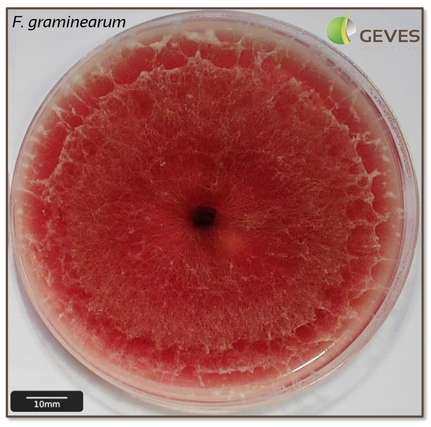
Development of a tool for *Fusarium* species identification isolated from cereal based on DNA barcoding

Contact: mylene.ruh@geves.fr

Mylène Ruh[1], Quentin Beaurepère[1], Laurence Landais[1], Amandine Lê Van[1], Céline Le Guisquet[1], Isabelle Sérandat[1], Heidi Udnes Aamot[2], Erik Lysøe[2], Ilaria Alberti[3], Mauro Dal Prà[4], Jessie O'Shaughnessy[5], Alex Reid[5], Marian McEwan[5], Eduardo Galvez[6], Gabriela Medina[6], Kimberly Kenward[7], Alex Playdon[7], Nicole Calliou[8] and Lorenzo Lombard[9]

[1] GEVES, France. [2] NIBIO, Norway. [3] CREA – CI, Italy. [4] CREA – DC, Italy. [5] SASA, United Kingdom. [6] Vitalab, Chile. [7] 20/20 SeedLabs Inc., Canada. [8] SGS - Crop Science, Canada. [9] NAK, Netherlands.

Background



Fusarium Head Blight, caused by several *Fusarium* species in mono- or co-infections, reduces yields by up to 70% worldwide and can contaminate grains with harmful mycotoxins, raising food safety concerns.

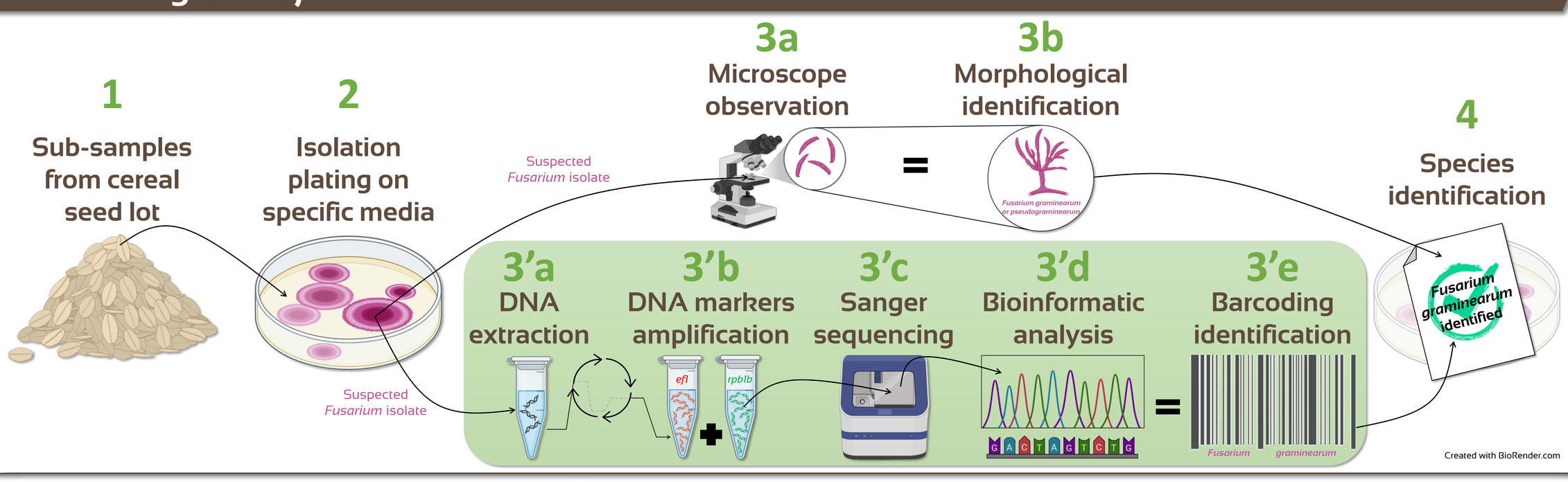
With fewer chemical treatments, phytosanitary testing demands are rising, requiring robust methods.

