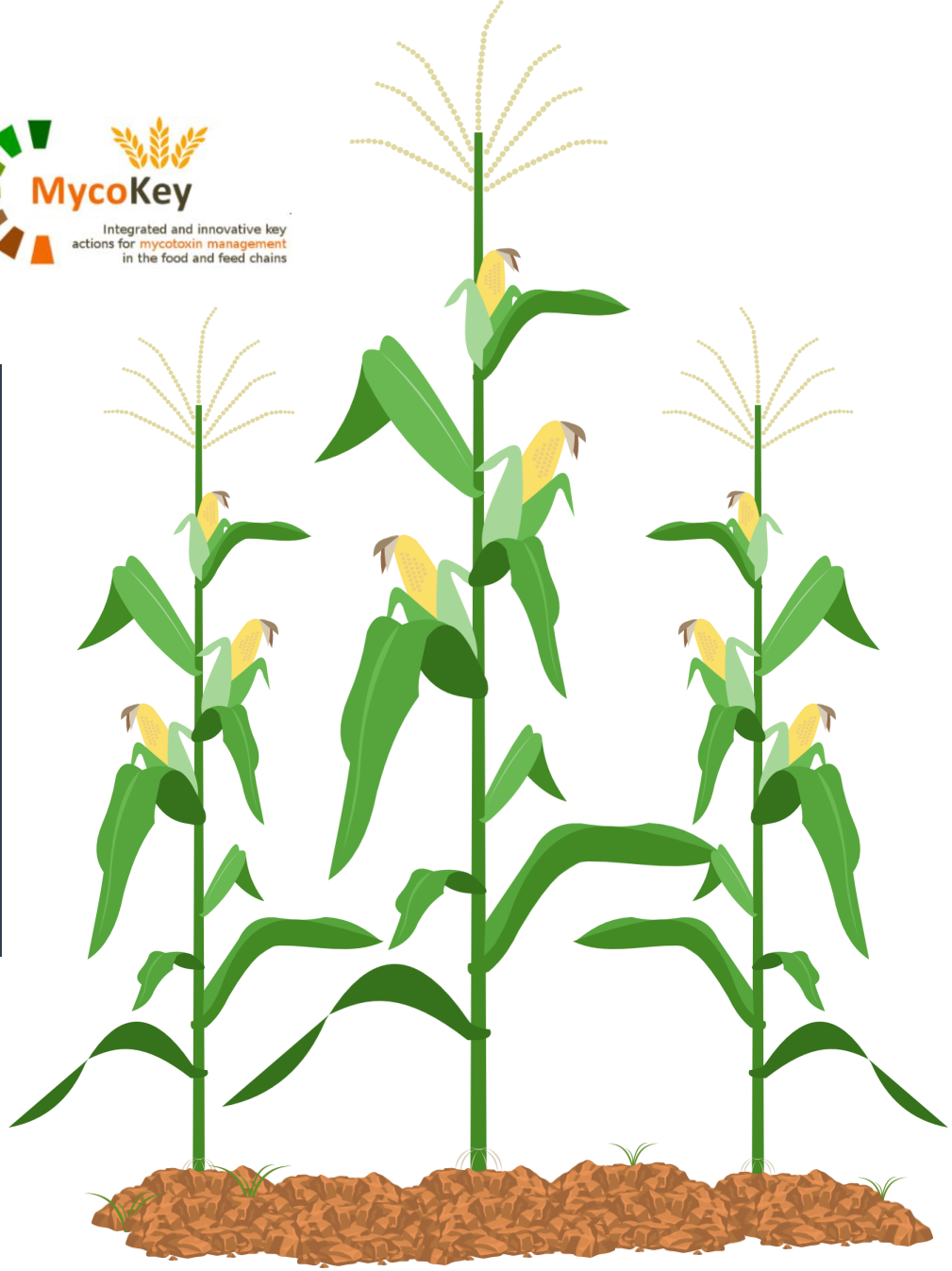
Fungal volatile organic compounds, can be used to develop aflatoxin-specific sensors?



MycoTWIN-MycoKey INTERNATIONAL CONFERENCE

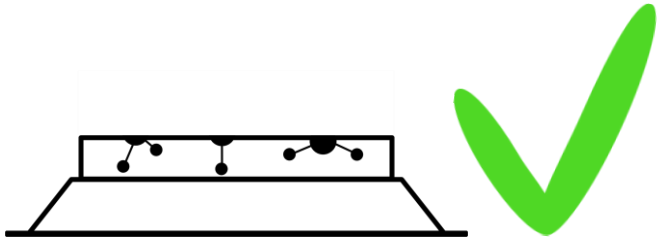
Bari, 9 to 12 November 2021

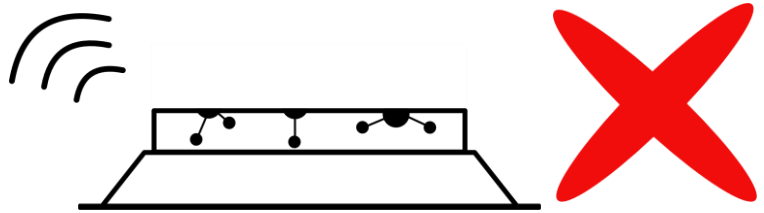
Laurie Josselin - Ph.D.

