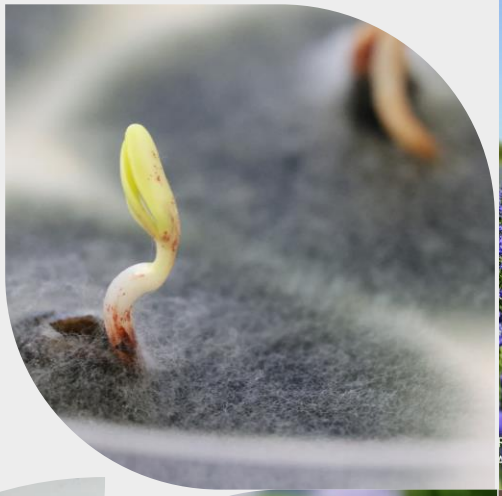
GEVES
Expertise & Performance
Groupe d'Étude et de contrôle des Variétés Et des Semences

Detection of five different fungal taxa on flax seeds with one method





Presented by Justine FOUCHER

Project team: Claire Granon, Céline Le Guisquet, Gaël Cesbron, Lorine Le Daré, Quentin Beaurepere, Mylène Ruh, Isabelle Sérandat, Nicolas Denancé, Jaiana Malabarba & Justine Foucher.

EPPO Conference on Diagnostics, Vienna
2026-04-22/24

Introduction

Flax is an important crop
for food and textiles

5 major fungi are pathogens of flax :
Alternaria linicola, Botrytis cinerea, Colletotrichum linicola, *Boeremia exigua* and *Fusarium* spp

→ Transmitted by seeds

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European Union : maximum permitted infection rate at 1 or 5% of the seeds.

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ISTA rule 7-007
Alternaria linicola
Botrytis cinerea
Colletotrichum linicola

ISTA: international seed testing association

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ISTA rule 7-007

Alternaria linicola
Botrytis cinerea
Colletotrichum linicola

Aim: addition of 2 pathogens in the ISTA method

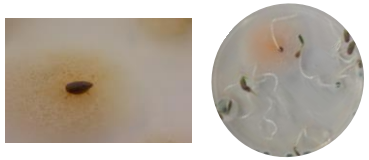
→ To modify the ISTA method: it is necessary to validate modification by evaluating performance criteria of the method

ISTA: international seed testing association

Method proposed

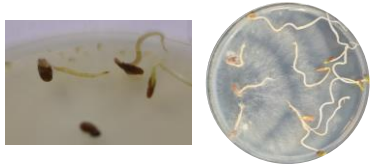
→ **Similar to the current ISTA 7-007 method: morphological identification of fungi**

Boeremia exigua



Colletotrichum linicola

Fusarium spp.



Botrytis cinerea

Alternaria linicola

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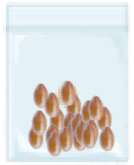
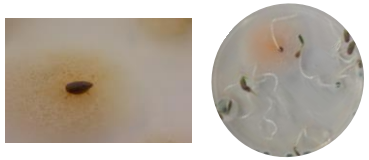


Seed sample

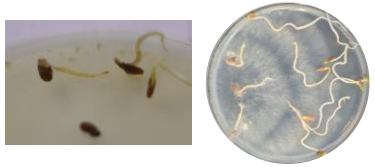
Sample size:
400 seeds

Boeremia exigua



Colletotrichum linicola

Fusarium spp.



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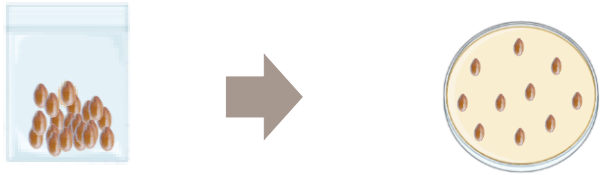


Seed sample

Sample size:
400 seeds

Plating seeds on media

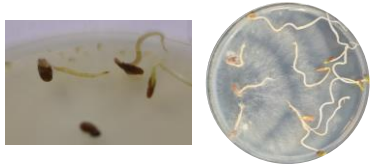
Malt-Agar (MA)
or
Potatoes Dextrose Agar (PDA)
with streptomycin 50 mg/L

10 seeds per plate

Boeremia exigua

Colletotrichum linicola

Fusarium spp.



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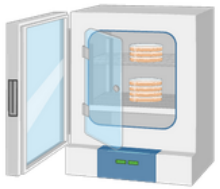


Incubation

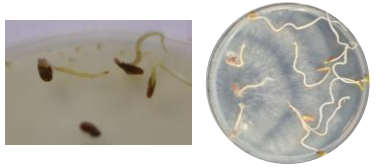
7 days at 20°C
in darkness
or
12h dark/12h NUV

Instead of 9 days

Boeremia exigua

Colletotrichum linicola

Fusarium spp.



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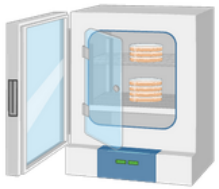

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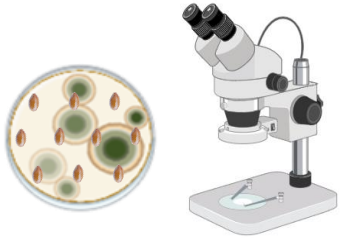
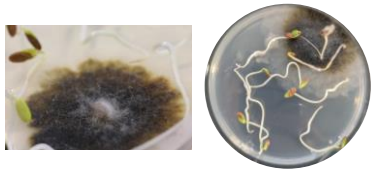
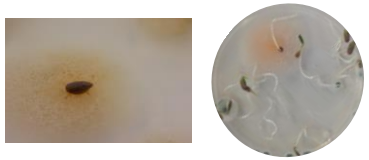


Morphological identification

Record the number of infected seeds for each pathogen *Botrytis cinerea, Alternaria linicola, Colletotrichum linicola,* *Boeremia exigua, Fusarium* spp.

