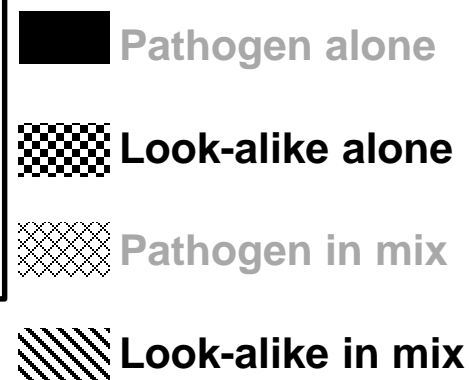
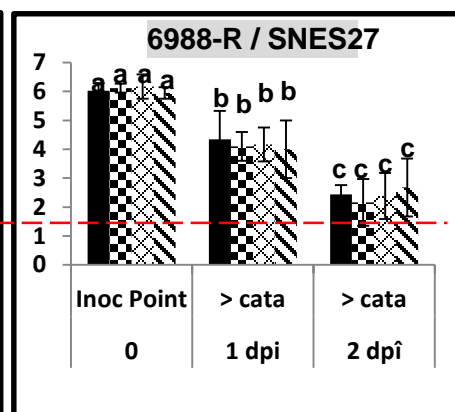
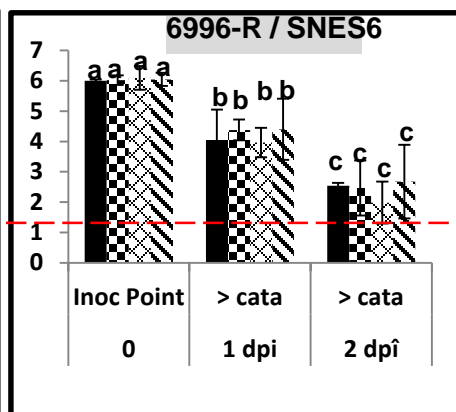
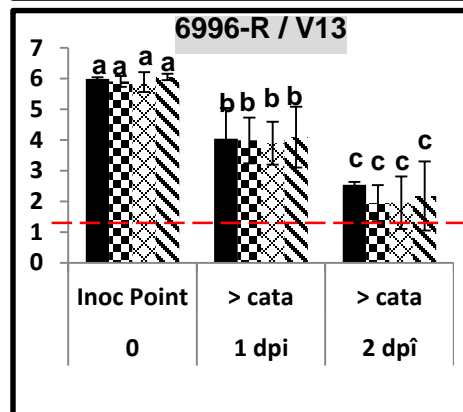
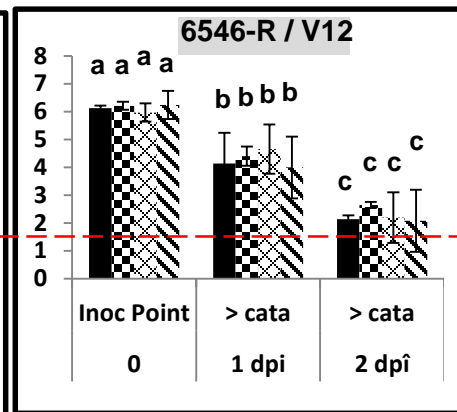
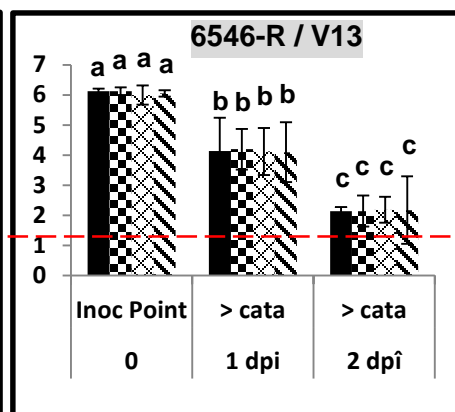
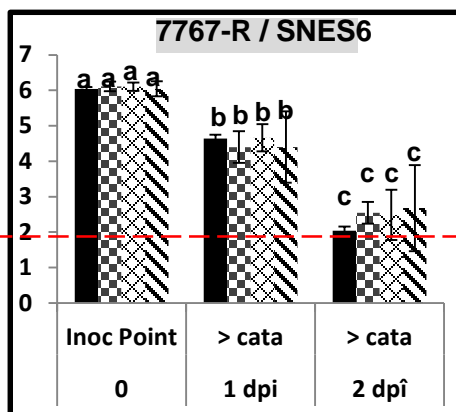
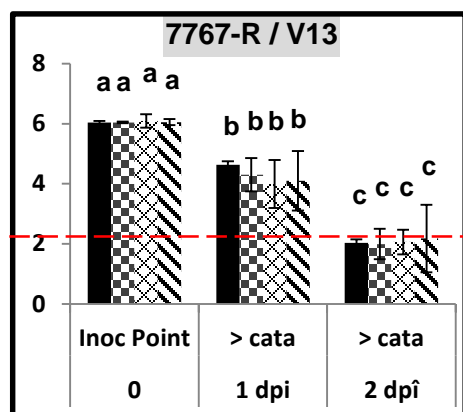
Pathogen in mix

