



Journées Jean Chevaugeon – Aussois 15-19 janvier 2024

Fighting mycotoxin contaminations by studying the compositional and metabolomic dynamics of a *Meta-Fusarium* exposed to abiotic and biotic stress

Valentin FIEVET

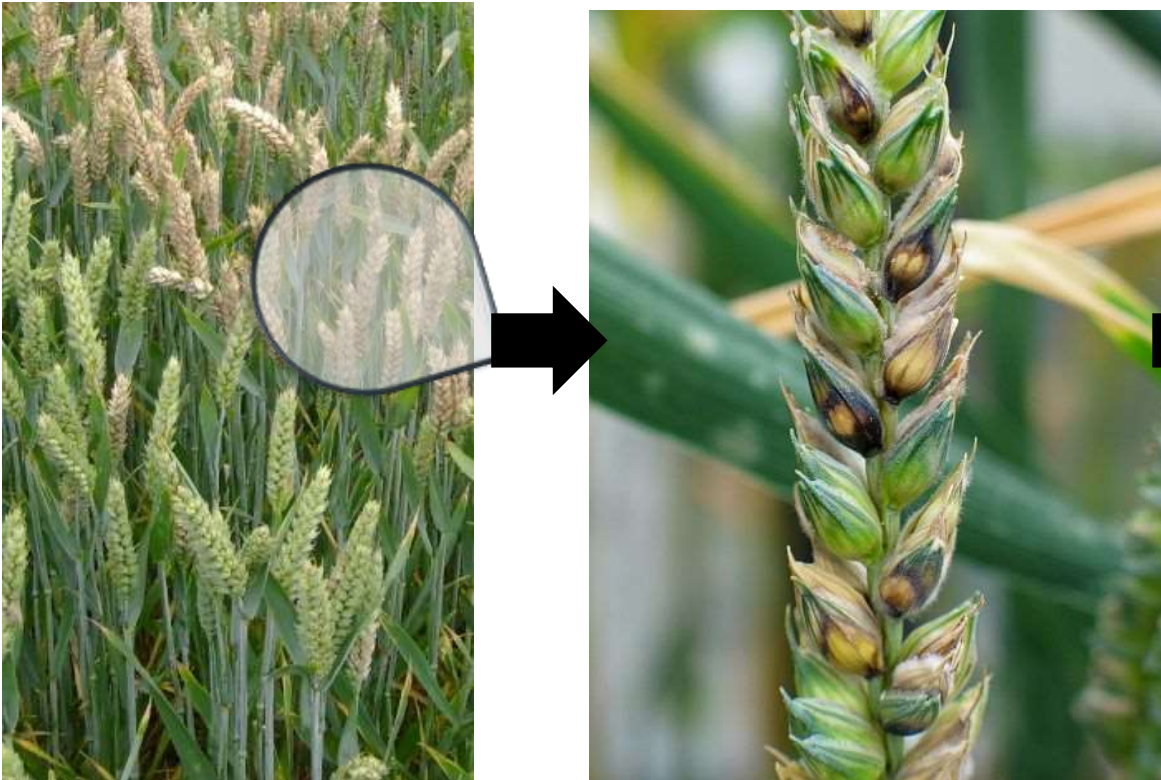
Supervised by:

- **Florence FORGET** (thesis supervisor)
- **Louis CARLES** (co-supervisor)

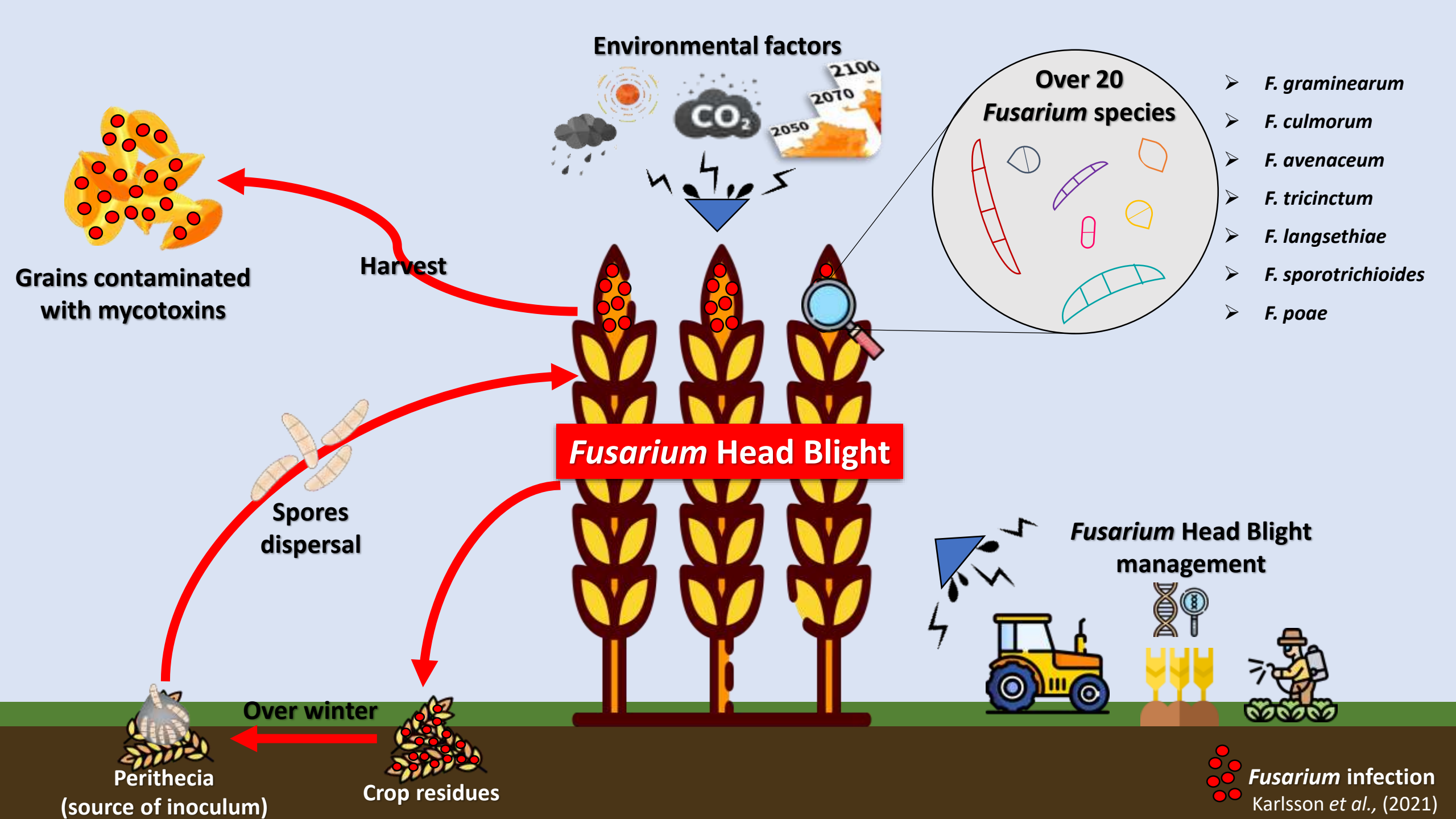


➤ Introduction & Context

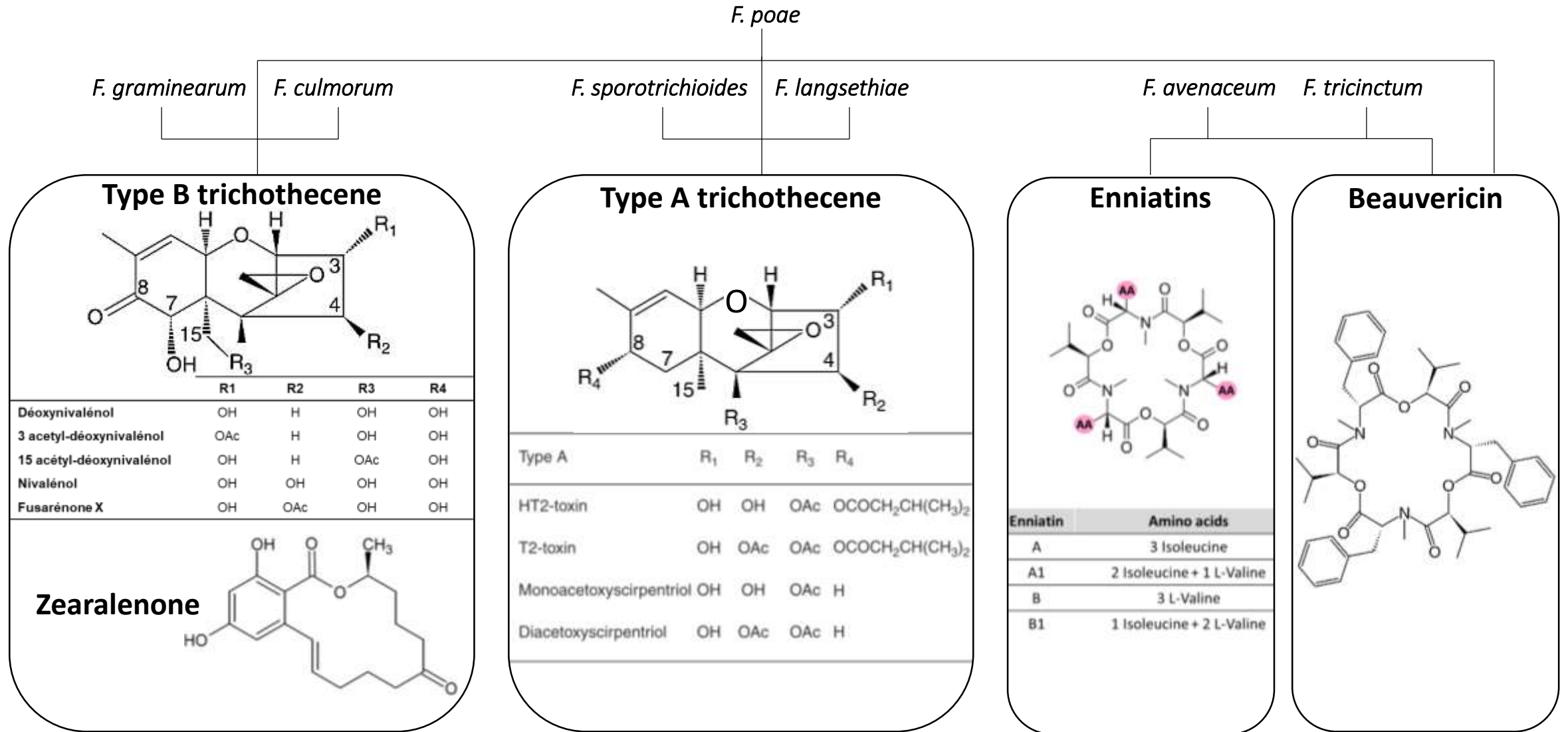
Fusarium Head Blight (FHB):