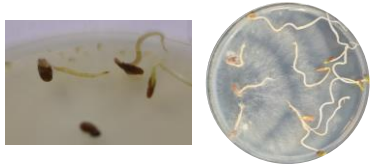


Boeremia exigua



Colletotrichum linicola

Fusarium spp.



Botrytis cinerea

Alternaria linicola

Evaluation of the performance criteria

Criteria	Definition

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Analytical specificity	The ability of a method to detect target strains (inclusivity) while not detecting non-targets strains (exclusivity)

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Repeatability	The ability of a method to produce the same results when repeated on identical samples under the same conditions (equipment, laboratory, time, etc.).
Reproducibility	The ability of a method to produce the same results when repeated on identical samples under different conditions (equipment, laboratories, time, etc.).

Evaluation of the analytical specificity

Criteria	Definition	Way to evaluate
Analytical specificity	The ability of a method to detect target strains (inclusivity) while not detecting non-targets strains (exclusivity)	Morphological criteria of a set of target and non-target strains were compared with the expected morphological criteria

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Target collection - 39

Species	Quantity of strains tested	Isolation host	Collection date
Alternaria linicola	9	Flax seed	1999 - 2025
Colletotrichum linicola	4	Flax seed	1999-2024
Botrytis cinerea	10	Flax seed, radish, Faba bean, Chickpea, Cabbage	2010-2025
Boeremia exigua	6	Flax seed and Lettuce	2014-2024
Fusarium avenaceum	2	Flax seed	2024-2025
Fusarium poae	2	Flax seed	2018-2024
Fusarium sporotrichioides	1	Unknown	2019
Fusarium graminearum	3	Flax seed and wheat	2002-2025
Fusarium culmorum	1	Flax seed	2024
Fusarium cerealis	1	Flax seed	2024

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Fusarium cerealis	1	Flax seed	2024

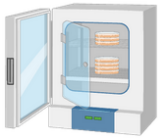
Non-target collection - 10

Species	Isolation host	Collection date
Phoma valerianellae	Lettuce	2020
Alternaria dauci	Carrot	1997
Epicoccum nigrum	NA	2011
Colletotrichum capsici	NA	2019
Trichoderma trixiae	Flax seed	2022
Alternaria alternata	NA	2011
Colletotrichum graminicola	Corn	2009
Rhizoctonia sp.	Flax seed	2021
Alternaria brassicicola	Cabbage	2000
Colletotrichum lupini	Lupin	2023

Evaluation of the analytical specificity

Establish a target and non-target collection

Grow target and non-target pathogen on MA media



Incubation 7 days at 20°C in darkness

Morphological identification
Identified target and non-target strains

Evaluation of the analytical specificity

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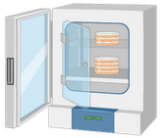
Grow target and non-target pathogen on MA media



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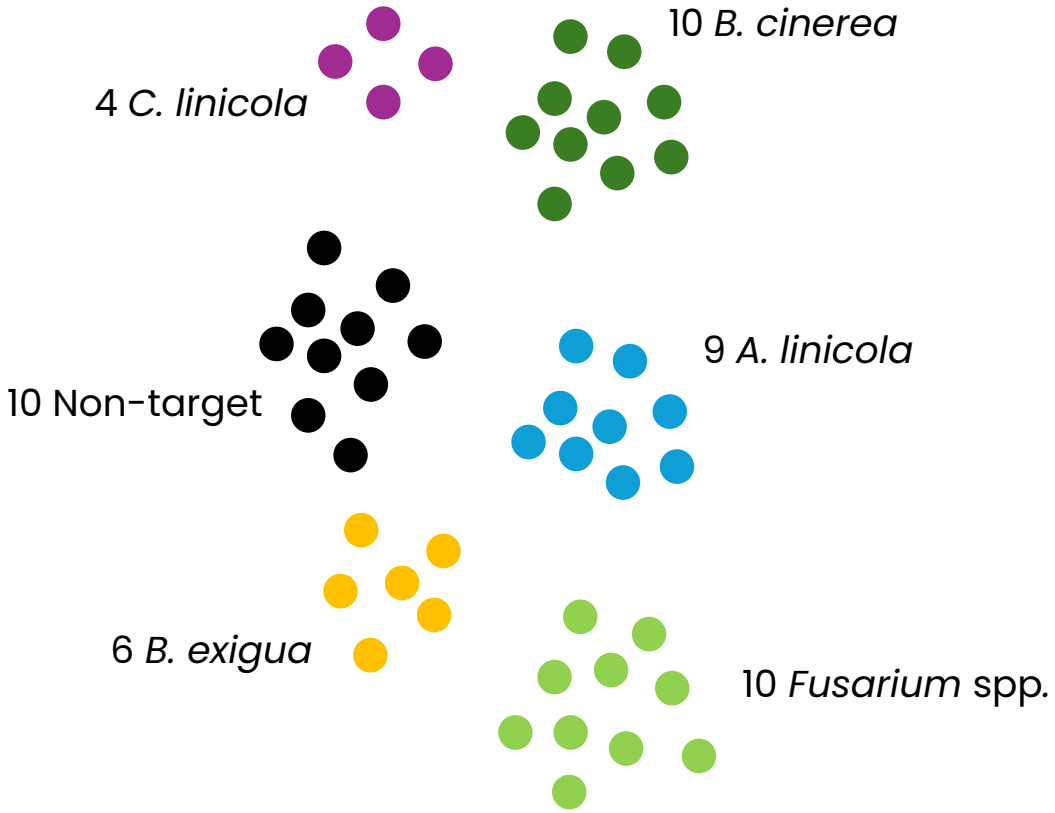