**Look-alike in mix**

# Xylem colonization by look-alikes and pathogenic strains

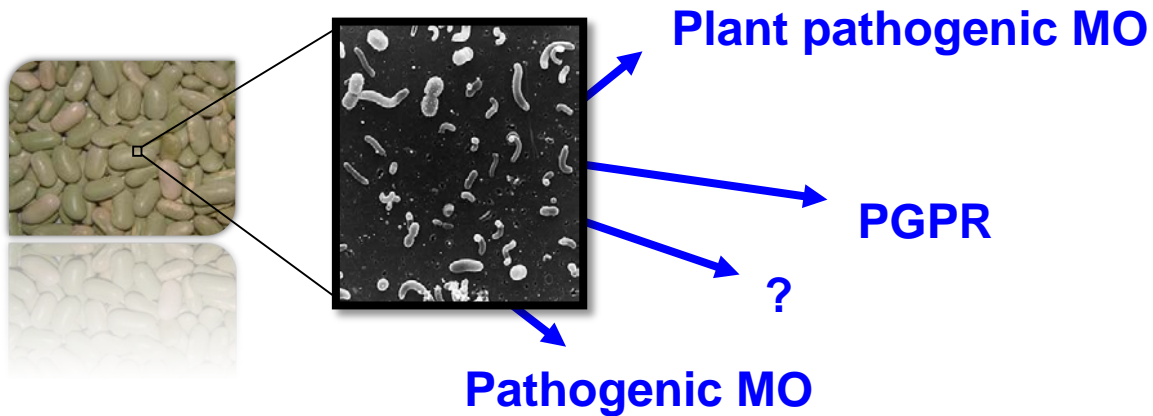


0 1 and 2 jpi



**No difference between pathogens and look-alikes**

# Look-alikes and transmission to seed of CBB agents



Not possible to monitor transmission to seeds for look-alikes

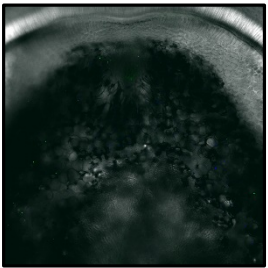
But look-alikes are isolated from seeds

Do not seem to interfere with pathogen transmission to seed

Not good candidates for biocontrol