- Genus *Fusarium*
- Affect **cereal crops** worldwide (wheat, maize, barley, ...)
- **Yield losses** and **reduced grain quality**
- Grains contamination with **mycotoxins**

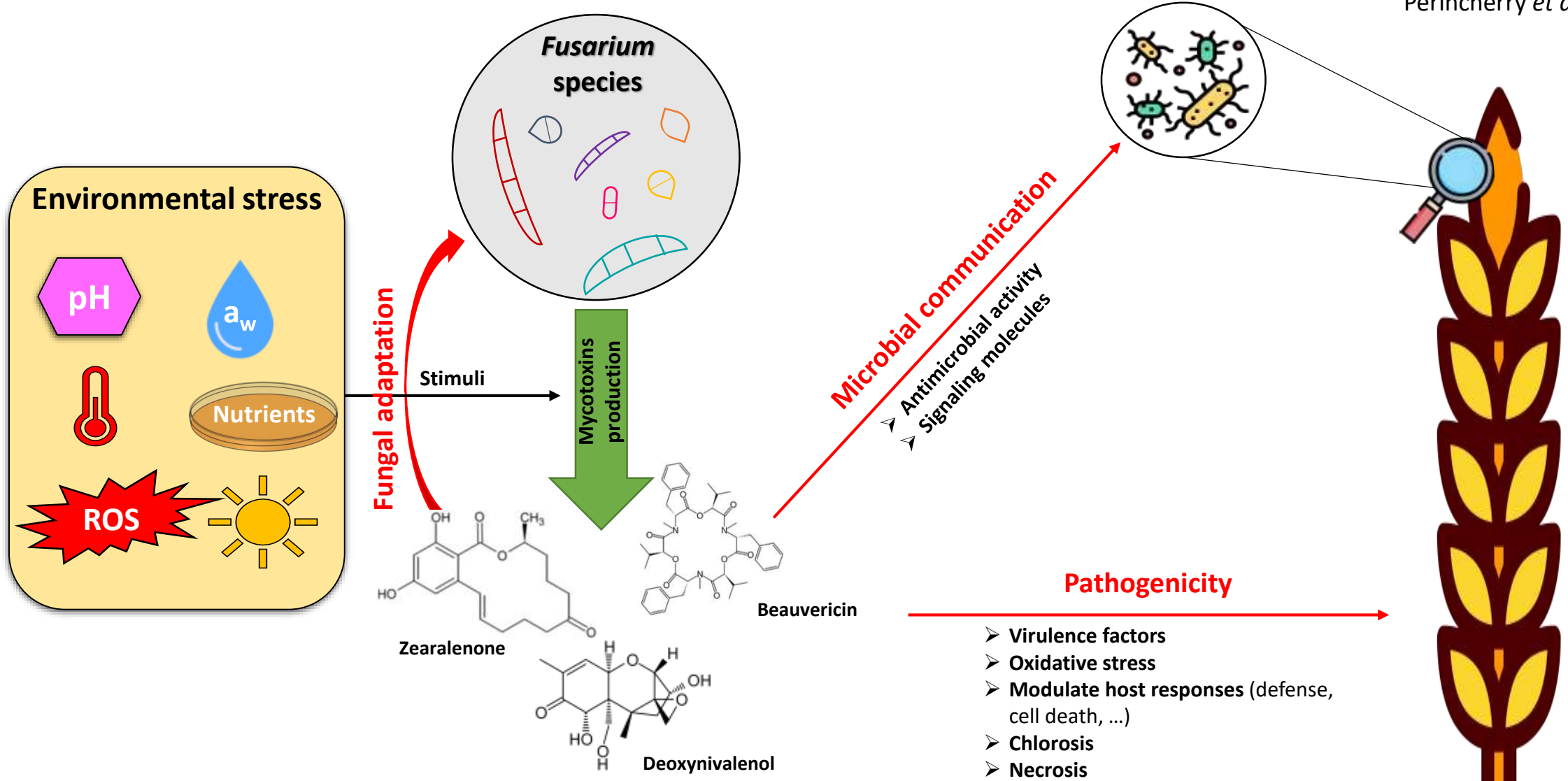


➤ Wide variety of mycotoxins



➤ Ecological roles of mycotoxins?

Venkatesh et Keller, (2019)
Perincherry et al., (2023)



➤ Problematic & Hypothesis

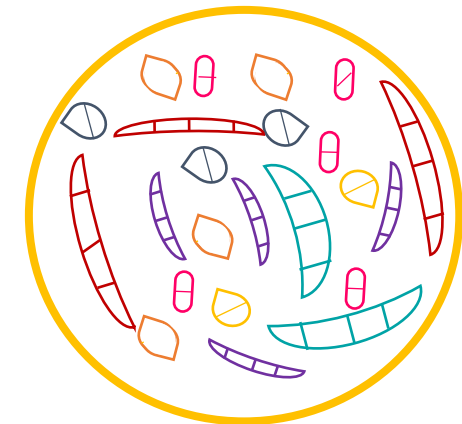
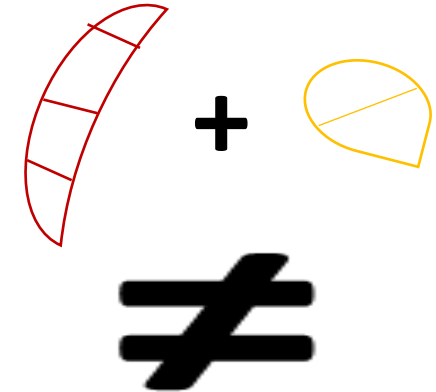
Problematic

- Recommended **agronomic practices** are insufficient to ensure **mycotoxin levels** compliant with EU regulation
- Research focused on “**1 pathogen=1 disease**”

Hypothesis

Observed **mycotoxin contaminations** result from a **global metabolism** caused by a **blend of *Fusarium* species** considered as a single **complex entity**

Single species level

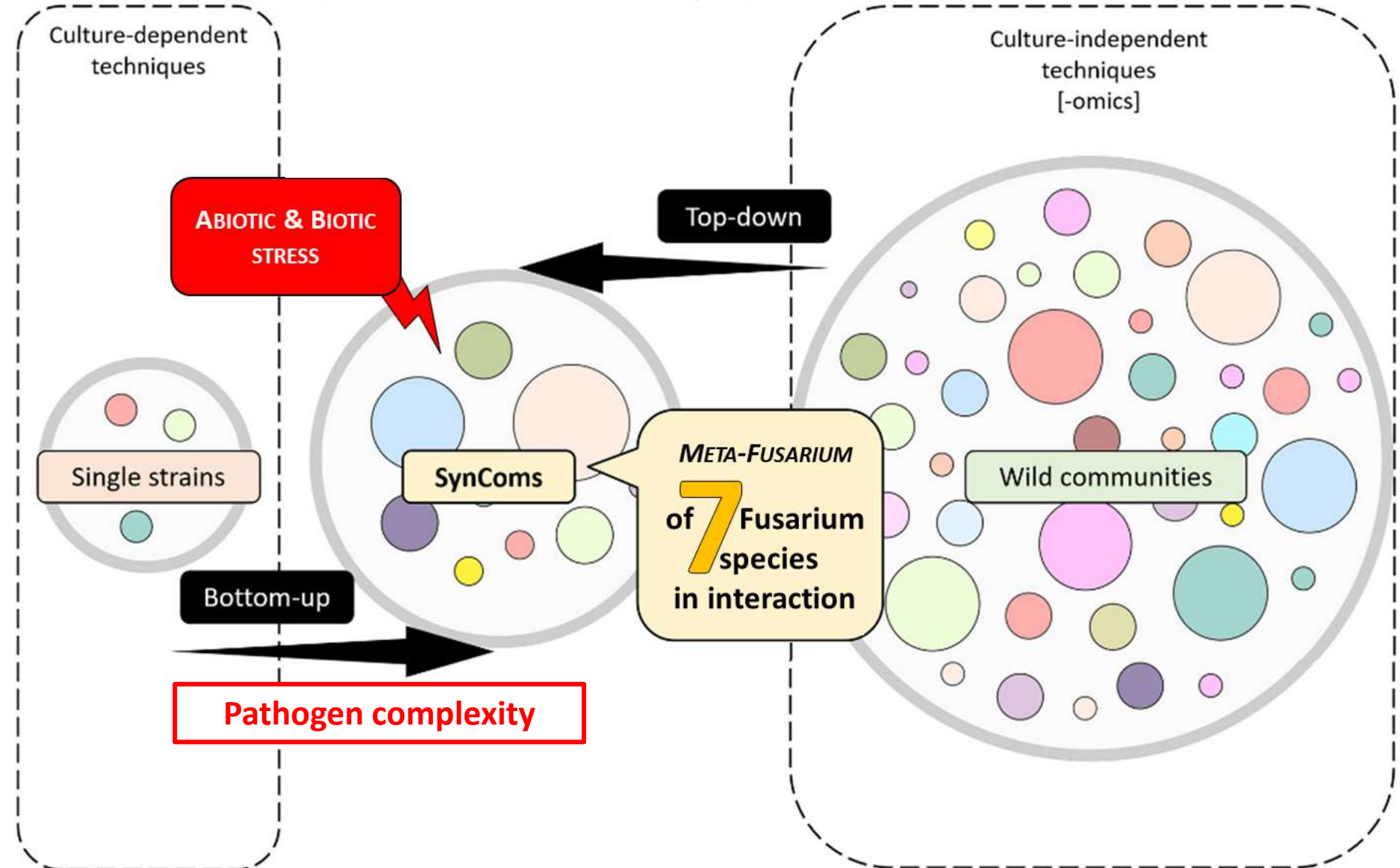


Complex entity level

➤ PhD subject

Studying a *Meta-Fusarium* in order to fight contaminations with mycotoxins

Objectives



➤ PhD subject

Research questions

Question 1

How environmental conditions can change patterns of species composition, mycotoxin production and interactions in a complex pathogen represented by the *Meta-Fusarium*?