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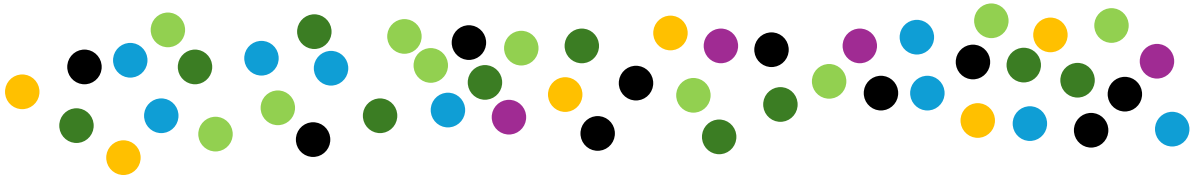
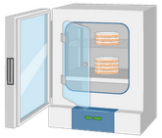


Establish a target and non-target collection

Grow target and non-target pathogen on MA media



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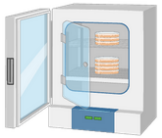
Morphological identification
Identified target and non-target strains

B. cinerea *A. linicola* *C. linicola* *B. exigua* *Fusarium* spp. Non-target

Evaluation of the analytical specificity

Establish a target and non-target collection

Grow target and non-target pathogen on MA media



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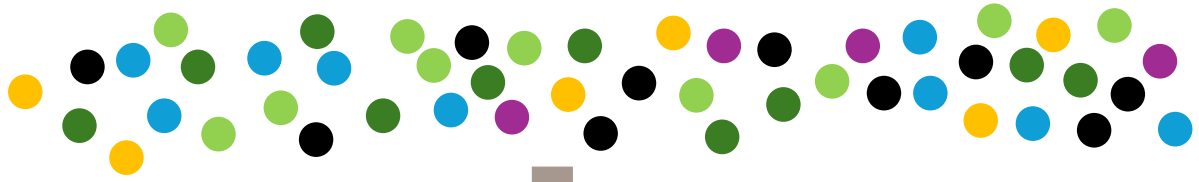


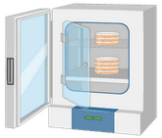
Detection of *B. cinerea*	Detection of *A. linicola*	Detection of *C. linicola*	Detection of *B. exigua*	Detection of *Fusarium* spp.

B. cinerea *A. linicola* *C. linicola* *B. exigua* *Fusarium* spp. Non-target

Evaluation of the analytical specificity

Establish a target and non-target collection

Grow target and non-target pathogen on MA media



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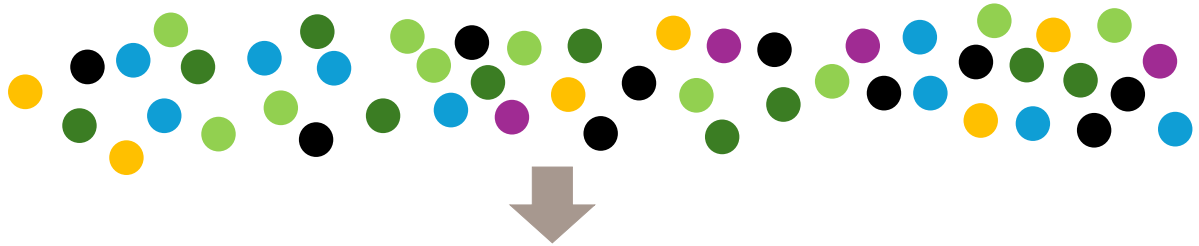


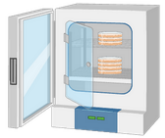
Detection of *B. cinerea*	Detection of *A. linicola*	Detection of *C. linicola*	Detection of *B. exigua*	Detection of *Fusarium* spp.
Targets				
● 10+/10				

● *B. cinerea* ● *A. linicola* ● *C. linicola* ● *B. exigua* ● *Fusarium* spp. ● Non-target

Evaluation of the analytical specificity

Establish a target and non-target collection

Grow target and non-target pathogen on MA media



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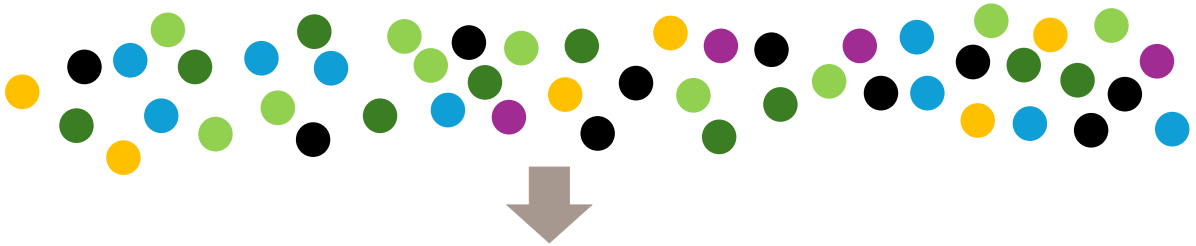

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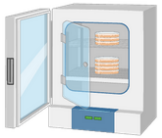
Targets				
10+/10				
Non-targets				
0+/39				

B. cinerea *A. linicola* *C. linicola* *B. exigua* *Fusarium* spp. Non-target

Evaluation of the analytical specificity

Establish a target and non-target collection

Grow target and non-target pathogen on MA media



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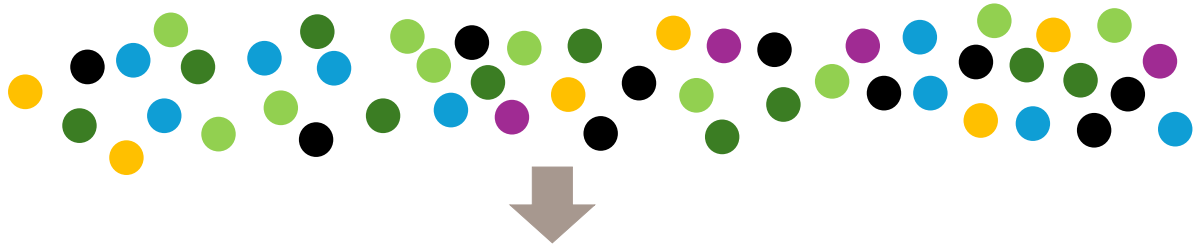

