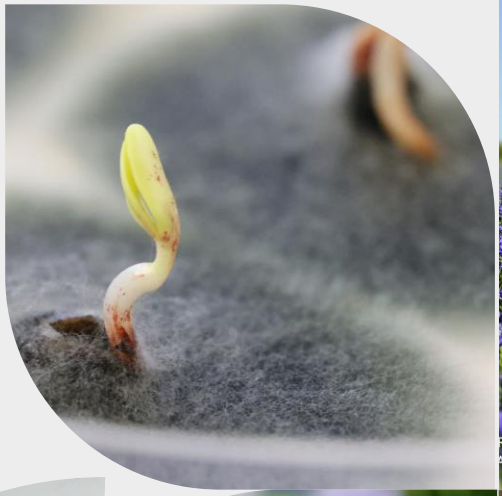
GEVES
Expertise & Performance
Groupe d'Étude et de contrôle des Variétés Et des Semences

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





Presented by Justine FOUCHER

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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

To limit the spread of these fungi : regulated as quarantine or as regulated non quarantine pest in several countries

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

A detection method on seeds is essential to limit the spread of these pathogens

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

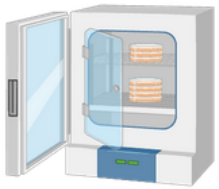
Seed sample

Sample size:
400 seeds

Plating seeds on media

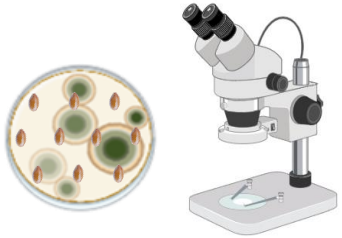
Malt-Agar (MA)
or
Potatoes Dextrose Agar (PDA)
with streptomycin 50 mg/L

10 seeds per plate



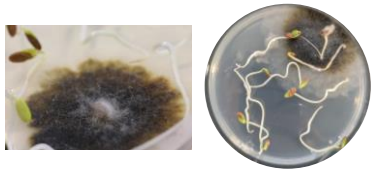
Incubation

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



Morphological identification

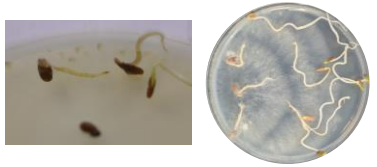
Record the number of infected seeds for each pathogen *Botrytis cinerea, Alternaria linicola, Colletotrichum linicola, Boeremia exigua, Fusarium* spp.



Boeremia exigua

Colletotrichum linicola

Fusarium spp.



Botrytis cinerea

Alternaria linicola

Evaluation of the performance criteria

Criteria	Definition
Analytical specificity	The ability of a method to detect target strains (inclusivity) while not detecting non-targets strains (exclusivity)
Analytical sensitivity (Limit of detection)	Smallest amount of the target pathogen that can be detected
Diagnostic sensitivity	The ability of a method to give correct positive results and not produce false negatives
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.).