An ongoing ISTA project develops a detection and morphological identification method, but its complexity highlights the need for a complementary approach for identifying *Fusarium* species.

ISTA project (2022-2026) bringing together nine partner laboratories from around the world to develop and validate a method for identifying *Fusarium* isolates using barcoding.

Once validated, an optional barcode identification step will be added to the ISTA method for the detection of *Fusarium* spp. in cereal seeds.

Methodological objective



Materials and Methods

Isolates collection:

- **69 target isolates** → 16 target species
- **23 non-target isolates** → 18 non-target species
- **Characterisation** → morphological criteria + PCR

Target			Non-target		
F. species complex / Genus	Species	Nb. of isolate	*F.* species complex / Genus	Species	Nb. of isolate
F. fujikuroi	*F. fujikuroi*	2	*Alternaria*	*A. tenuissima*	1
	F. proliferatum	7	*Bipolaris*	*B. sorokiniana*	1
	F. subglutinans	3	*Cladosporium*	sp.	1
	F. verticillioides	6	*Epicoccum*	*E. nigrum*	1
F. sambucinum	*F. cerealis*	2	*F. incarnatum-equiseti*	*F. equiseti*	2
	F. culmorum	5	*F. lateritium*	*F. lateritium*	1
	F. graminearum	5	*F. oxysporum*	*F. oxysporum*	2
	F. langsethiae	5	*F. sambucinum*	*F. venenatum*	1
	F. poae	5	*F. tricinctum*	*F. acuminatum*	2
	F. pseudograminearum	3	*Neocosmospora*	*N. solani*	2
	F. sambucinum	4	*Parastagonospora*	*Pa. avenae*	1
	F. sporotrichioides	4		*Pa. nodorum*	1
F. tricinctum	*F. avenaceum*	4	*Penicillium*	sp.	1
	F. tricinctum	4	*Pyrenophora*	*Py. avenae*	1
Microdochium	*M. majus*	6		*Py. graminea*	1
	M. nivale	4		*Py. teres*	1
			Trichothecium	sp.	1
			Unclear status	*F. caeruleum*	2

DNA extraction:

- **Sample preparation** → freeze-drying for 24h at -50°C
- **Extraction kit** → Qiagen DNeasy Plant Pro kit

Barcoding markers:

- ***efl***: translation elongation factor 1

Modified primers from Boutigny *et al.*, 2019

- ***rpb1b***: second part of the largest subunit of DNA-dependent RNA polymerase II

Modified primers from O'Donnel *et al.*, 2010 (semi-nested PCR)

Barcoding identification:

- **Sanger sequencing**
- **Consensus sequence preparation** (EPPO Bulletin PM7/129)
- **Identification analysis** (database - method) :
  - **NCBI - Nucleotide BLAST** (unsuitable for *Microdochium* sequences)
  - **Fusarioid-ID - Pairwise DNA Aligments** (unsuitable for *Microdochium* sequences)
  - **Fusarioid-ID - Polyphasic identification** (unsuitable for *Microdochium* sequences)
  - **Sequences from NCBI** (*Fusarium* genomes) **and NIBIO** (*Microdochium* genomes) **- MLSA**

Results and Final validation

Analytical specificity:

- Inclusivity → identification of the expected target species
- Exclusivity → identification of a species not belonging to the target species