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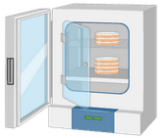
Targets	Targets			
10+/10	9+/9			
Non-targets	Non-targets			
0+/39	0+/40			

B. cinerea *A. linicola* *C. linicola* *B. exigua* *Fusarium* spp. Non-target

Evaluation of the analytical specificity

Establish a target and non-target collection

Grow target and non-target pathogen on MA media



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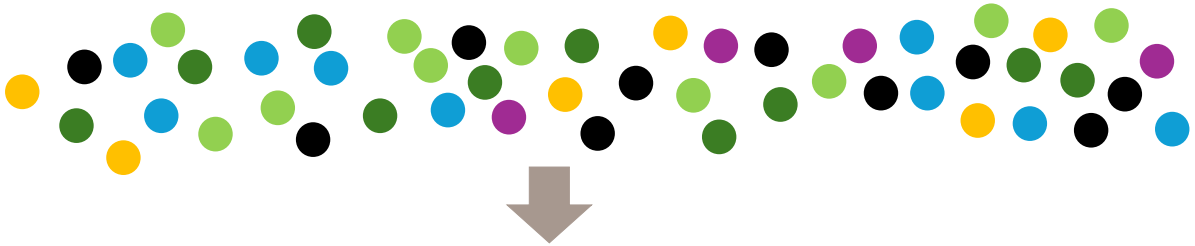

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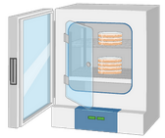
Targets	Targets	Targets		
10+/10	9+/9	4+/4		
Non-targets	Non-targets	Non-targets		
0+/39	0+/40	0+/45		

B. cinerea *A. linicola* *C. linicola* *B. exigua* *Fusarium* spp. Non-target

Evaluation of the analytical specificity

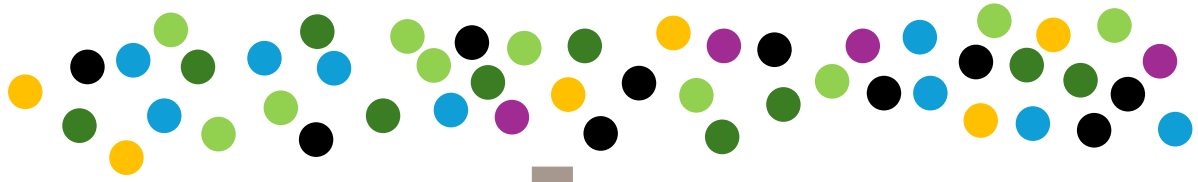
Establish a target and non-target collection

Grow target and non-target pathogen on MA media



Incubation 7 days at 20°C in darkness

Morphological identification
Identified target and non-target strains



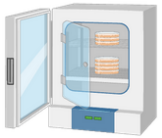
Detection of *B. cinerea*	Detection of *A. linicola*	Detection of *C. linicola*	Detection of *B. exigua*	Detection of *Fusarium* spp.
Targets	Targets	Targets	Targets	
10+/10	9+/9	4+/4	6+/6	
Non-targets	Non-targets	Non-targets	Non-targets	
0+/39	0+/40	0+/45	0+/43	

B. cinerea *A. linicola* *C. linicola* *B. exigua* *Fusarium* spp. Non-target

Evaluation of the analytical specificity

Establish a target and non-target collection

Grow target and non-target pathogen on MA media



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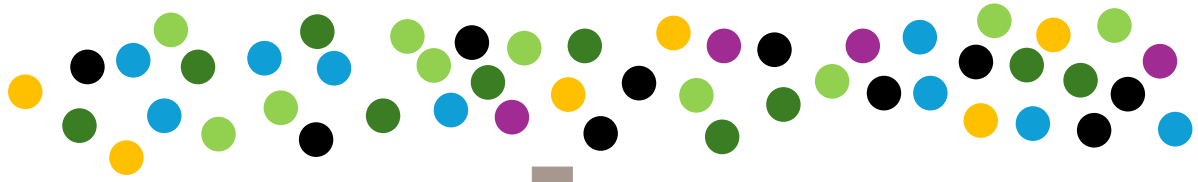

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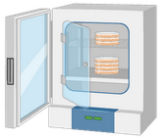

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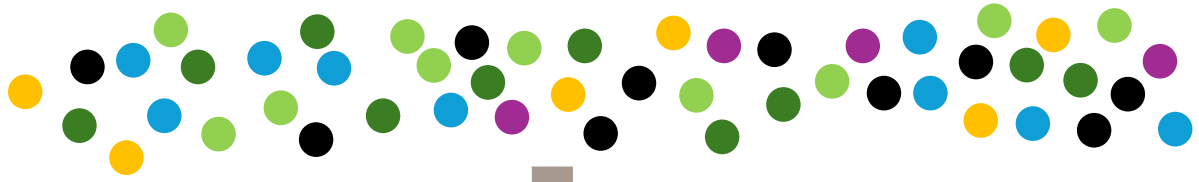

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Inclusivity: 100% Exclusivity: 100%
for each pathogen

B. cinerea *A. linicola* *C. linicola* *B. exigua* *Fusarium* spp. Non-target

Evaluation of the analytical sensitivity

Criteria	Definition	Way to evaluate
Analytical sensitivity (Limit of detection)	Smallest amount of the target pathogen that can be detected	10 samples will be tested, each sample contained 1 contaminated seed for each pathogen and 395 healthy seeds

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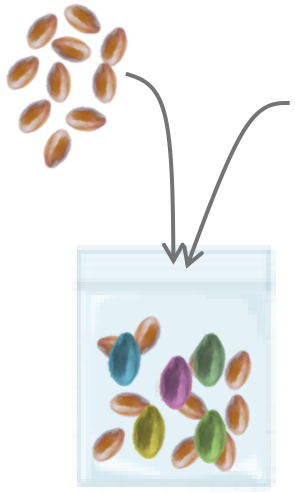