Role of look-alikes in transmission of *Xff* to bean seeds



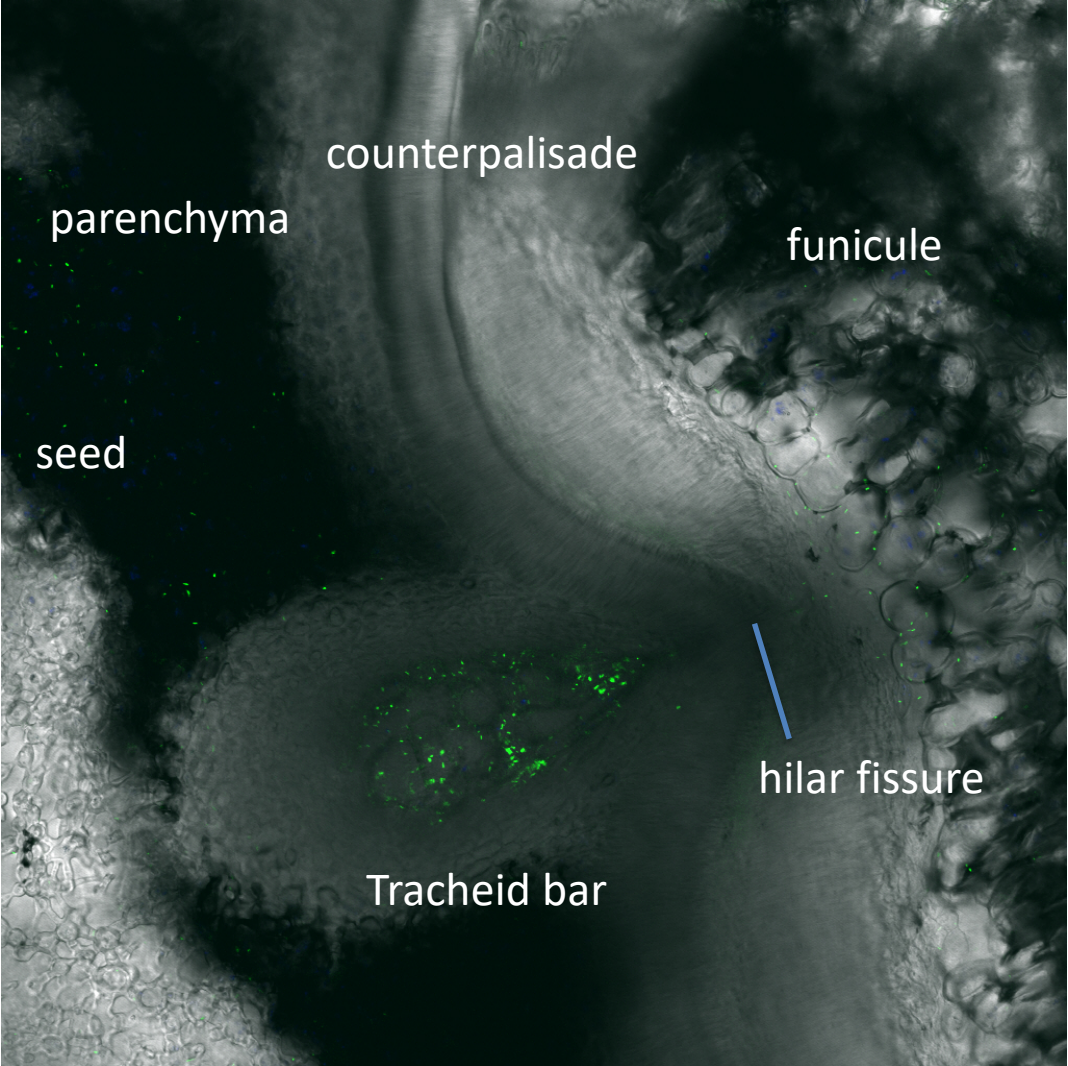
Translocation of *gfp*-tagged strain of *Xff* to seeds using confocal scanning-laser microscopy



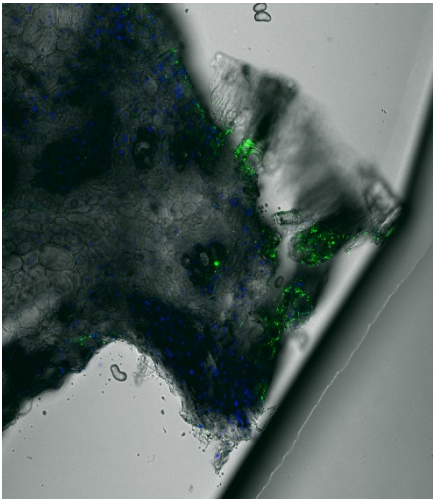
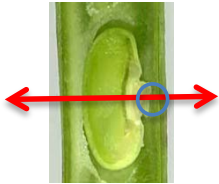
Efficiencies of transmissions of CBB agents from seeds to plantlets.

# Localization of Xff during seed formation on bean plants

*Xff gfp*-tagged cell following spray inoculation of bean plants at flower bud stage