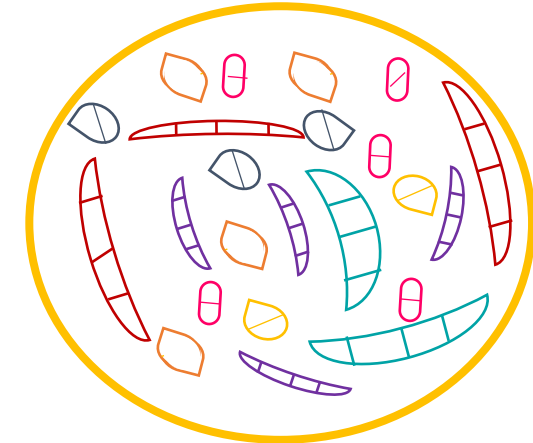
Question 2

What is the added value of the *Meta-Fusarium* approach to implement new eco-responsible solutions to fight *Fusarium* head blight and mycotoxin contaminations in crops?

Synthetic community

META-FUSARIUM

7 *Fusarium* species



- *F. graminearum*
- *F. culmorum*
- *F. avenaceum*
- *F. tricinctum*
- *F. langsethiae*
- *F. sporotrichioides*
- *F. poae*

➤ Research strategy

1

Selection and phenotyping of the strains of the 7 species that compose the *Meta-Fusarium*

2

Development of tools to study and characterize the *Meta-Fusarium*

3

Characterization of the *Meta-Fusarium* under different abiotic pressure and in the presence of biosolutions

1








Selection and phenotyping of the strains that compose the *Meta-Fusarium*

A. Strain selection

Selection criteria:

- Strain isolation (wheat, France)
 - Sporulating ability (inoculum)
 - Fungal development
 - Mycotoxin production
- } *In vitro* standard conditions



	Species		Strain ID
	> <i>F. graminearum</i>	->	Fgr149
	> <i>F. culmorum</i>	->	Fcu337
	> <i>F. avenaceum</i>	->	Fav498
	> <i>F. tricinctum</i>	->	Ftr521
	> <i>F. sporotrichioides</i>	->	Fsp101
	> <i>F. langsethiae</i>	->	Fla509
	> <i>F. poae</i>	->	Fpo073



Phenotyping:

- Fungal development (germination, growth)
- Mycotoxin pattern
- Metabolomic profile

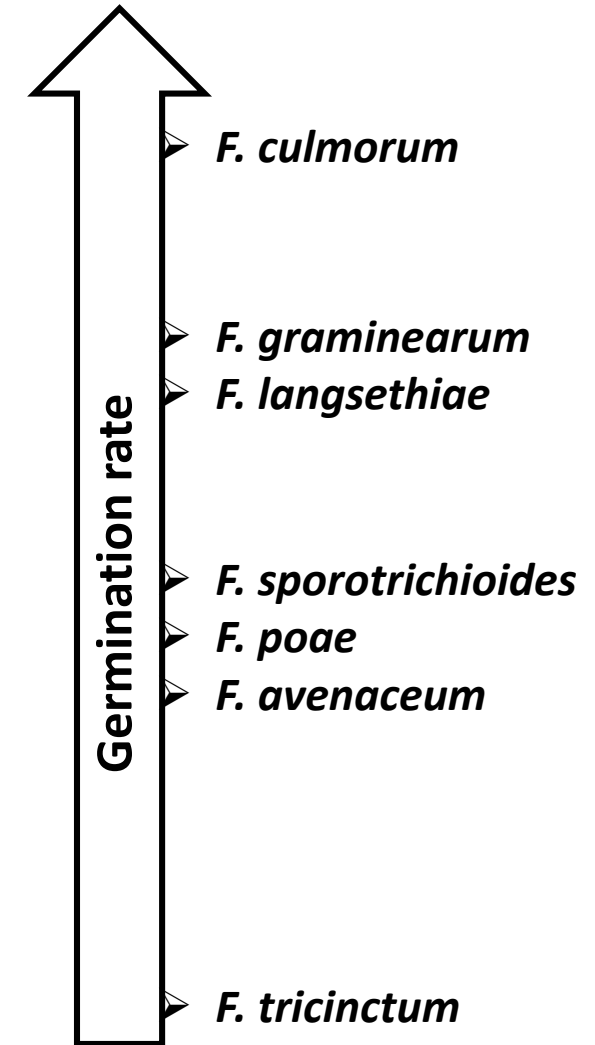
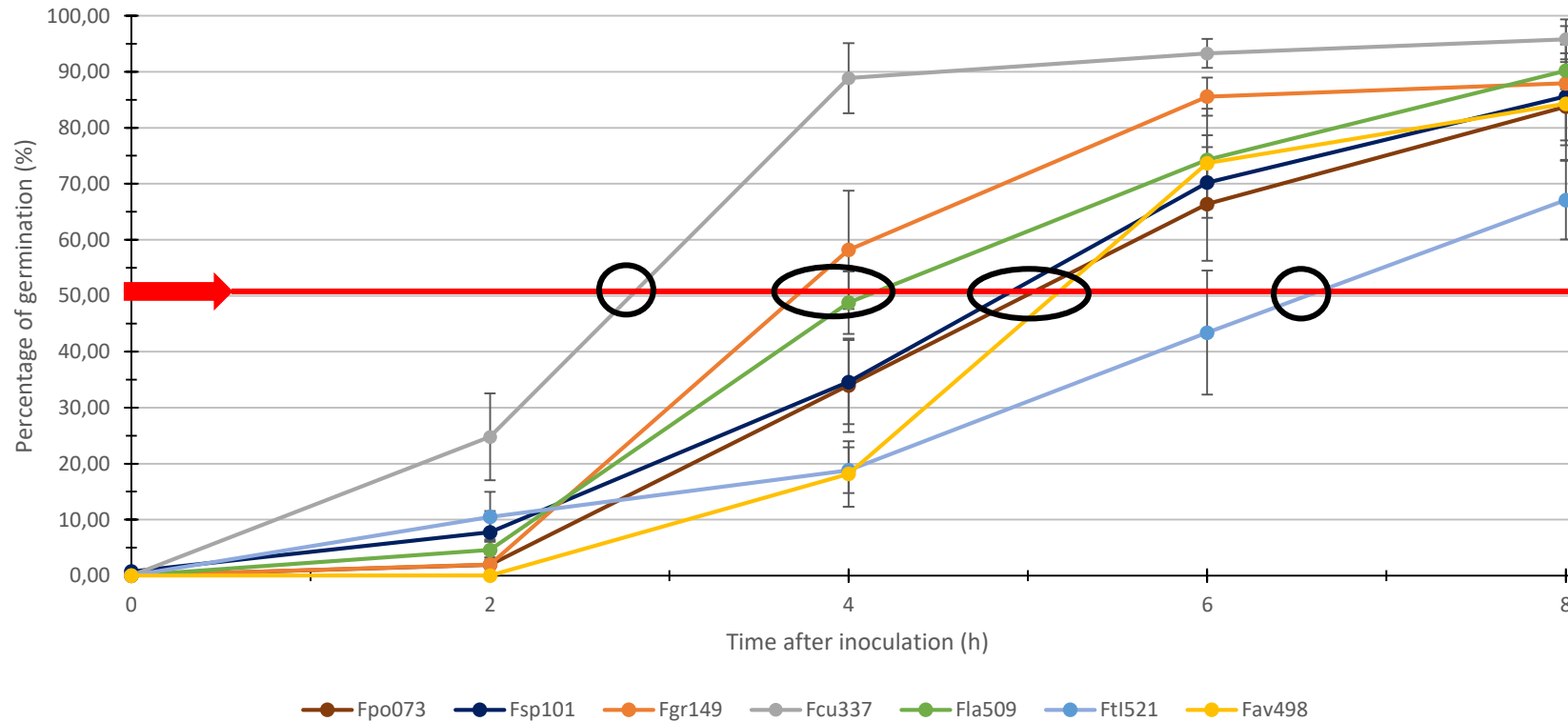
1

Selection and phenotyping of the strains that compose the *Meta-Fusarium*

B. Phenotyping characterization:

➤ Germination kinetics

Germination kinetics of *Meta-fusarium* strains, separated, in MS sucrose, at 25°C