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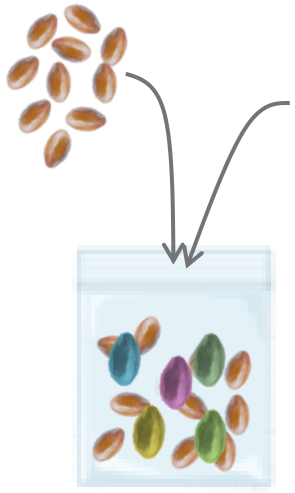

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→ Need to produce artificial contaminated seeds

Evaluation of the analytical sensitivity

Development of a method for artificial contamination

Machado *et al.,* 2004, Sousa *et al.,* 2006

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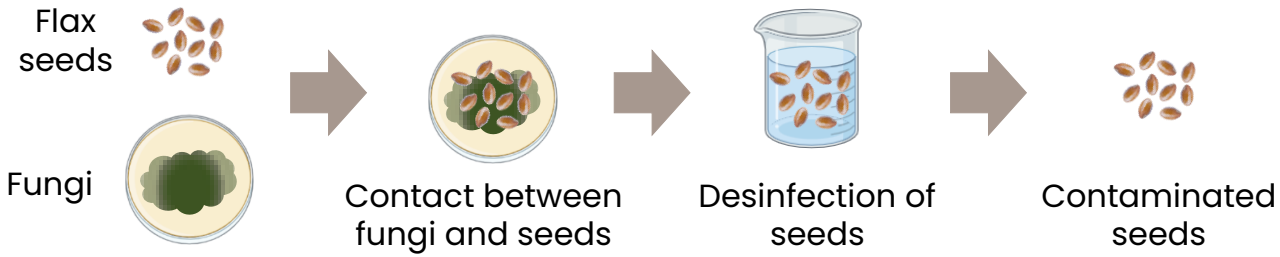

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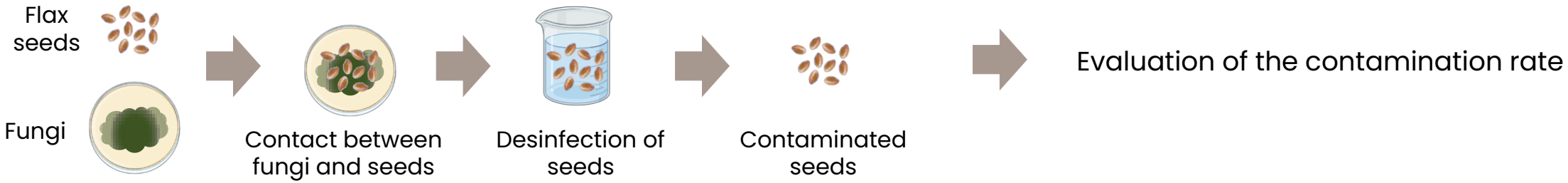