Transversal cut at the hile



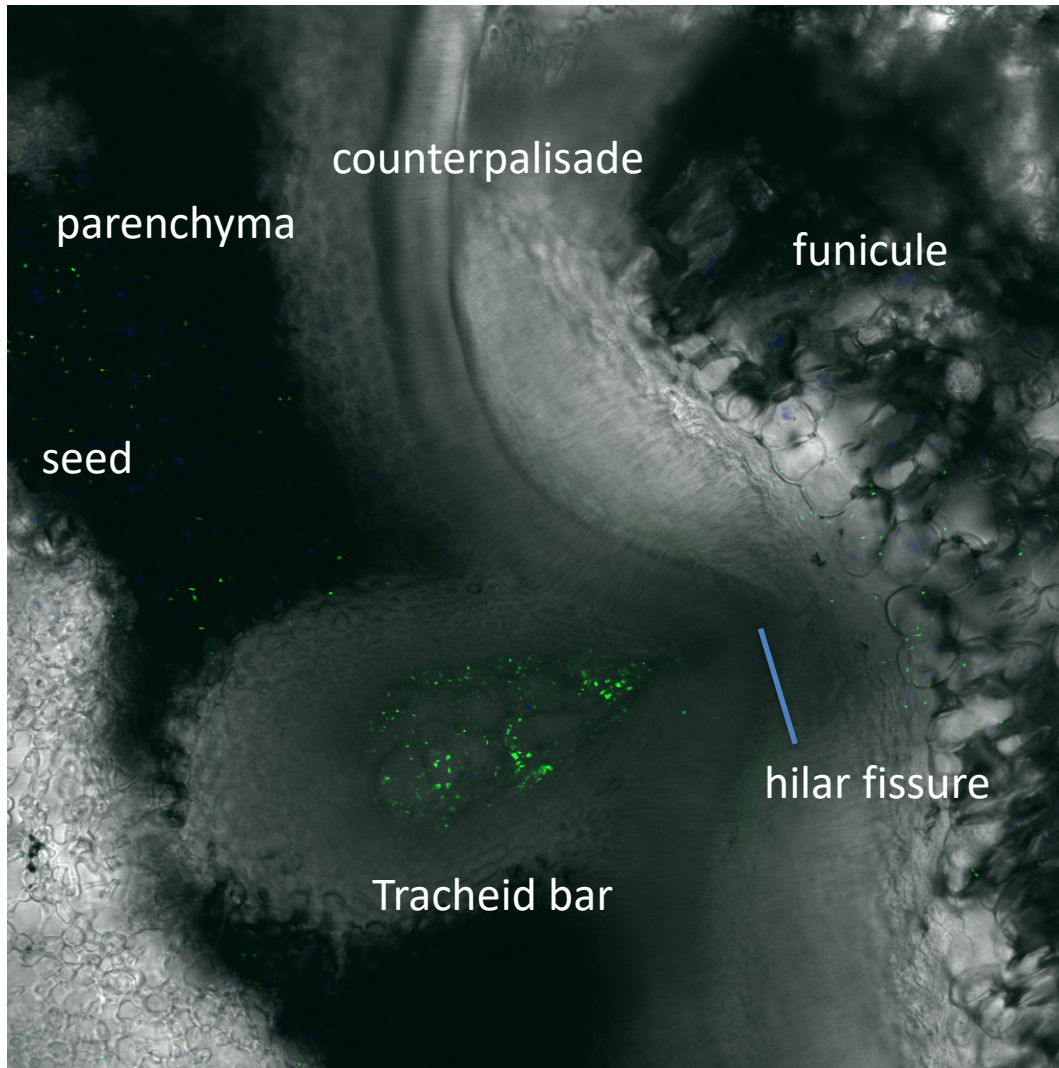
28 dai

Seed

Mother plant

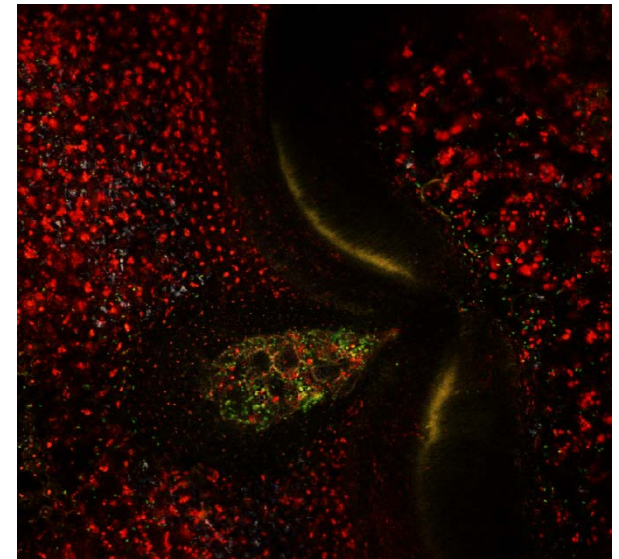
# Localization of Xff during seed formation on bean plants

*Xff* gfp-tagged cell following spray inoculation of bean plants at flower bud stage