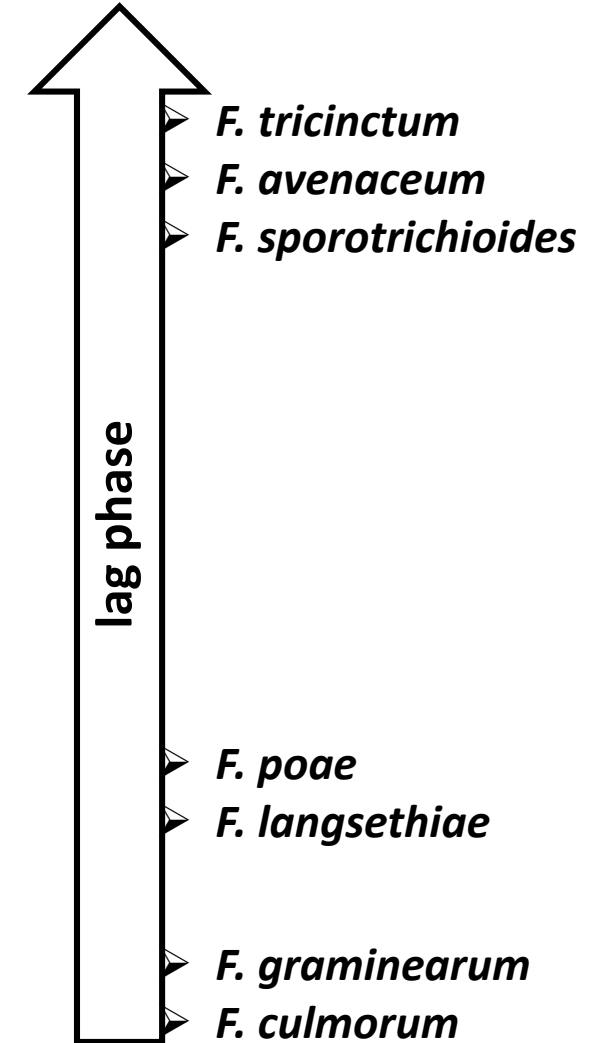
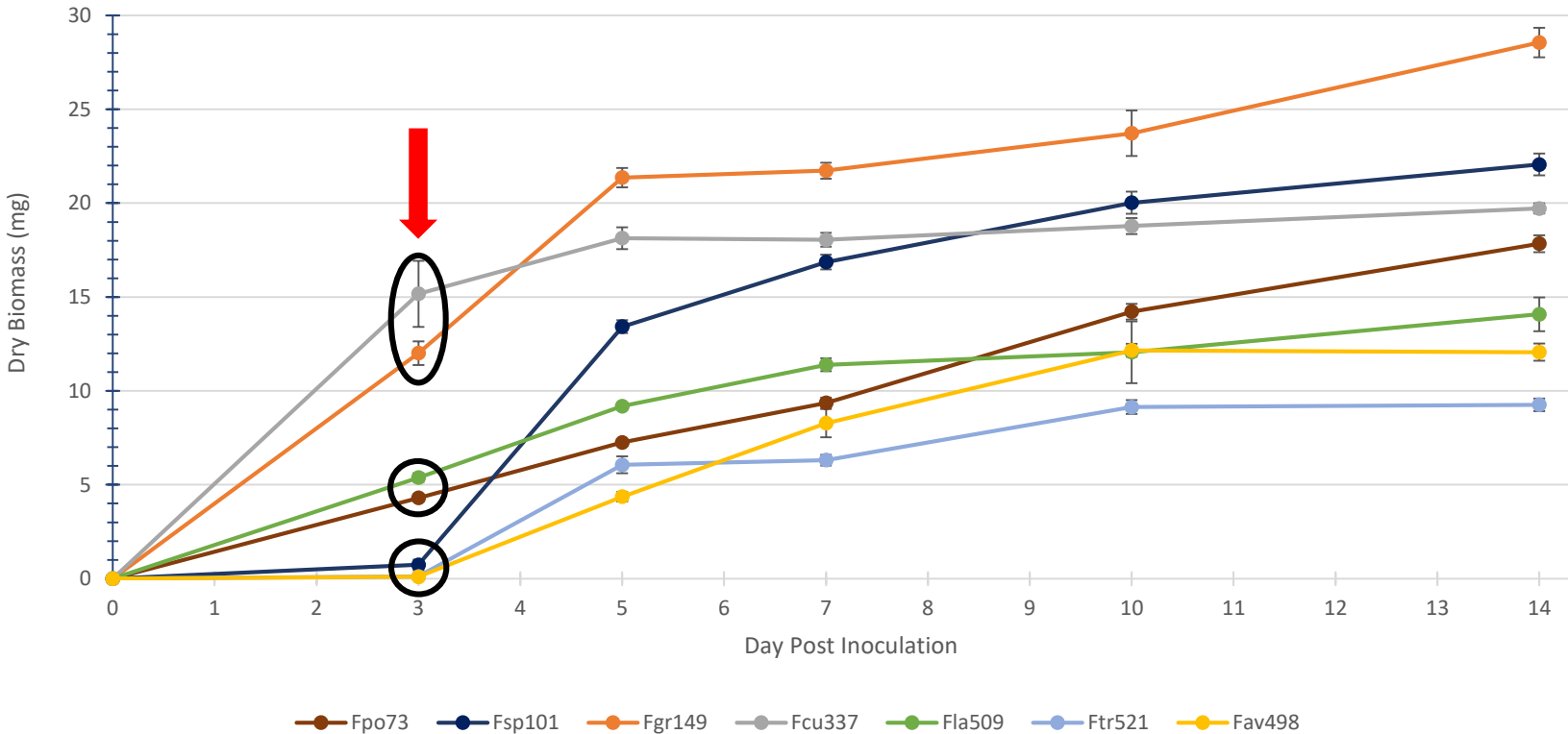
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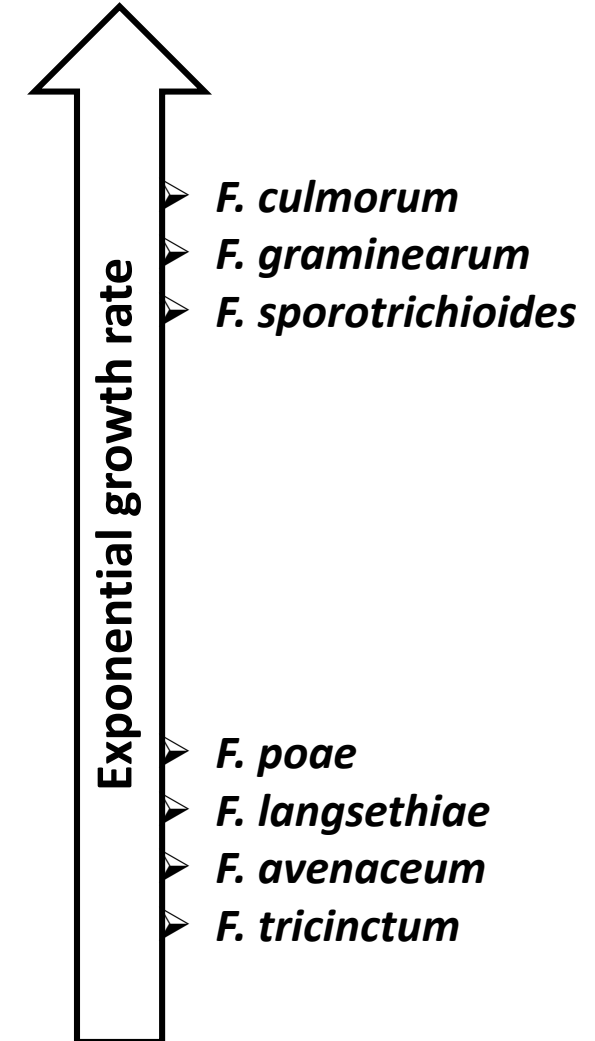
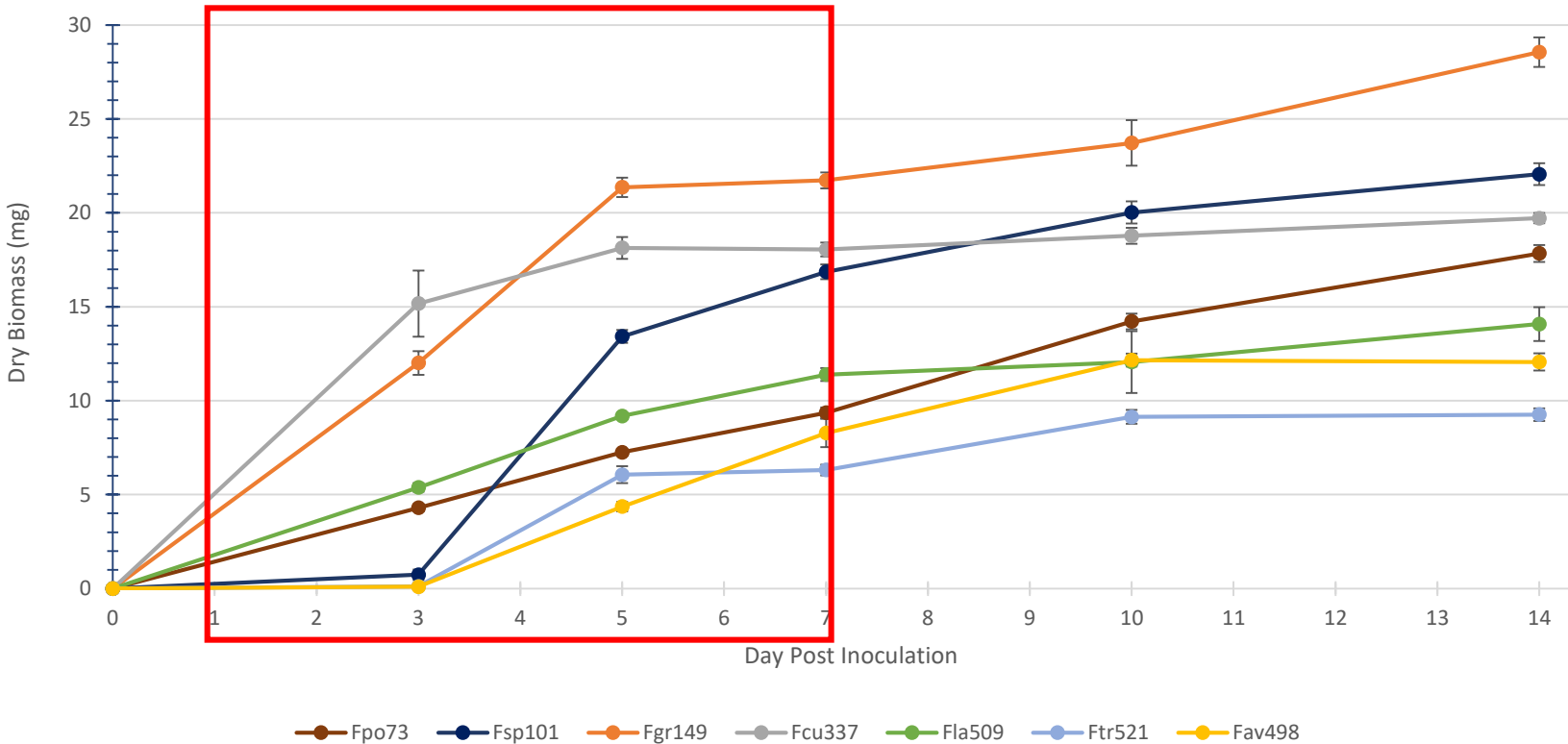
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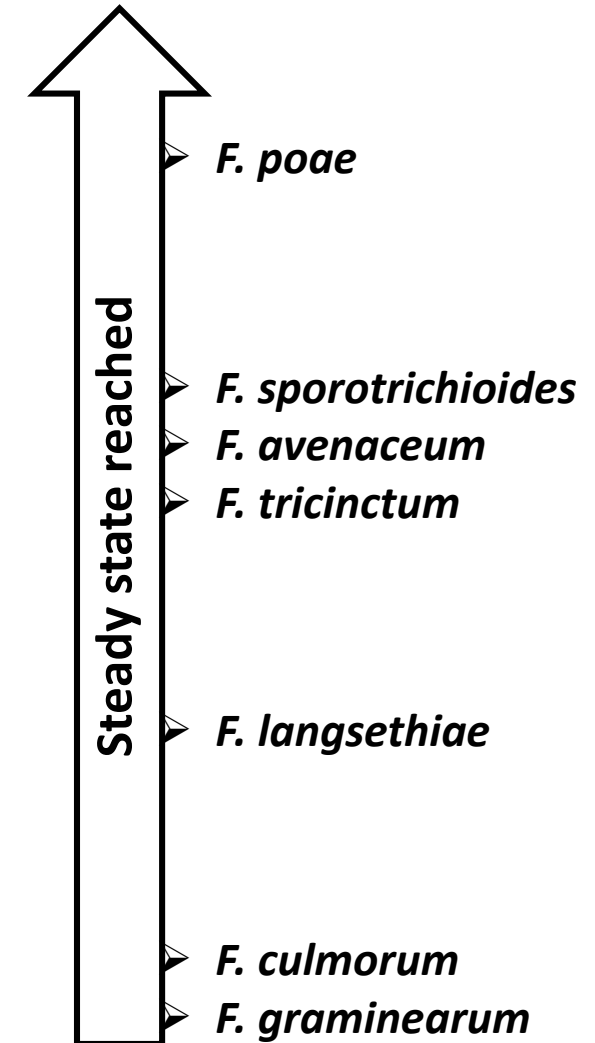
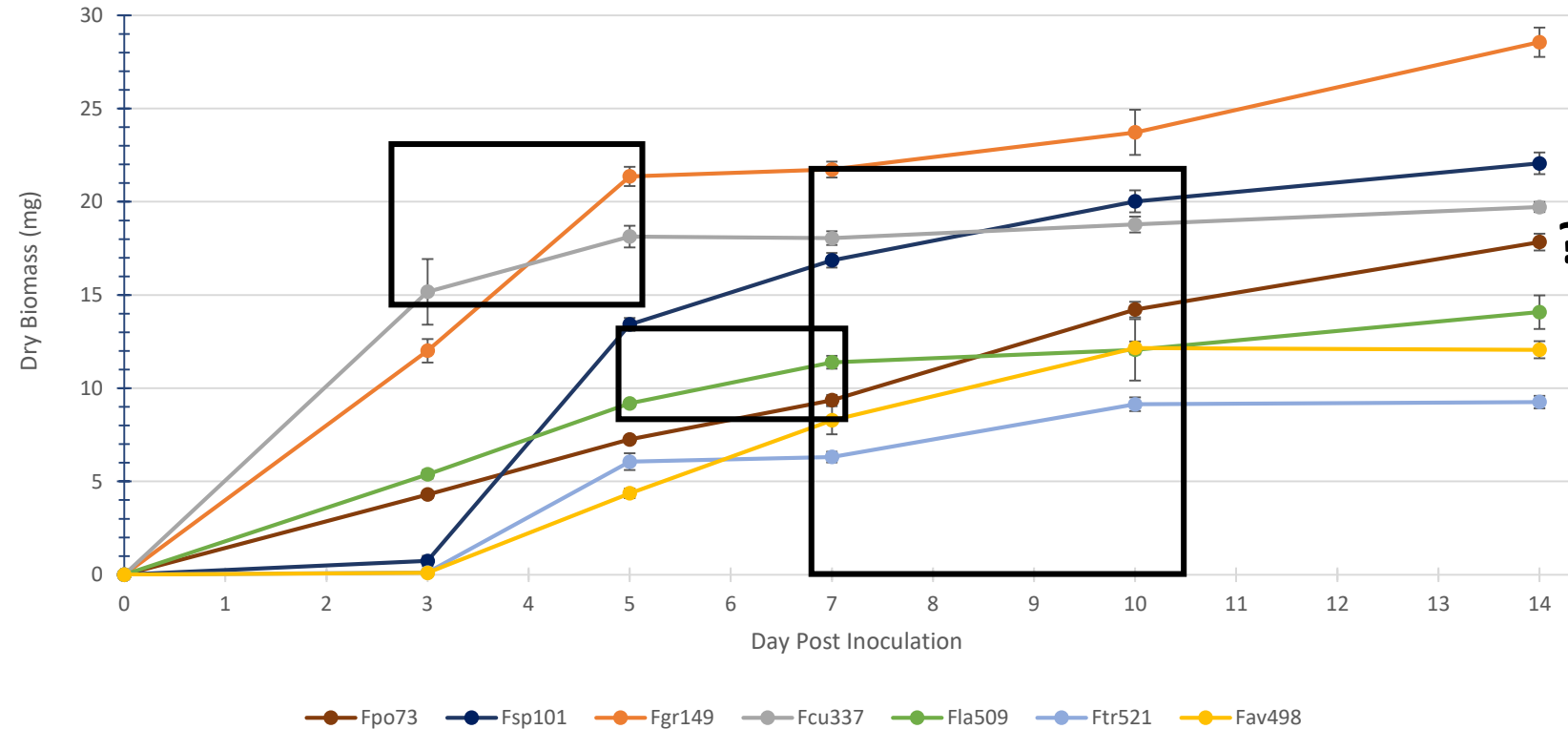
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Selection and phenotyping of the strains that compose the *Meta-Fusarium*