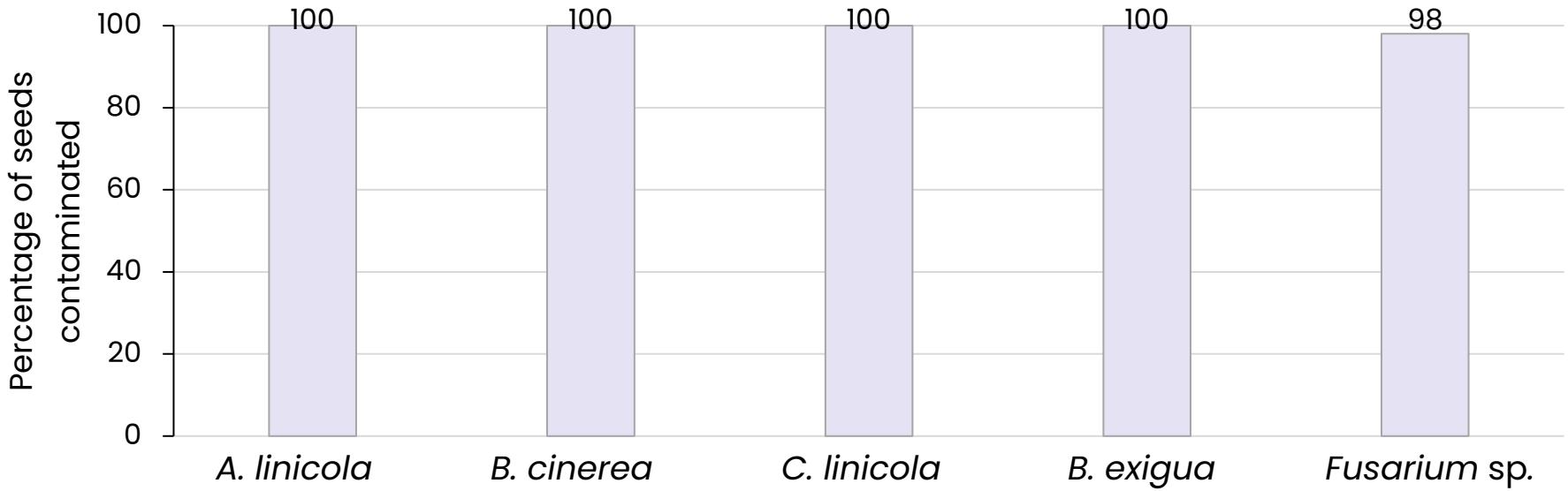

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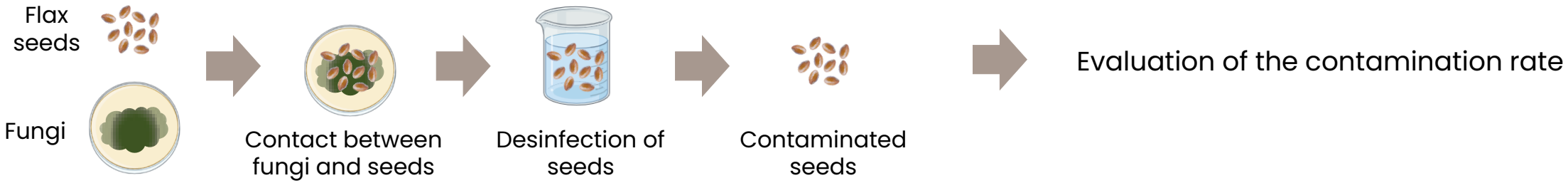
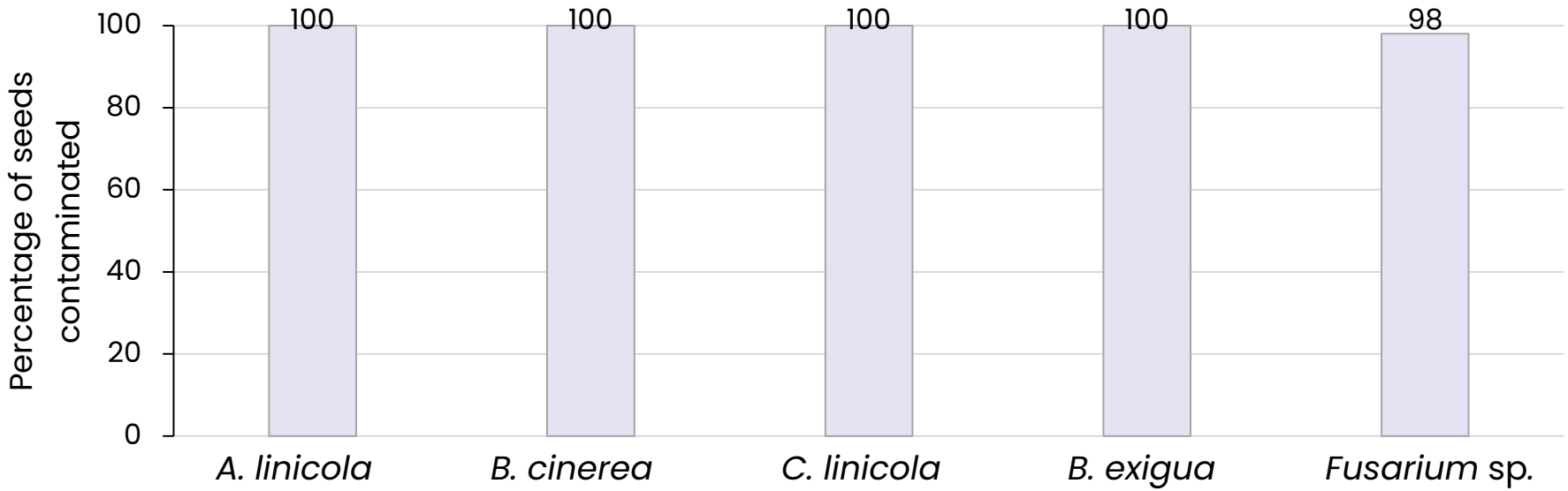


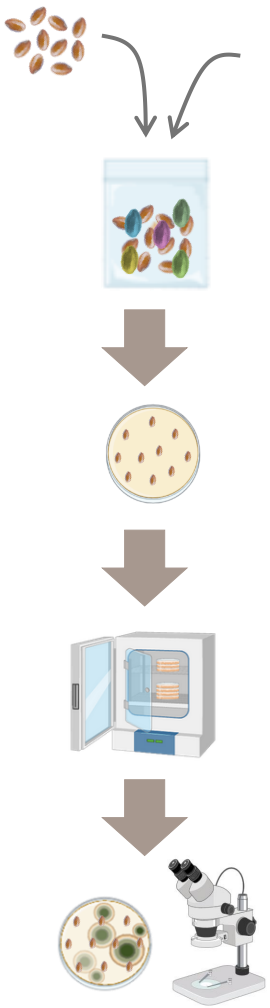


Contamination rates are at 100-98%

These seeds can be used for the evaluation of the analytical sensitivity

Evaluation of the analytical sensitivity

395 healthy seeds



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Plating seeds on media
Malt-Agar (MA)
with streptomycin 50 mg/L
10 seeds per plate

Incubation
7 days at 20°C in darkness

Morphological identification
Record the number of seeds infected by *Botrytis cinerea, Alternaria linicola, Colletotrichum linicola, Boeremia exigua, Fusarium* spp.

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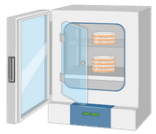

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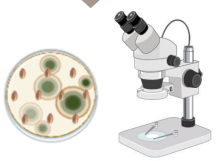

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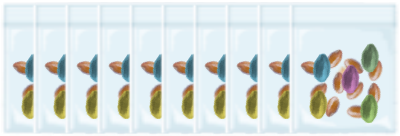

Test performed on 10 samples

Evaluation of the analytical sensitivity

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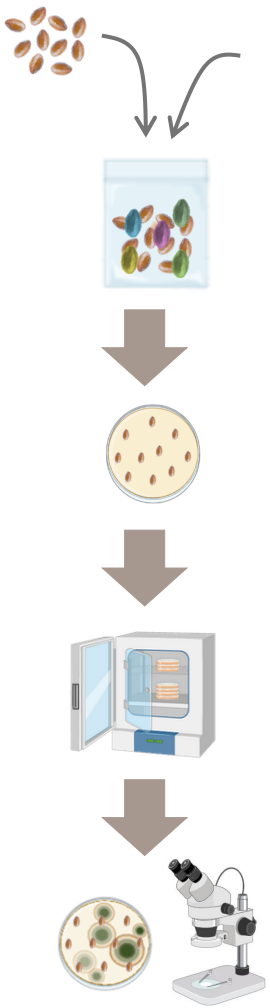