Seed

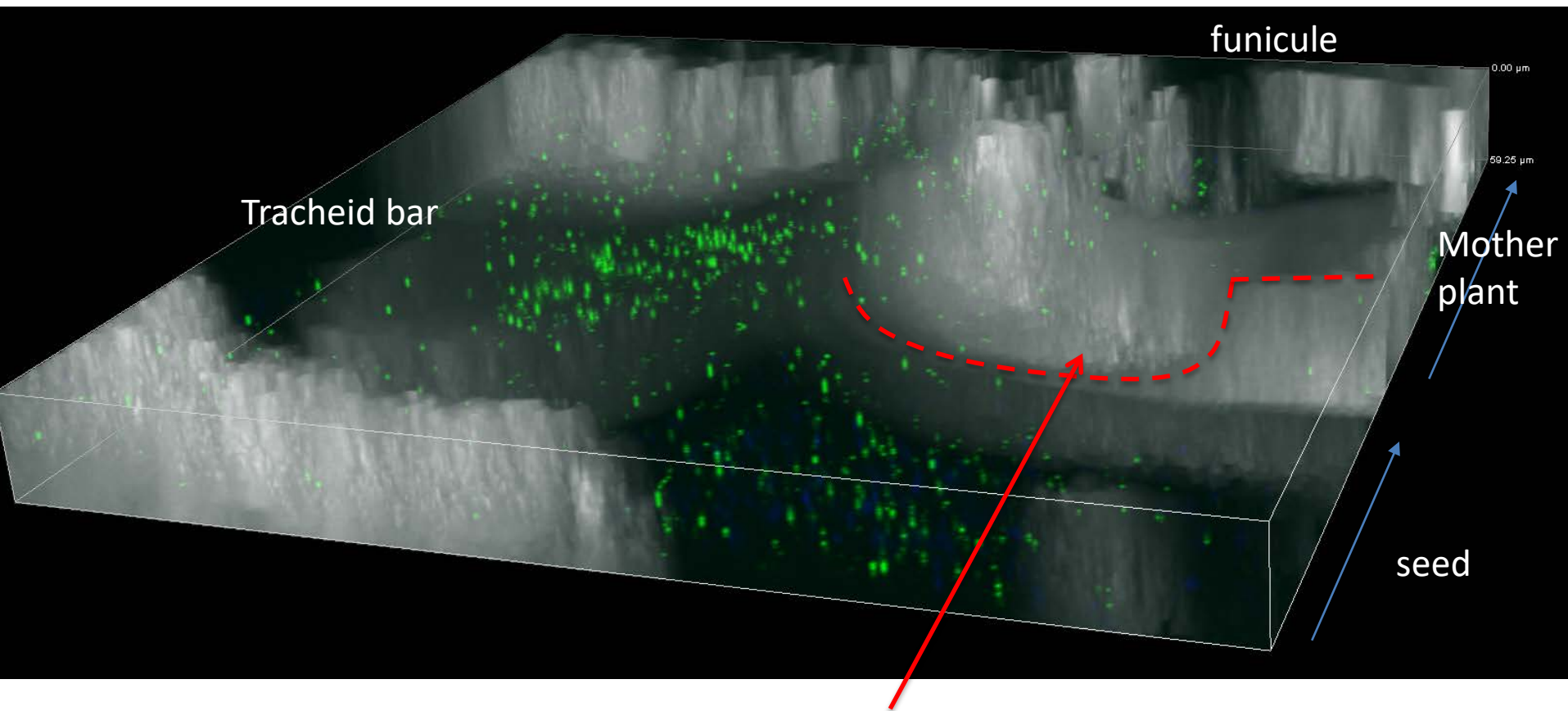
Mother plant





# Localization of Xff during seed formation on bean plants

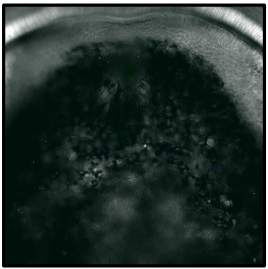
*Xff* gfp-tagged cell following spray inoculation of bean plants at flower bud stage



Aperture in the counterpalisade of the hile



Role of look-alikes in transmission of *Xff* to bean seeds



Translocation of *gfp*-tagged strain of *Xff* to seeds using confocal scanning-laser microscopy