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

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

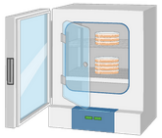
Non-target collection - 10

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

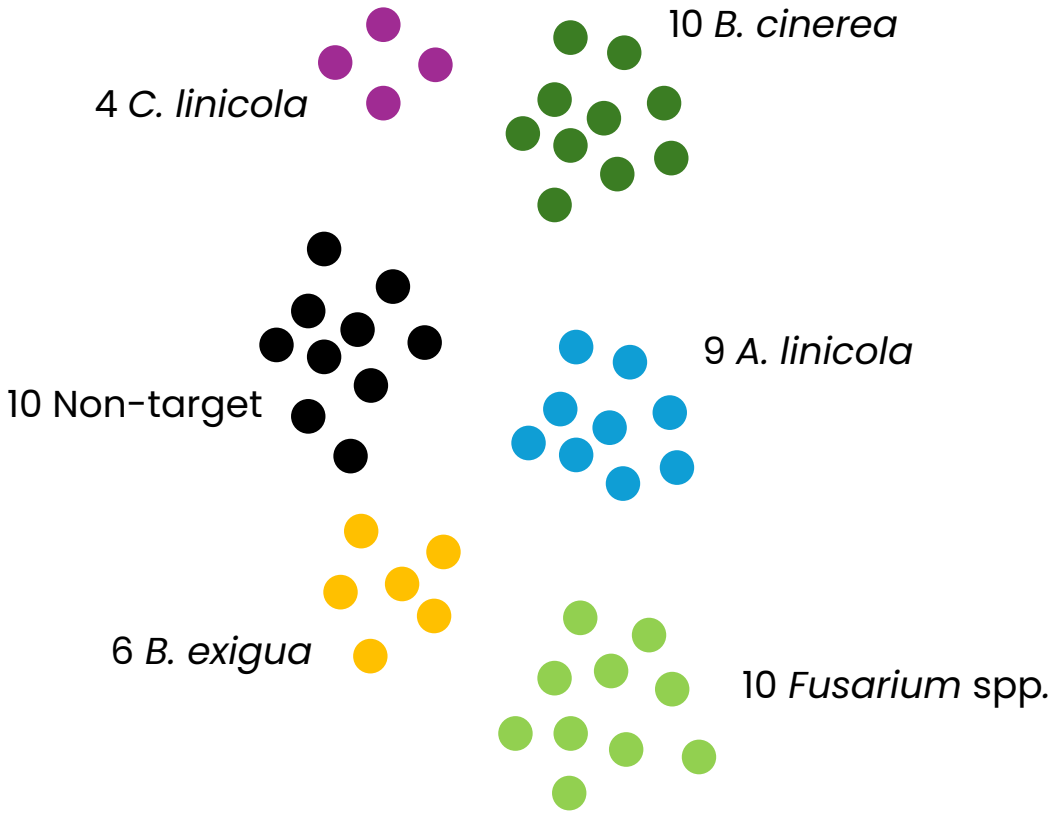
Establish a target and non-target collection

Grow target and non-target pathogen on MA media



Incubation 7 days at 20°C in darkness

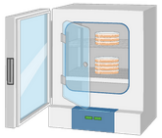
Morphological identification
Identified target and non-target strains



Evaluation of the analytical specificity

Establish a target and non-target collection

Grow target and non-target pathogen on MA media



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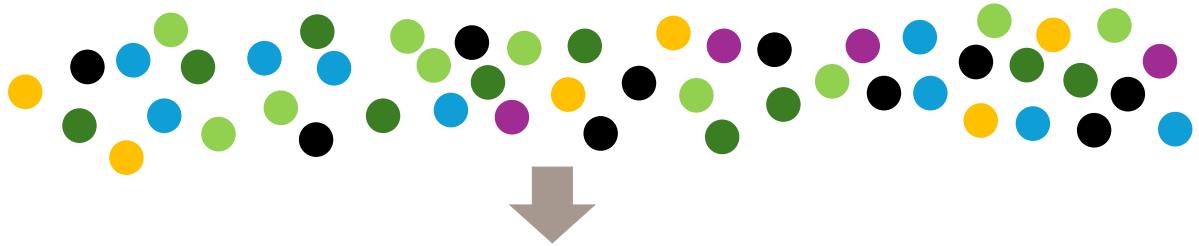
Morphological identification
Identified target and non-target strains



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

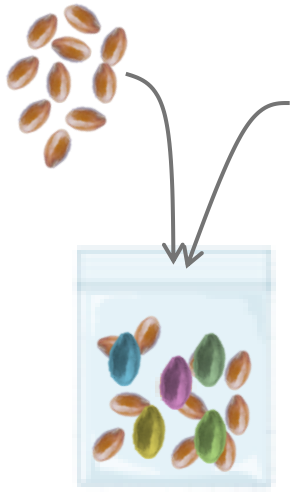
Inclusivity: 100% Exclusivity: 100%
for each pathogen

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

395 healthy seeds



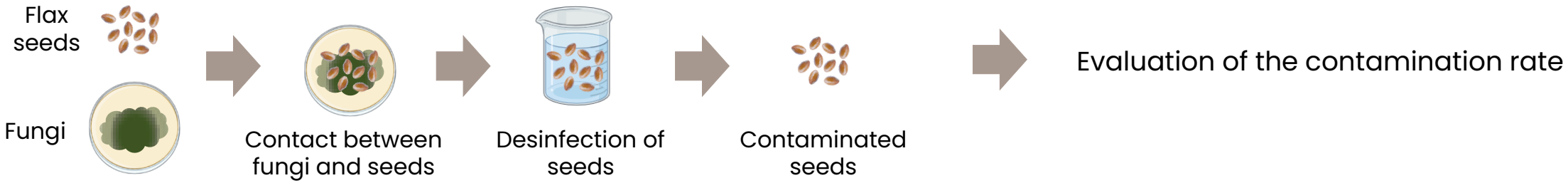
- 1 seed contaminated by *B. cinerea*
- 1 seed contaminated by *A. linicola*
- 1 seed contaminated by *C. linicola*
- 1 seed contaminated by *B. exigua*
- 1 seed contaminated by *Fusarium* sp.