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Samples	% of seeds contaminated by				
	A. linicola	*C. linicola*	*B. cinerea*	*B. exigua*	*Fusarium* spp.
1	0.25	0.25	0.25	0.25	0.25
2	0.25	0.25	0.25	0.25	0.25
3	0.25	0.25	0.25	0.25	0.25
4	0.25	0.25	0.25	0.25	0.25
5	0.25	0.25	0.25	0.25	0.25
6	0.25	0.25	0.25	0.25	0.25
7	0.25	0.25	0.25	0.25	0.25
8	0.25	0.25	0.25	0.25	0.25
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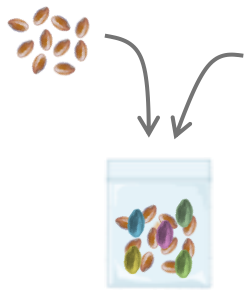

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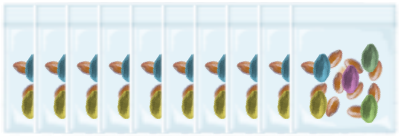

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8	0.25	0.25	0.25	0.25	0.25
9	0.25	0.25	0.25	0.25	0.25
10	0.25	0.25	0.25	0.25	0.25

All contaminated seeds were detected

Analytical sensitivity is validated at 1 contaminated seed in a sample of 400 seeds (corresponding to 0.25% of contamination)

Evaluation of the performance criteria

Criteria	Definition	Way to evaluate
Diagnostic sensitivity	The ability of a method to give correct positive results and not produce false negatives	Validated through a TPS with at least 3 laboratories (ideally 8) Samples with 3 levels of infection (healthy, medium and highly infected)
Diagnostic specificity	The ability of a method to give correct negative results and not produce false positives	
Repeatability	The ability of a method to produce the same results when repeated on identical samples under the same conditions (equipment, laboratory, time, etc.).	
Reproducibility	The ability of a method to produce the same results when repeated on identical samples under different conditions (equipment, laboratories, time, etc.).	

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Organization of a test performance study

Several qualified laboratories

Panel of codified samples
Infected and healthy

Evaluation of the performance criteria

Organized with ISTA

Evaluation of the performance criteria

Organized with ISTA

Selection of qualified laboratories

Organization of a pre comparative test

Preliminary test to evaluate stability of the artificial contamination and laboratories expertise on fungi for the method

11 participants
Panel of 9 samples of 100 seeds
Healthy and artificially contaminated samples

Results of the different laboratories will be compared using boxplot, and statistical test will be used.

Evaluation of the performance criteria

Organized with ISTA

Selection of qualified laboratories

Find healthy and infected seed lots

Evaluation of the performance criteria

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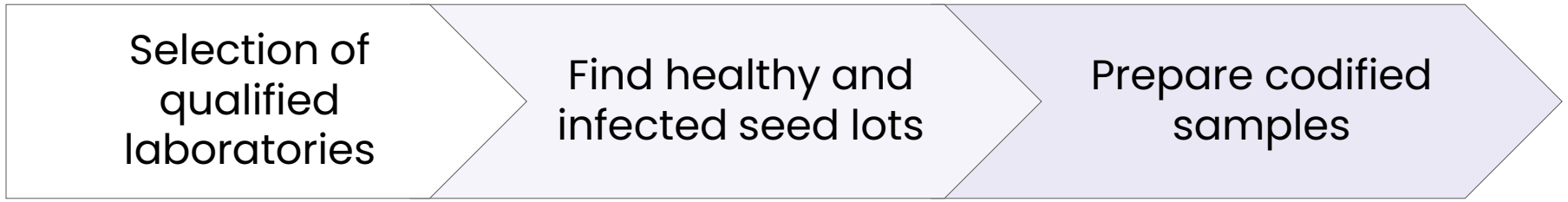

Evaluation of the performance criteria

Organized with ISTA

- Selection of qualified laboratories
- Find healthy and infected seed lots
- Prepare codified samples
- Evaluation of the homogeneity of samples

Evaluation of the performance criteria

Organized with ISTA

- Selection of qualified laboratories
- Find healthy and infected seed lots
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- Send samples

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- Proposal to update the ISTA method

Conclusion

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An ISTA method for five-fungi detection in flax seeds: validation in progress.

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Ensure the viability of fungi

But...

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reliable worldwide tomorrow.

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Validated internationally today,
reliable worldwide tomorrow.

A practical tool to improve global plant health and support regulatory control.