**Efficiencies of transmissions of CBB agents from seeds to plantlets.**

# Transmission rates of seed borne bacteria under optimal conditions



**Spray-inoculation of R5-beans**

**Harvest of seeds 6 wk later**

**4 days after sowing in germ boxes**



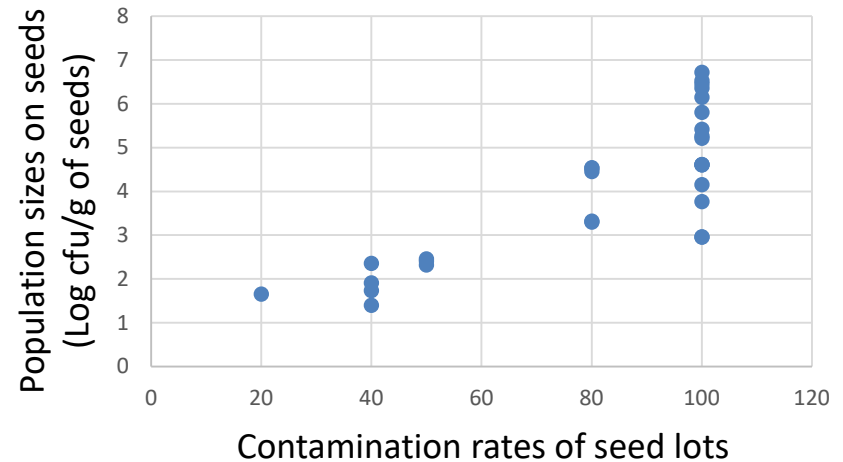
**7 days after sowing in soil**



# Transmission rates of seed borne bacteria under optimal conditions

Results from 29 bean seed lots harvested from plants inoculated with CBB agents

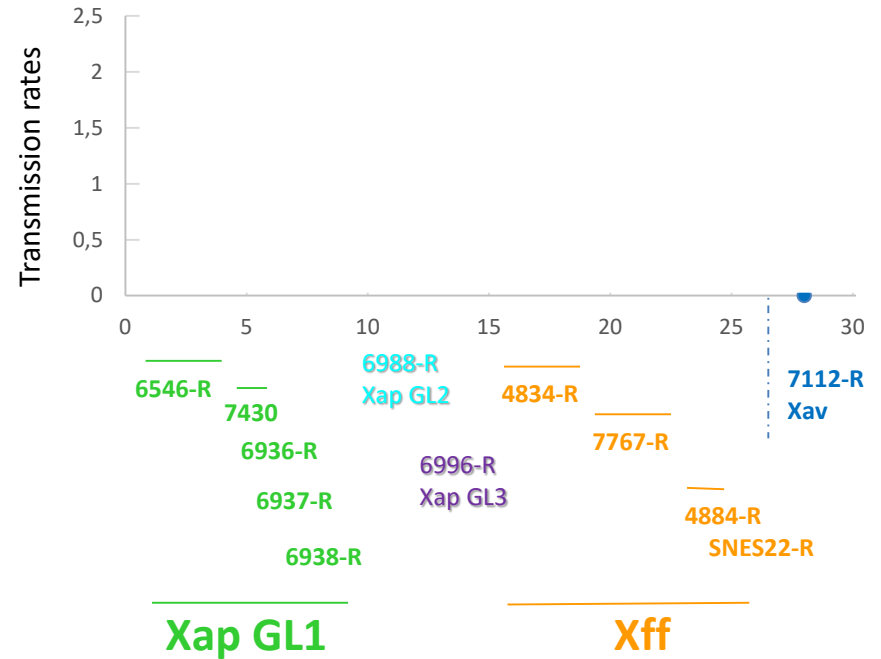
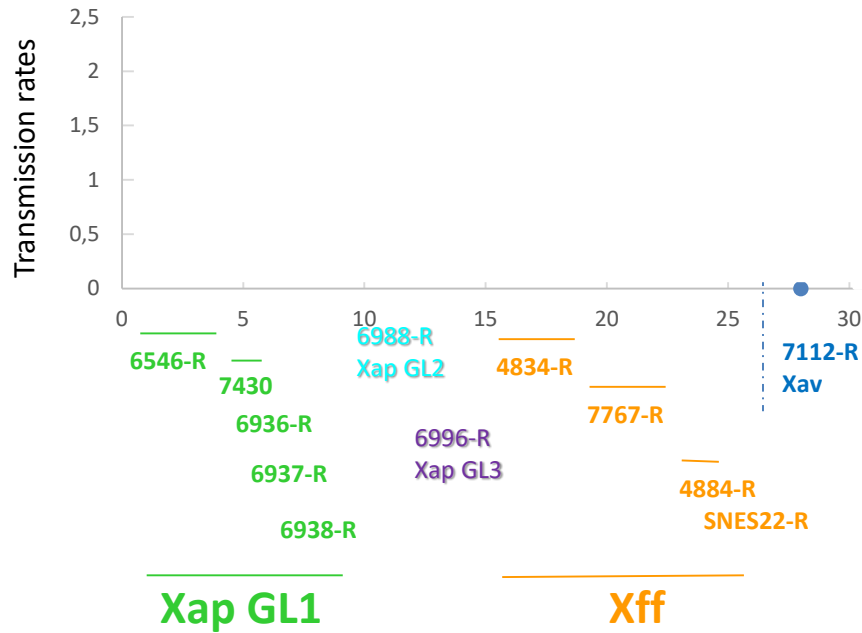
Large range of Contamination Rates of seed lots  
And of associated population sizes