B. Phenotyping characterization:

➤ Growth kinetics

Growth kinetics of *Meta-fusarium* strains, separated, in MS sucrose, at 25°C



1

Selection and phenotyping of the strains that compose the *Meta-Fusarium*

B. Phenotyping characterization:

➤ Mycotoxin pattern

		Type B trichothecene				Type A trichothecene			Enniatins			
		DON	15ADON	NIV	FX	T2	HT2	DAS	ENNA	ENNA1	ENNB	ENNB1
Strains	F. graminearum	+	+++	nd	nd	-	-	-	-	-	-	-
	F. culmorum	nd	nd	nd	+	-	-	-	-	-	-	-
	F. poae	-	-	-	-	nd	nd	++	-	-	-	-
	F. sporotrichioides	-	-	-	-	+++	+++	++	-	-	-	-
	F. langsethiae	-	-	-	-	++	+	+	-	-	-	-
	F. tricinctum	-	-	-	-	-	-	-	nd	+	+++	++
	F. avenaceum	-	-	-	-	-	-	-	nd	nd	+++	+

1

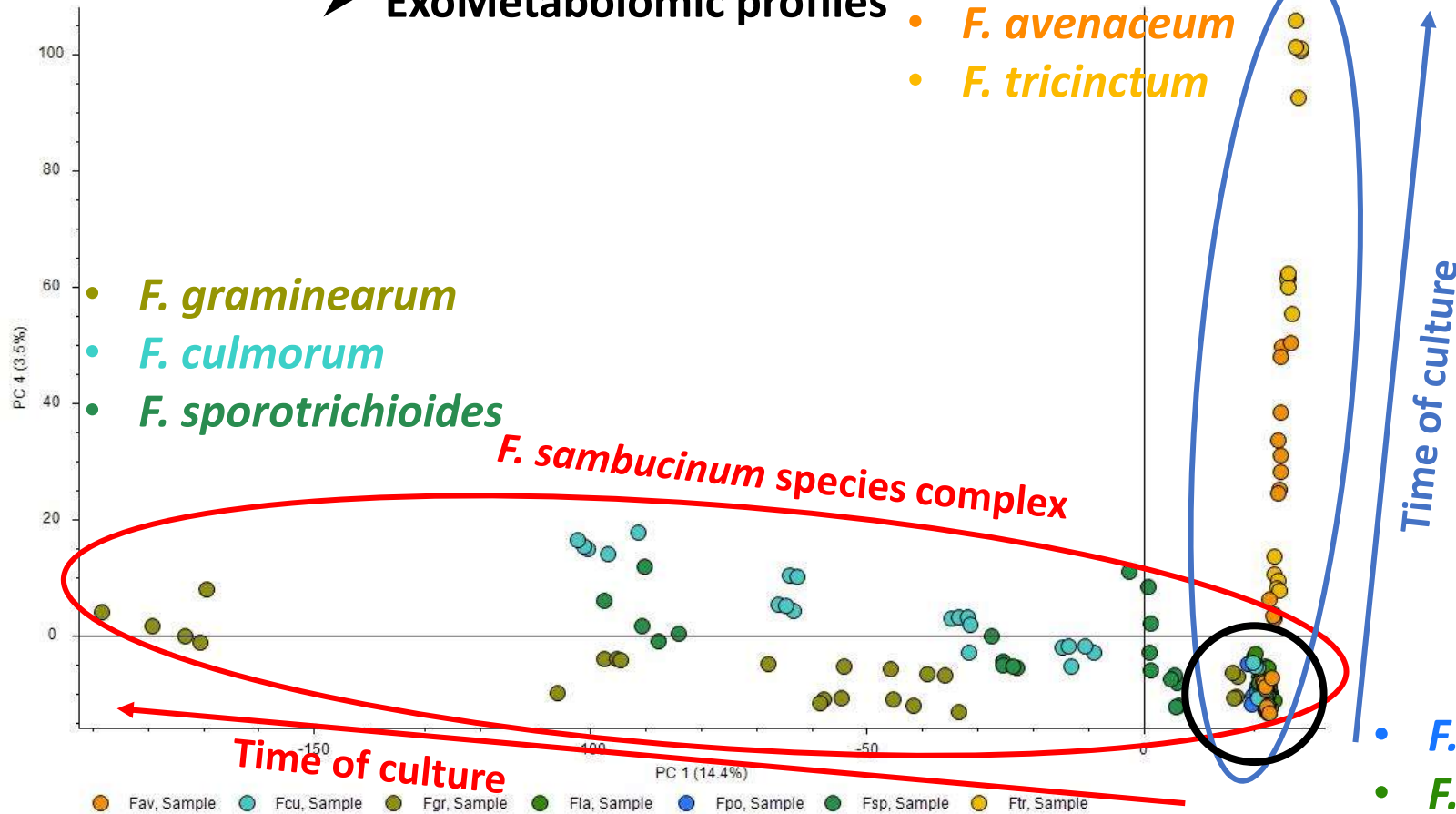
Selection and phenotyping of the strains that compose the *Meta-Fusarium*