Thank you
for your attention


Claire Granon


Céline Le Guisquet


Gaël Cesbron


Lorine Le Daré


Isabelle Sérandat

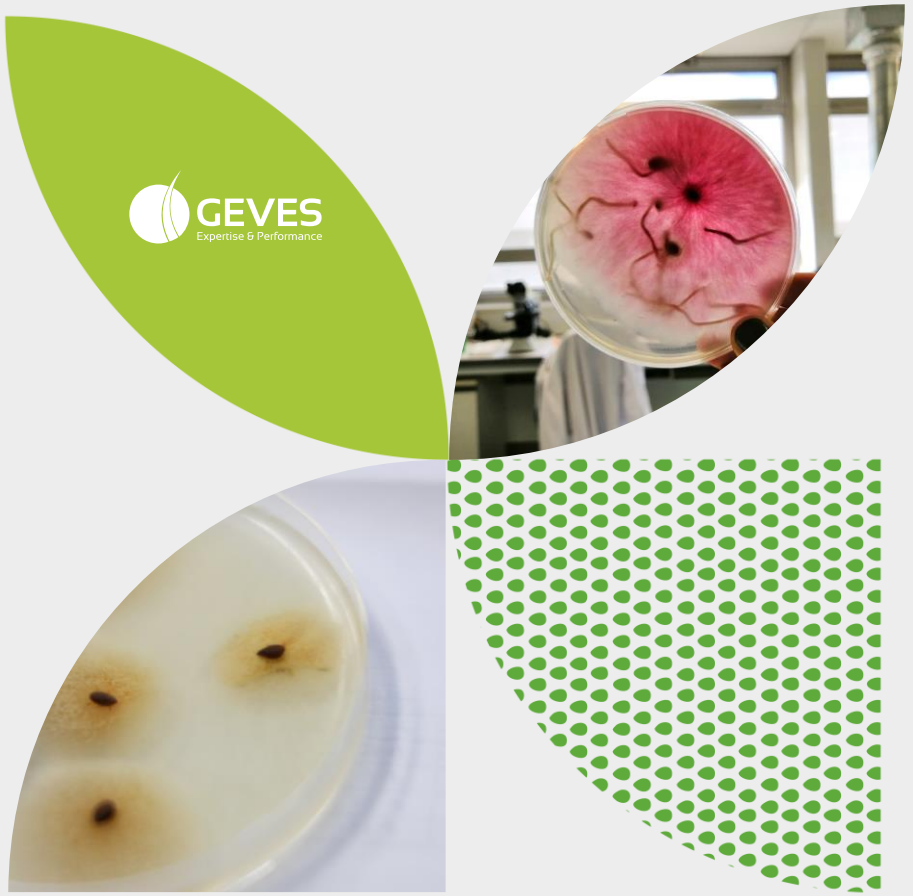

Justine Foucher


Nicolas Denancé


Jaiana Malabarba


Mylène Ruh


Quentin Beaurepere



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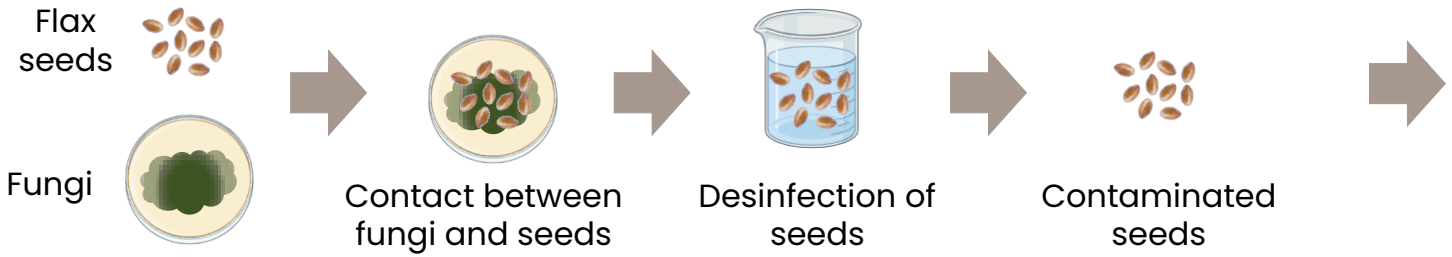

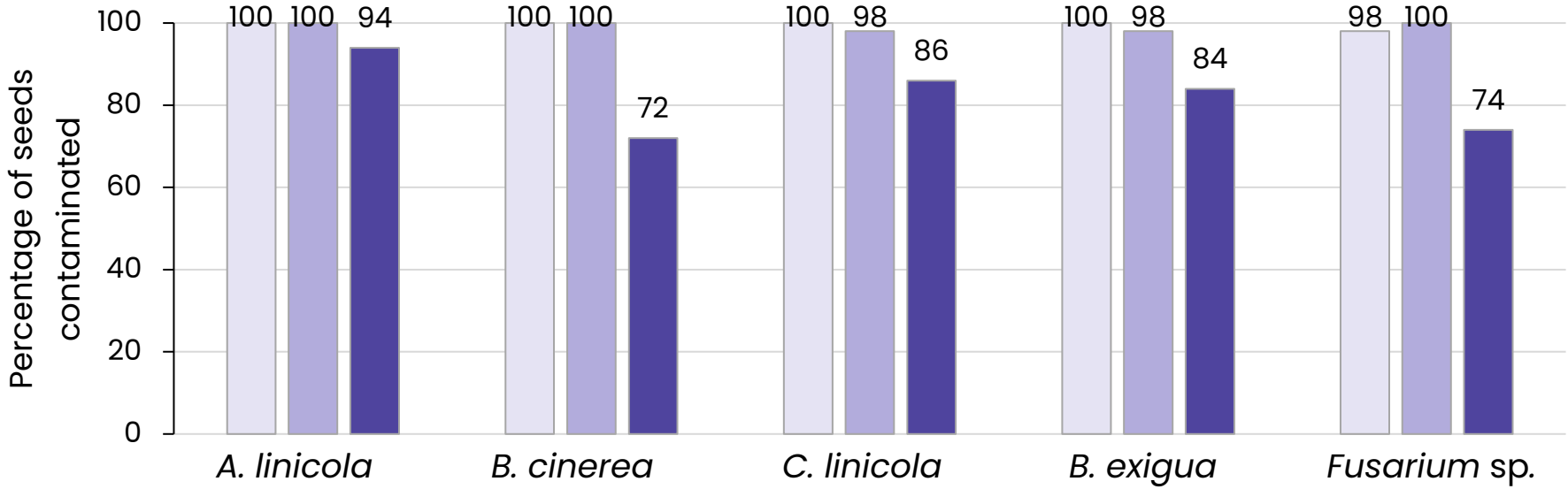

Evaluation of the performance criteria

Criteria	*ISTA validation study 2014*	*Aim of this project:*
	Detection of *B. cinerea, C. linicola* and *A. linicola*	
Analytical specificity	Not evaluated	Integrate *Boeremia exigua* and *Fusarium* spp.
Analytical sensitivity (Limit of detection)	Evaluated only on MA Samples of 100 seeds containing one infected seed ***1% detectable***	Evaluate the analytical specificity
Diagnostic sensitivity	***73% B. cinerea, 77% C. linicola, 100% A. linicola***	Use samples of 400 seeds to re-evaluate performance criteria
Diagnostic specificity	***100% for the three***	
Repeatability Reproducibility	Evaluated during a TPS with 3 laboratories on samples of 100 seeds ***OK for C. linicola and A. linicola*** ***Not OK for B. cinerea***	Use stable level of infection