# Transmission rates of seed borne bacteria under optimal conditions

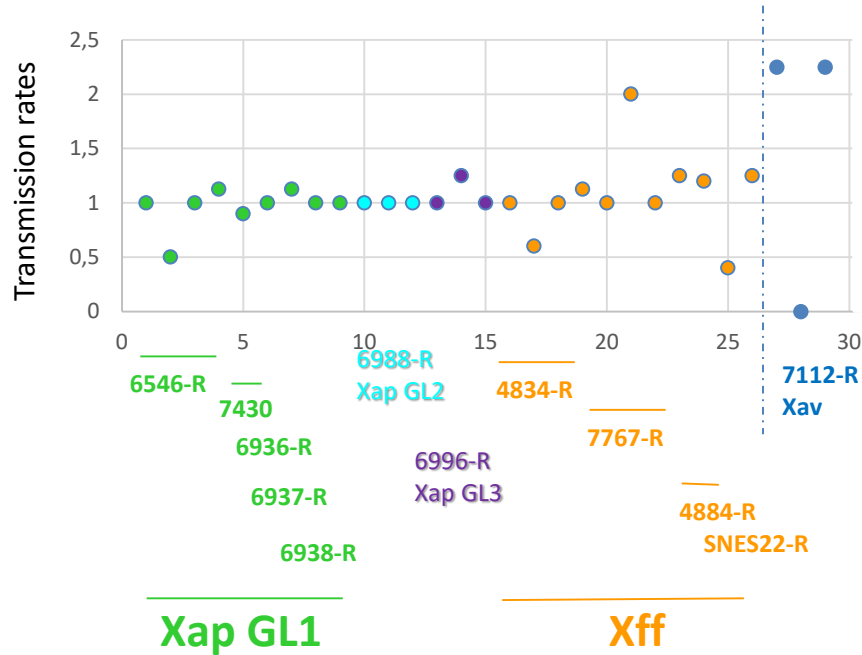
TR 4: CR plantlet lots 4 dai/ CR seed lots