B. Phenotyping characterization:

➤ ExoMetabolomic profiles

F. tricinctum species complex

- *F. avenaceum*
- *F. tricinctum*



- Metabolomic profiles show some differences according to :
 - *The Fusarium* species complex
 - Time of culture
- **Mycotoxin impact?**
- Metabolomic profiles of *F. poae* and *F. langsethiae* can not be differentiated

Selection and phenotyping of the strains that compose the *Meta-Fusarium*

Conclusion & hypothesis

- *F. culmorum* and *F. graminearum* -> faster germination rate and earlier biomass development
 - Will they **predominate** in the *Meta-Fusarium*?
- *F. tricinctum*, *F. avenaceum* and *F. sporotrichioides* -> slower early stage development
 - Will they be **inhibited/outdone** by strains growing faster?
- **Differential mycotoxins/metabolites production** over time between strains
 - What are the roles of these secondary metabolites?
 - Fungal adaptation
 - Pathogenicity
 - Interactions

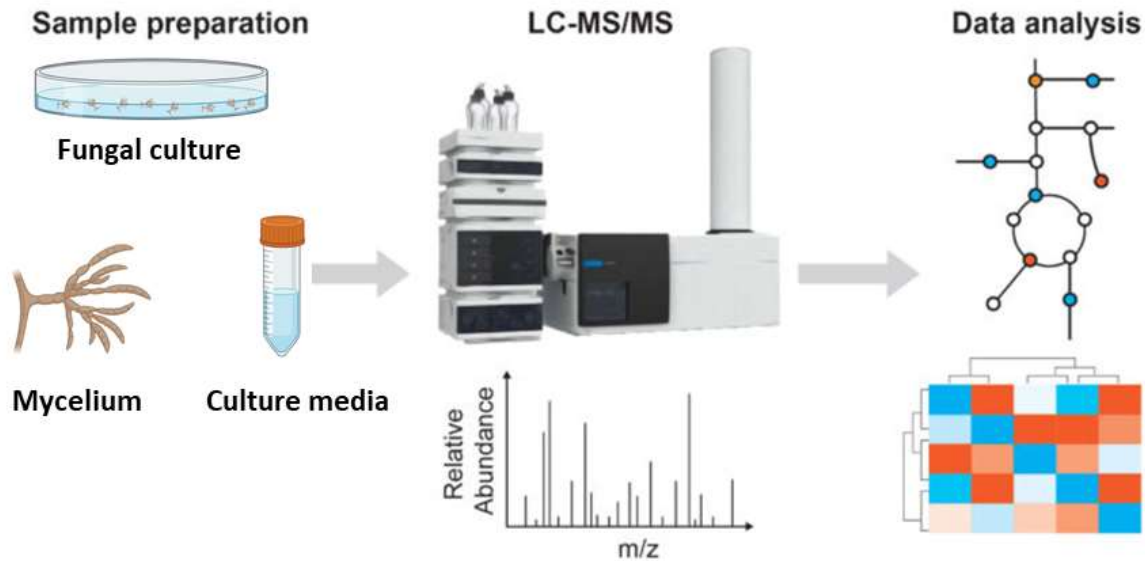
2

Development of tools to study and characterize the *Meta-Fusarium*

Metabolomic profile analysis



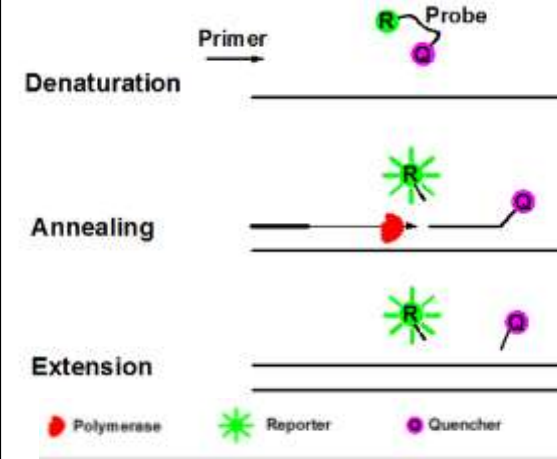
Workflow



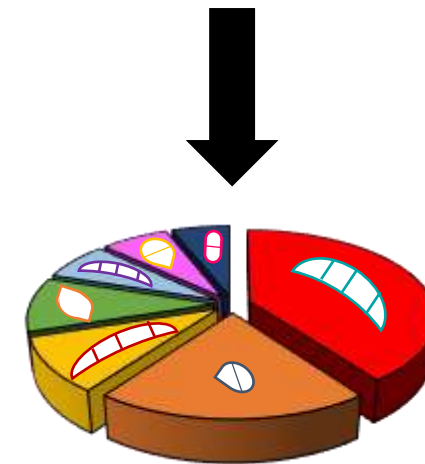
Species composition characterization



Real Time Polymerase Chain Reaction (qPCR)



- Targeting the **single copy gene** *EF1α*
- **Species specific**
- **Hydrolysis probe**



Characterization of the *Meta-Fusarium* under different abiotic & biotic pressure

Compositional dynamics of the *Meta-Fusarium* and its production of secondary metabolites under various environmental conditions

Environmental conditions

20°C ; +/- H₂O₂ 0.5mM

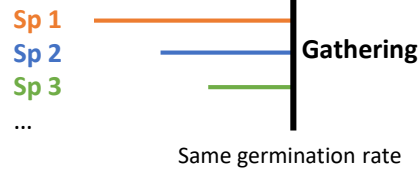
25°C ; +/- H₂O₂ 0.5mM

Inoculum: 7 *Fusarium* species

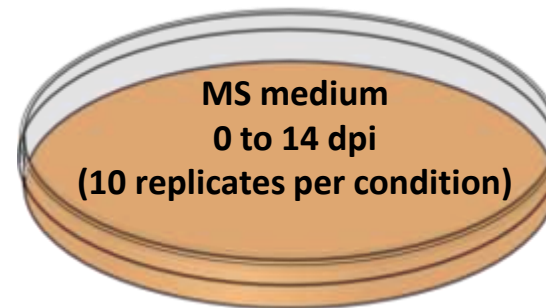
= ***Meta-Fusarium***

Type 1: Not germinated conidia

Type 2: Germinated conidia



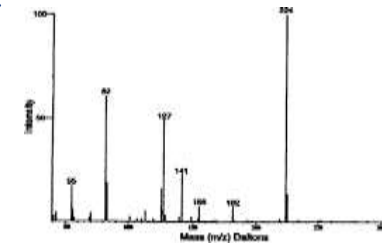
Cultivation



Analysis



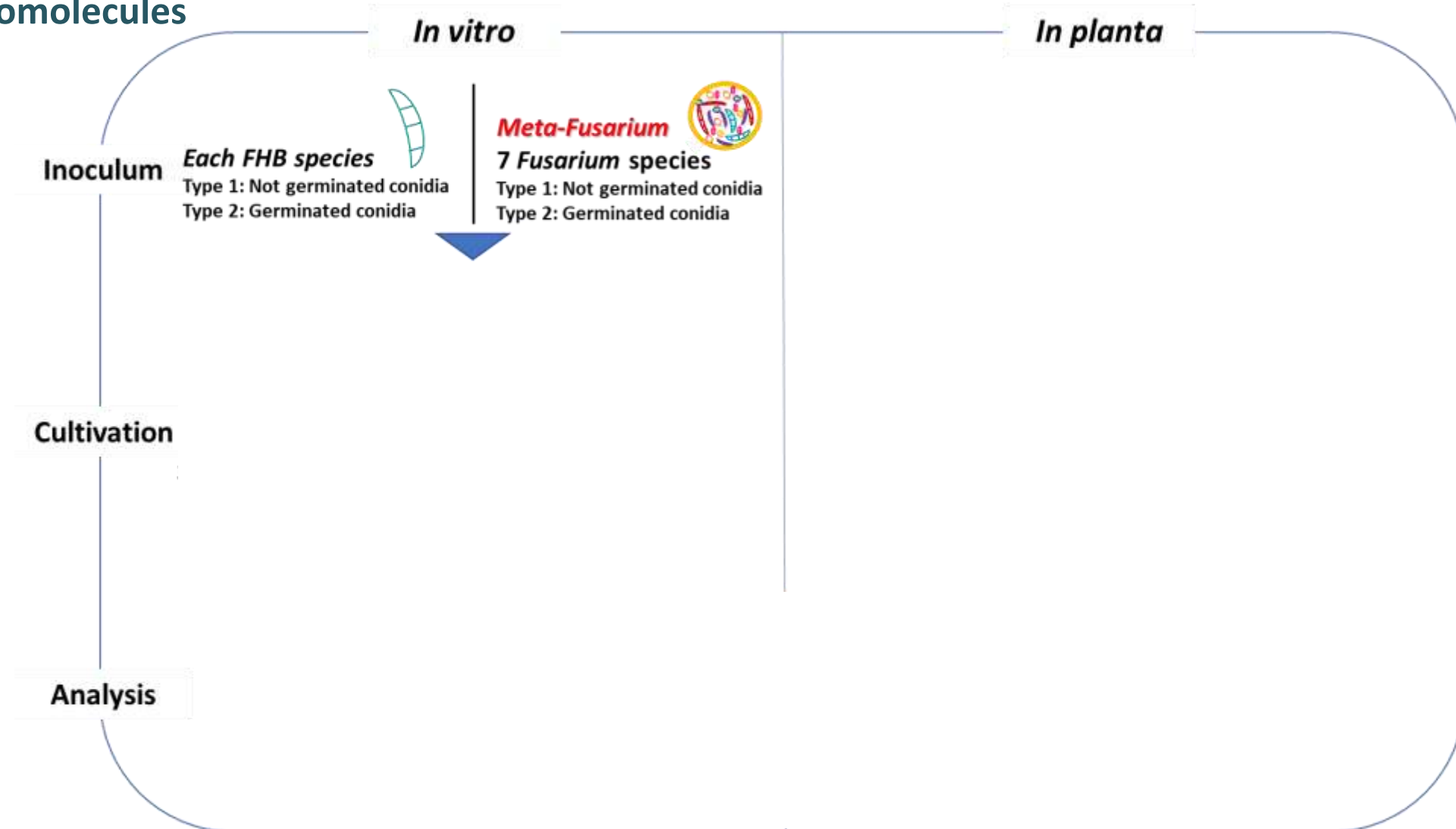
Species composition (qPCR)