Expected = Obtained
Expected among Obtained
Expected ≠ Obtained

Expected species	Species obtained					
	NCBI - Nucleotide BLAST		Fusarioid-ID - Pairwise DNA Aligments		Fusarioid-ID - Polyphasic identification	Sequences from NCBI and NIBIO - MLSA
	efl	*rpb1b*	*efl*	*rpb1b*	*efl* + *rpb1b*	*efl* + *rpb1b*
F. avenaceum	*F. avenaceum*	*F. avenaceum* (3) *F. arthrosporioides* (1)	*F. avenaceum* (1) *F. arthrosporioides* (3)	*F. avenaceum* (3) *F. arthrosporioides* (1)	*F. avenaceum*	*F. avenaceum*
F. cerealis	*F. cerealis*	*F. cerealis*	*F. cerealis*	*F. cerealis*	*F. cerealis*	*F. cerealis*
F. culmorum	*F. culmorum*	*F. culmorum*	*F. culmorum*	*F. culmorum*	*F. culmorum*	*F. culmorum*
F. fujikuroi	*F. fujikuroi*	*F. fujikuroi*	*F. fujikuroi*	*F. fujikuroi*	*F. fujikuroi*	*F. fujikuroi*
F. graminearum	*F. graminearum*	*F. graminearum*	*F. graminearum*	*F. graminearum*	*F. graminearum*	*F. graminearum*
F. langsethiae	*F. langsethiae*	*F. langsethiae*	*F. langsethiae*	*F. langsethiae*	*F. langsethiae*	*F. langsethiae*
F. poae	*F. poae*	*F. poae*	*F. poae*	*F. poae*	*F. poae*	*F. poae*
F. proliferatum	*F. proliferatum* (5) *F. proliferatum/fujikuroi* (2)	*F. proliferatum*	*F. proliferatum*	*F. proliferatum*	*F. proliferatum*	*F. proliferatum*
F. pseudograminearum	*F. pseudograminearum*	*F. pseudograminearum*	*F. pseudograminearum*	*F. pseudograminearum*	*F. pseudograminearum*	*F. pseudograminearum*
F. sambucinum	*F. sambucinum*	*F. kyushuense*	*F. sambucinum*	*F. sambucinum*	*F. sambucinum*	*F. sambucinum*
F. sporotrichioides	*F. sporotrichioides*	*F. sporotrichioides*	*F. sporotrichioides*	*F. sporotrichioides*	*F. sporotrichioides*	*F. sporotrichioides*
F. subglutinans	*F. subglutinans*	*F. subglutinans/awaxi*	*F. subglutinans*	*F. subglutinans/awaxi*	*F. subglutinans*	*F. subglutinans*
F. tricinctum	*F. tricinctum* (2) *F. acuminatum* (1) *F. reticulatum* (1)	*F. tricinctum* (2) *F. campestre* (1) *F. reticulatum* (1)	*F. tricinctum* (2) *F. campestre* (1) *F. californicum* (1)	*F. tricinctum* (2) *F. campestre* (1) *F. californicum* (1)	*F. tricinctum* (2) *F. campestre* (1) *F. californicum* (1)	*F. tricinctum* (2) *F. campestre* (1) *F. californicum* (1)
F. verticillioides	*F. verticillioides* (5) *F. coicis* (1)	*F. verticillioides* (5) *F. musae* (1)	*F. verticillioides* (5) *F. coicis* (1)	*F. verticillioides* (5) *F. coicis* (1)	*F. verticillioides* (5) *F. coicis* (1)	*F. verticillioides* (5) *F. coicis* (1)
M. majus						*M. majus*
M. nivale						*M. nivale*
F. acuminatum	*F. acuminatum*	*F. acuminatum*	*F. acuminatum*	*F. acuminatum*	*F. acuminatum*	*F. acuminatum*
F. caeruleum	*F. coeruleum*	*F. torreyae*	*F. coeruleum*	*F. zanthoxyli*	*F. pulchrum*	*F. coeruleum*
F. equiseti	*F. equiseti*	*F. equiseti*	*F. equiseti* (1) *F. toxicum* (1)	*F. toxicum/ipomoeae*	*F. toxicum*	*F. equiseti/toxicum*
F. lateritium			*F. concentricum*	*N. plagianthi*		
F. oxysporum	*F. oxysporum*	*F. oxysporum*	*F. menglaense* *F. cugenangense*	*F. cugenangense*	*F. cugenangense*	*F. oxysporum/ curvatum*
F. venenatum	*F. venenatum*	*F. venenatum*	*F. venenatum*	*F. venenatum*	*F. venenatum*	*F. venenatum*
N. solani	*F. solani* *F. caeruleum*	*F. torreyae* *F. crassum*	*F. caeruleum* *N. cucurbitae*	*F. zanthoxyli* *N. cucurbitae*	*F. pulchrum* *N. cucurbitae*	*F. solani* *F. caeruleum*

Conclusions:

- **For non-target non-*Fusarium* isolates, result obtained always ≠ *Fusarium***
- **Unexpected species identified for 3 isolates**
  F. campestre, californicum and *coicis* instead of *F. tricinctum* and *verticillioides*
- ***efl*+*rpb1b*, various databases and identification methods are required to guarantee the identification results obtained**
  efl and *rpb1b* sequences from *M. majus* and *nivale* are absent from public database
- **Identification method potentially applicable to *F. acuminatum* and *venenatum***

Inclusivity = 100%

Exclusivity = 100%

Comparative test to come (2026):

- Evaluation of **Robustness** (different DNA extraction methods), **Repeatability** (same result obtained by the same lab) and **Reproducibility** (same result obtained by different labs)
- At least **6 participating laboratories**
- Identification by all participants of **selected and anonymised target and non-target isolates**

European Fusarium Seminar
October 21-24, 2025 Bordeaux France