TR 7: CR plantlet lots 7 dai/ CR seed lots

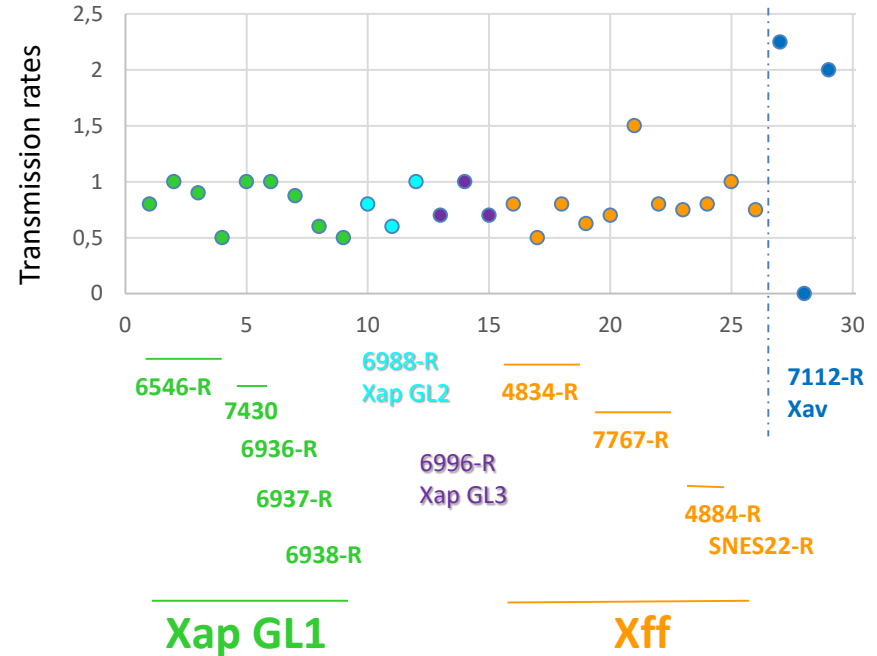


# Transmission rates of seed borne bacteria under optimal conditions

TR 4: CR plantlet lots 4 dai/ CR seed lots



TR 7: CR plantlet lots 7 dai/ CR seed lots

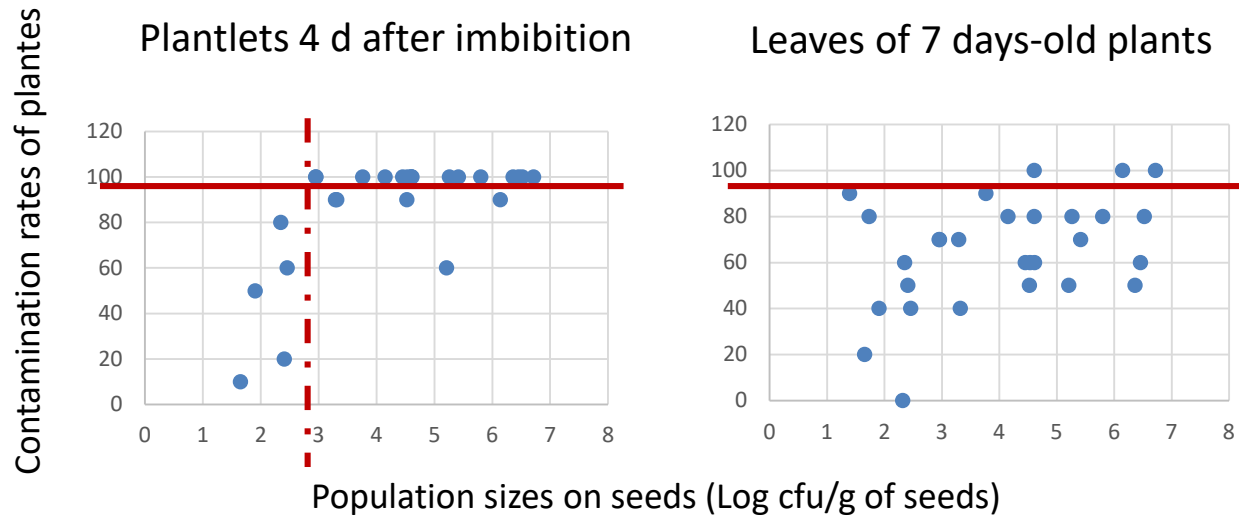


High TR from seeds to plantlets: 0.6 to 1.5 (2) for CBB agents

Transmission efficiency varies for a same strain (high variability for Xav strain CFBP 7112)

TR ~ 1 when analyzed 4 dai, more variable when determined 7 dai

# Transmission rates of seed borne bacteria under optimal conditions



All seed contaminations lead to plantlet contamination if analyzed 4 dai  
Contamination rates of plantlets lots are more or less predictable from mean population sizes in seeds

Population threshold ( $1 \times 10^3$  cfu/g of seed) on seeds for an efficient contamination of plantlets (4 dai)

If analyses are made on leaves of 7 days-old plantlets, contaminations are highly variable and not predictable from populations sizes determined on seeds.

# Conclusions

**How do look-alikes transmit to seeds?**

**Are they stopped by plant defenses in flower buds?**

**Bean bud inoculation lead to vascular transmission of Xff to seeds, and not only to ingress via the micropyle.**

**Analysis of plantlets 4 dai in germination boxes allows a good estimation of seed contaminations,**

**BUT tend to over-estimate efficient transmission to plants as determined by contamination of the cataphylls**



# Acknowledgments



**Armelle Darrasse**  
**Jean-François Guimbaud**  
**Anne Préveaux**



INEM

**Thank you for your attention**