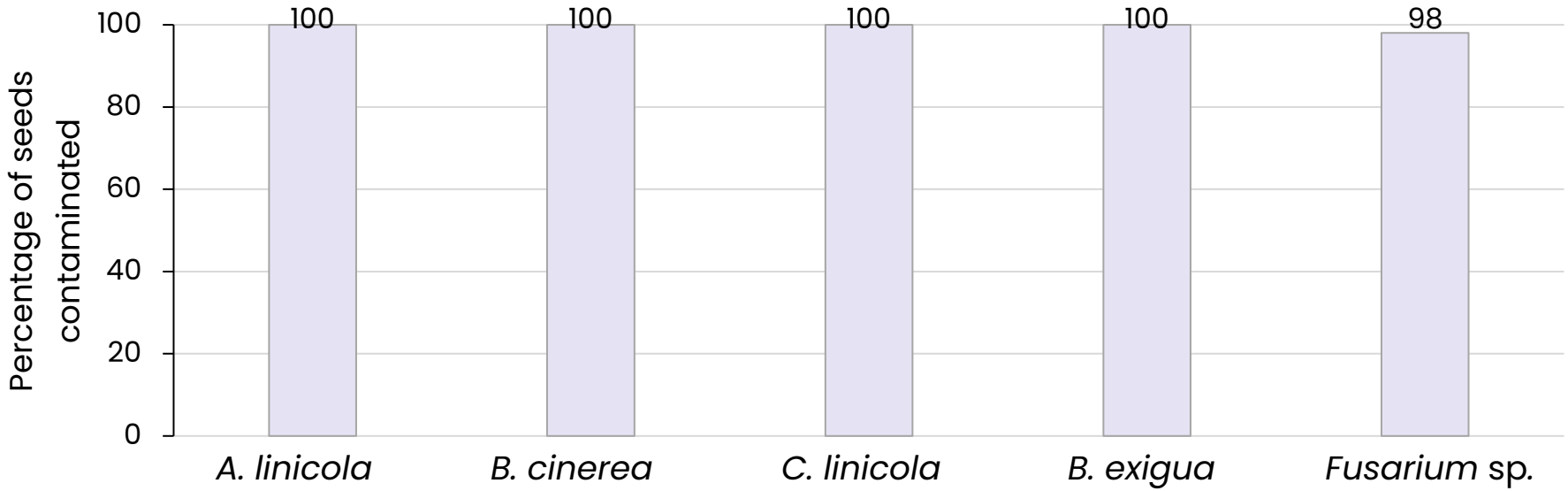
→ Need to produce artificial contaminated seeds

Evaluation of the analytical sensitivity

Development of a method for artificial contamination

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



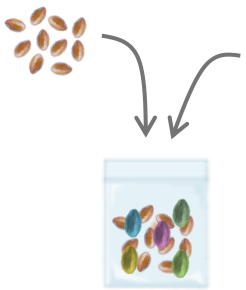


Contamination rates are at 100-98%

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

Evaluation of the analytical sensitivity

395 healthy seeds

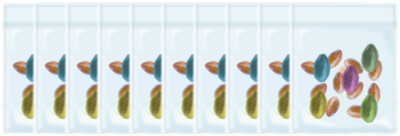


- 1 seed contaminated by *B. cinerea*
- 1 seed contaminated by *A. linicola*
- 1 seed contaminated by *C. linicola*
- 1 seed contaminated by *B. exigua*
- 1 seed contaminated by *Fusarium* spp.

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.



Test performed on 10 samples

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
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.).	

Organization of a test performance study

Several qualified laboratories

Panel of codified samples
Infected and healthy

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
- Prepare codified samples
- Evaluation of the homogeneity of samples
- Send samples
- Perfom the analysis by participants
- Evaluation of the stability of samples
- Collect participants' results
- Statistical analysis and evaluation of the performance criteria
- ISTA validation report
- Proposal to update the ISTA method

Conclusion

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

Quantitative method
Ensure the viability of fungi

But...
need of expertise in fungi identification by morphology

Validated internationally today,
reliable worldwide tomorrow.

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

Thank you
for your attention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Céline Le Guisquet


Gaël Cesbron


Lorine Le Daré


Isabelle Sérandat


Justine Foucher


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Jaiana Malabarba


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