Metabolomic profiles (LC-MS)

Characterization of the *Meta-Fusarium* under different abiotic & biotic pressure

Susceptibility of the *Meta-Fusarium* to biotic interactions involving microorganisms, antifungal biomolecules



➤ Expected results & Perspectives



Significant scientific advances



Economic and public health benefits

New knowledges on *Fusarium* Head Blight:

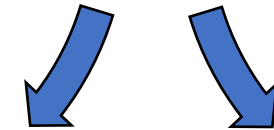
- Determinants of mycotoxin accumulation
- Interaction mechanisms between FHB species
- Regulation mechanisms of mycotoxin production
- Fungal composition/mycotoxin evolution and prediction on field



Proof of concept of the interest of studies on a plant meta-pathogen, transferable to other fungal diseases

- One species vs *Meta-Fusarium*
- Tripartite interactions studies (FHB species, plant, biosolutions)
- Development of global FHB control strategies

New tools, to reduce the consequences of *Fusarium* head blight



Reduction in economic losses

Decreased consumer exposure

Towards a decrease in synthetic fungicides

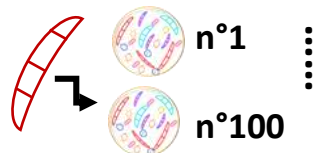


Thank you for your attention !

➤ Appendix

WP 1 COMPOSITIONAL DYNAMICS OF META-FUSARIUM SP. AND ITS PRODUCTION OF 2^{SD} METABOLITES UNDER VARIOUS ENVIRONMENTAL CONDITIONS

WP 3 IMPACT OF THE INTRA-SPECIFIC GENETIC DIVERSITY ON THE COMPOSITION AND FUNCTIONING OF META-FUSARIUM SP.



- *F. graminearum* strains diversity

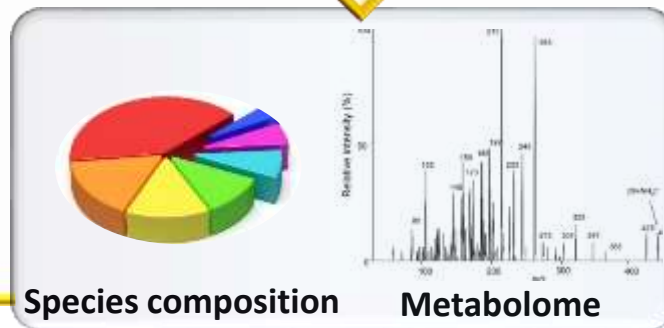
WP 4 SUSCEPTIBILITY OF META-FUSARIUM SP. TO BIOTIC INTERACTIONS WITH PLANT TISSUE, OUTGROUP MICROORGANISM & ANTIFUNGAL BIOMOLECULE

- Behaviour on a plant organ
- Testing biocontrol solutions

WP 2 REGULATION OF MYCOTOXIN PRODUCTIONS BY META-FUSARIUM SP. WHEN EXPOSED TO ENVIRONMENTAL CHANGES

- Gene expression dynamics
- Chromatin dynamics
- Communication (Metabolites, smRNA)

META-FUSARIUM SP.
of 7 Fusarium species



Species composition

Metabolome

WP 0

COORDINATION



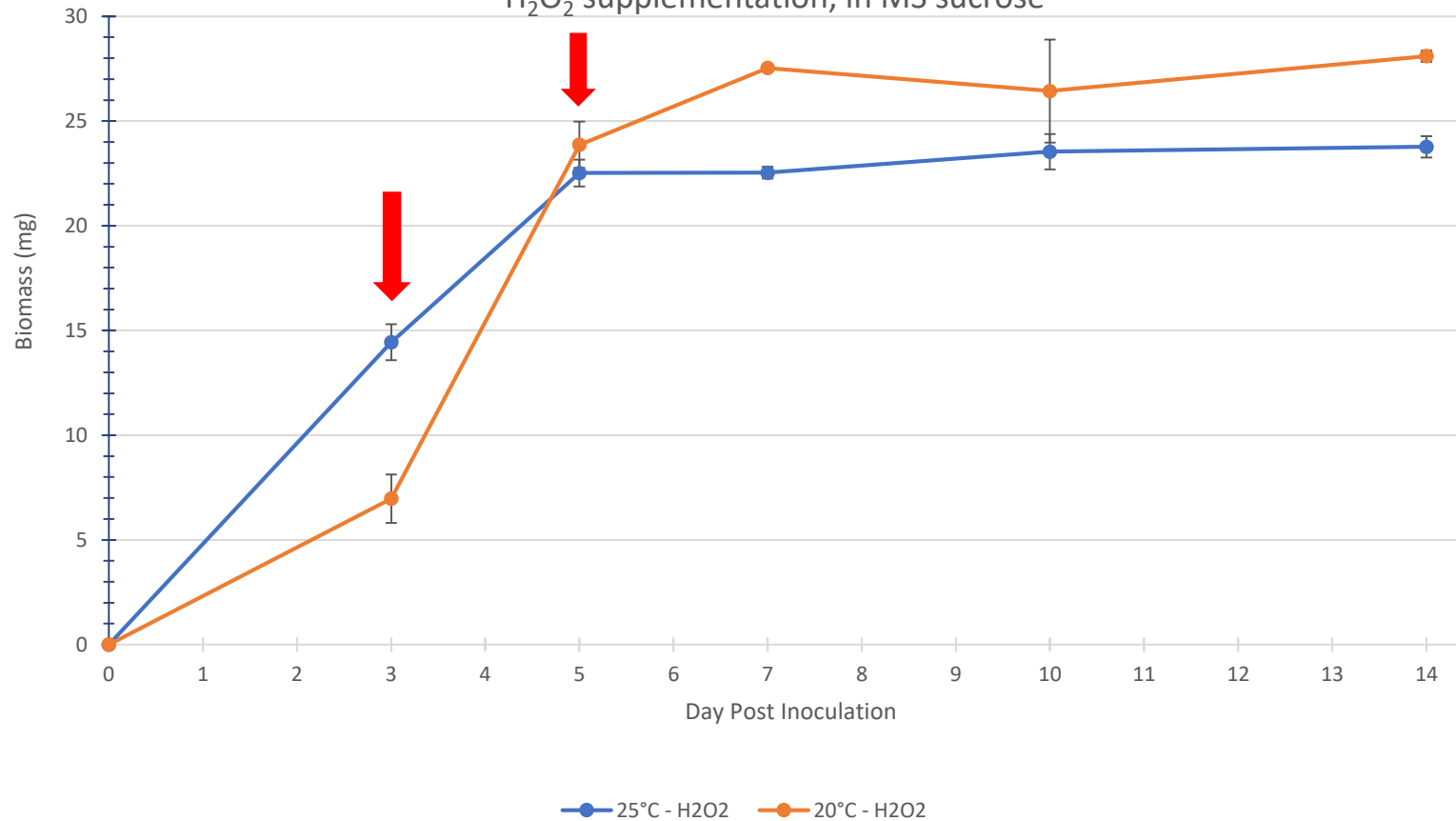
PROJECT MANAGEMENT

3

Characterization of the *Meta-Fusarium* under different abiotic & biotic pressure

Preliminary results

Growth kinetics of *Meta-fusarium* under different temperatures and with/without H₂O₂ supplementation, in MS sucrose



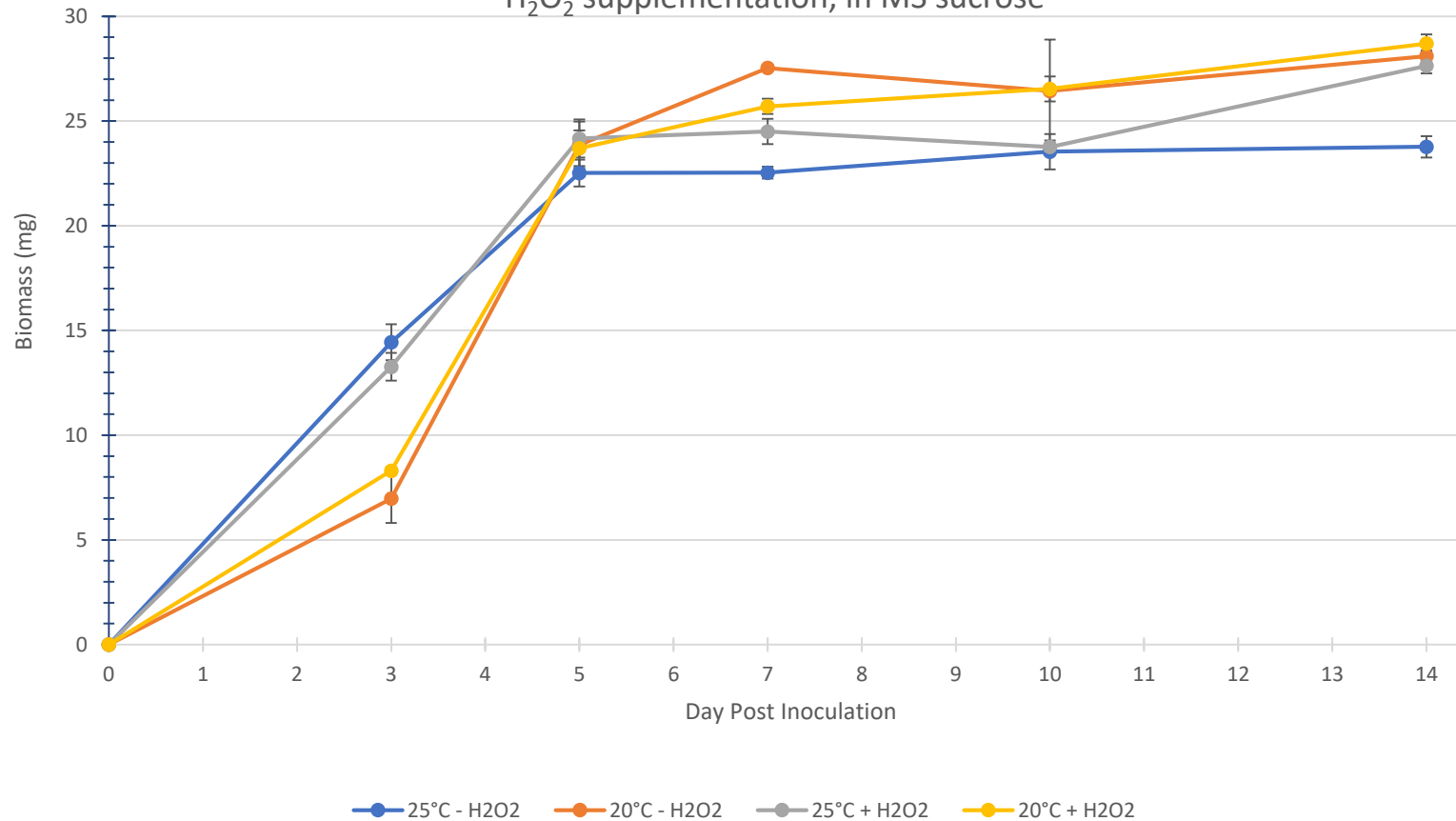
- **Temperature at 20°C seems to slow down the early growth (3days)**
- **No differences from 5 days (plateau)**

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Characterization of the *Meta-Fusarium* under different abiotic & biotic pressure

Preliminary results

Growth kinetics of *Meta-fusarium* under different temperatures and with/without H₂O₂ supplementation, in MS sucrose



- **Temperature at 20°C seems to slow down the early growth (3days)**
- **No differences from 5 days (plateau)**
- **H₂O₂ does not seem to impact growth**

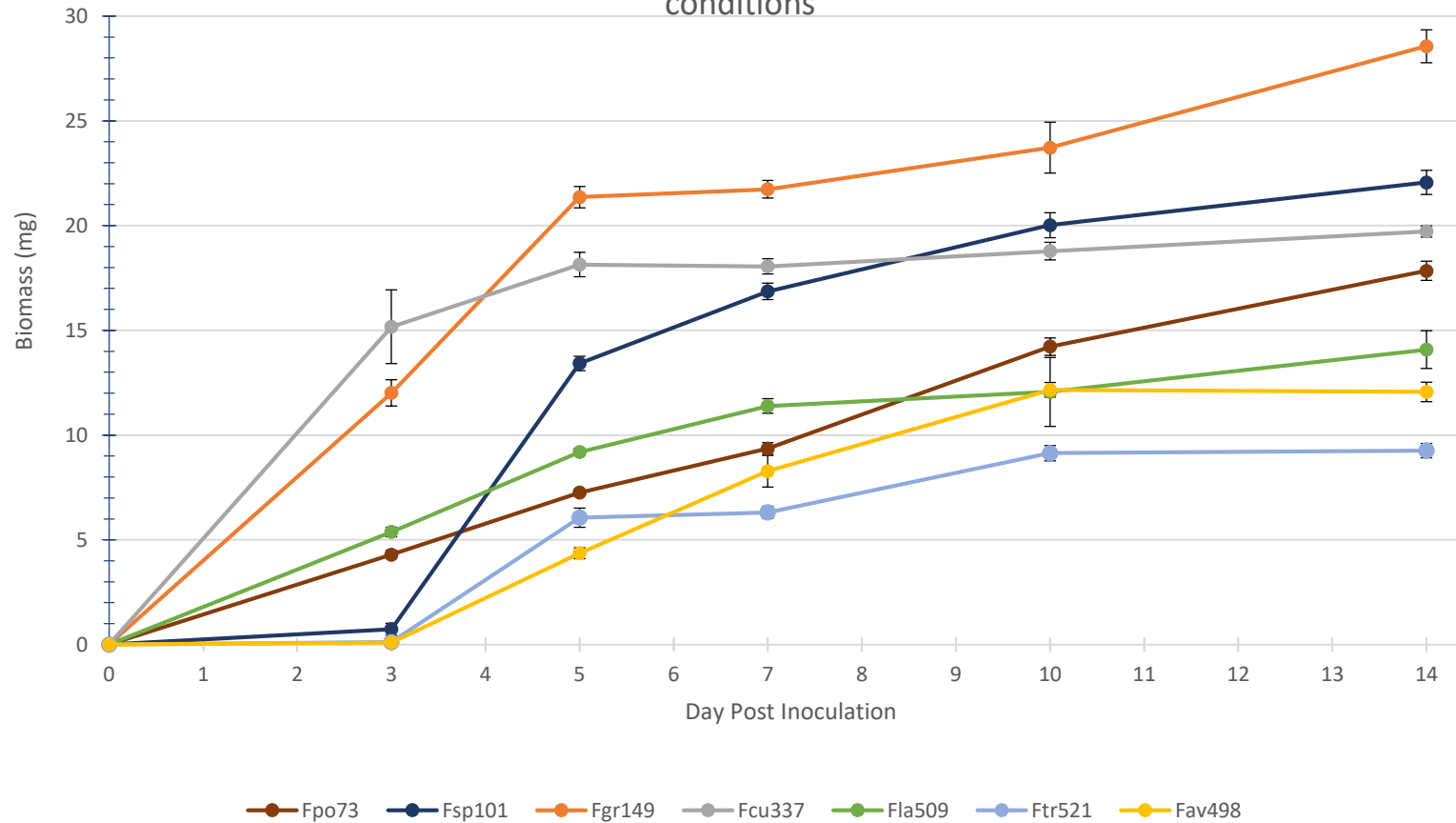
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Growth comparison between isolated strains and the *Meta-Fusarium*

Growth kinetics of *Meta-fusarium* compared to isolated strains in standard conditions



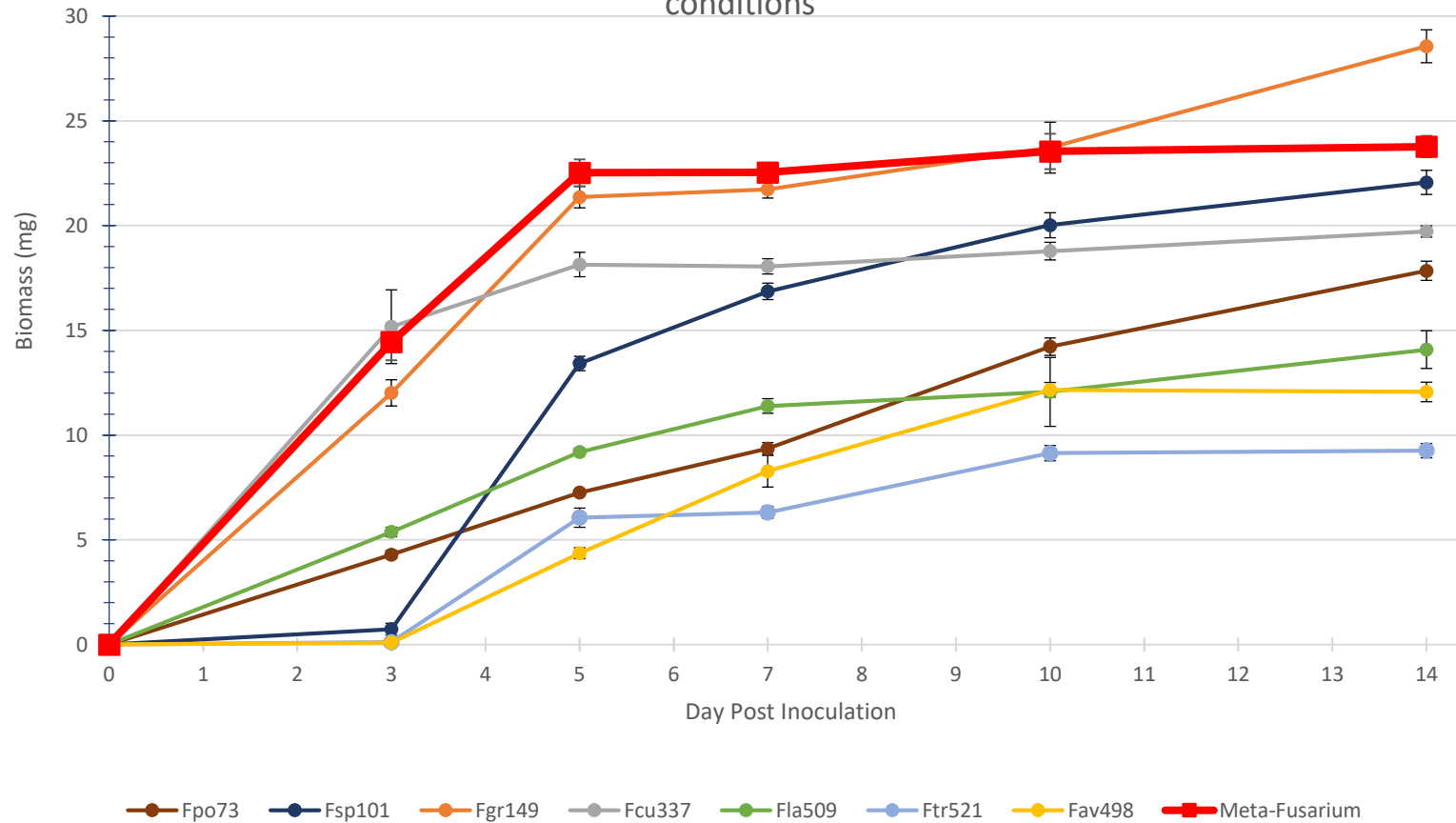
3

Characterization of the *Meta-Fusarium* under different abiotic & biotic pressure

Preliminary results

Growth comparison between isolated strains and the *Meta-Fusarium*

Growth kinetics of *Meta-fusarium* compared to isolated strains in standard conditions



- **Meta-Fusarium growth curve** similar to *F. culmorum* and *F. graminearum*
 - Predominant species?

- Perspectives:
 - Fungal composition of the *Meta-Fusarium*
 - Mycotoxin production by the *Meta-Fusarium*