NEW APPROACH & FINAL AIM

✓ Study of the VOCs emitted by fungi

✓ Study of the aflatoxin biosynthesis





→Presence of fungi

→Kind of genus

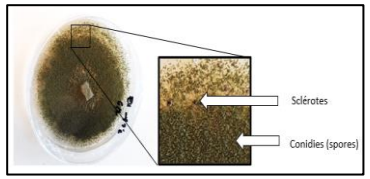
→Kind of species

→Production of aflatoxin

General information

Aspergillus flavus

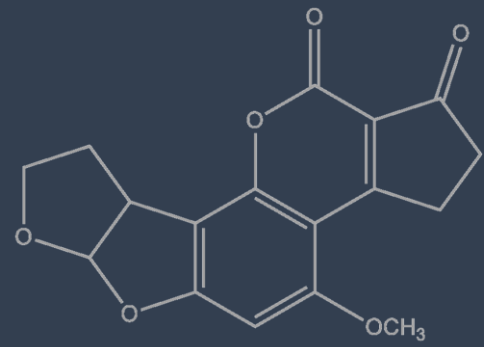
Two categories
(type S and L)



Toxigenic	Toxigenic	Non-toxigenic
High aflatoxin	*Variable aflatoxin*	*No aflatoxin*

Aflatoxin B1, B2, G1 and G2

Aflatoxin B1



Etymology

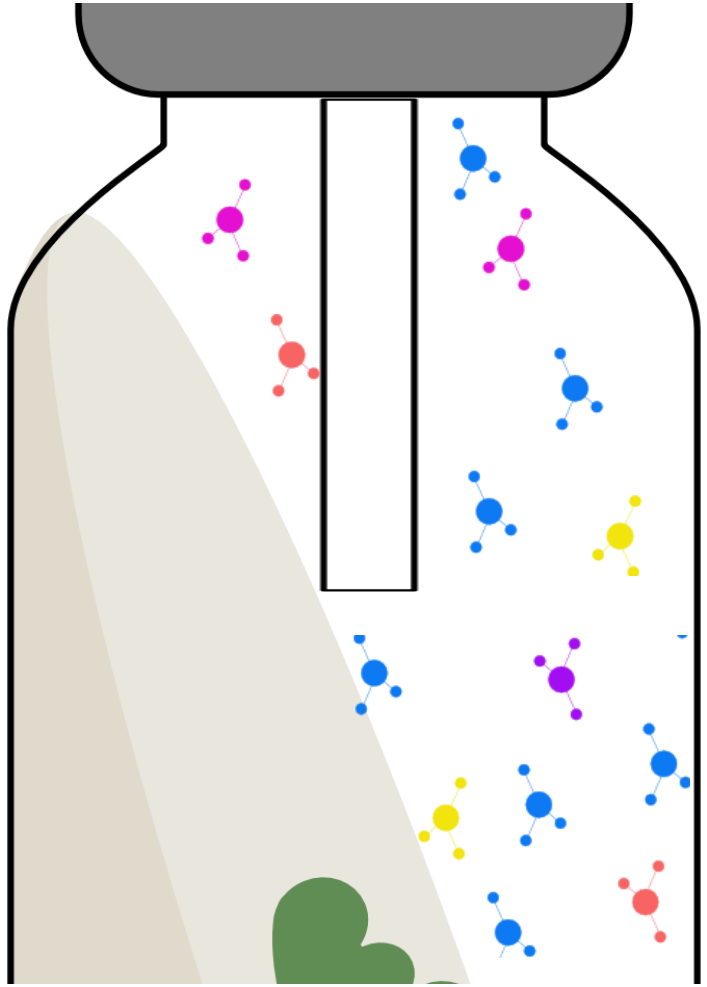
A. flavus

Fluorescence

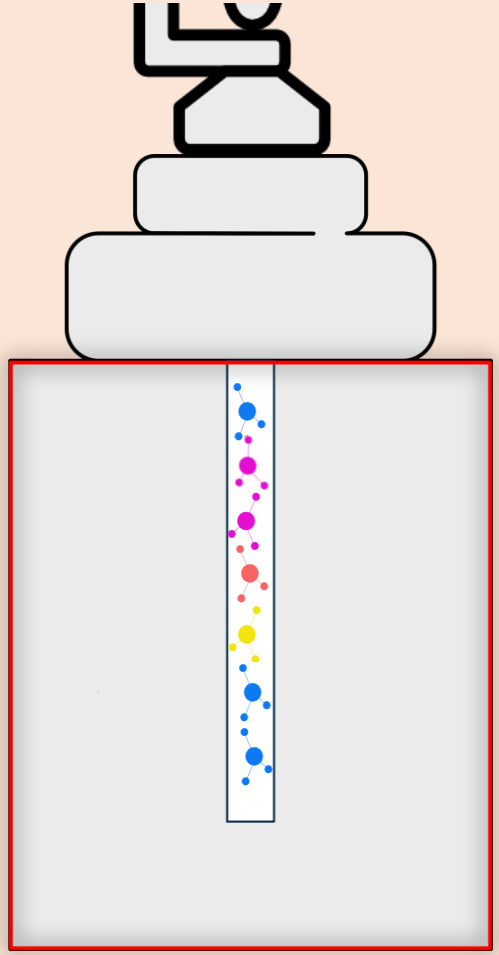
Thermostable

Method of analysis

EXTRACTION & INJECTION



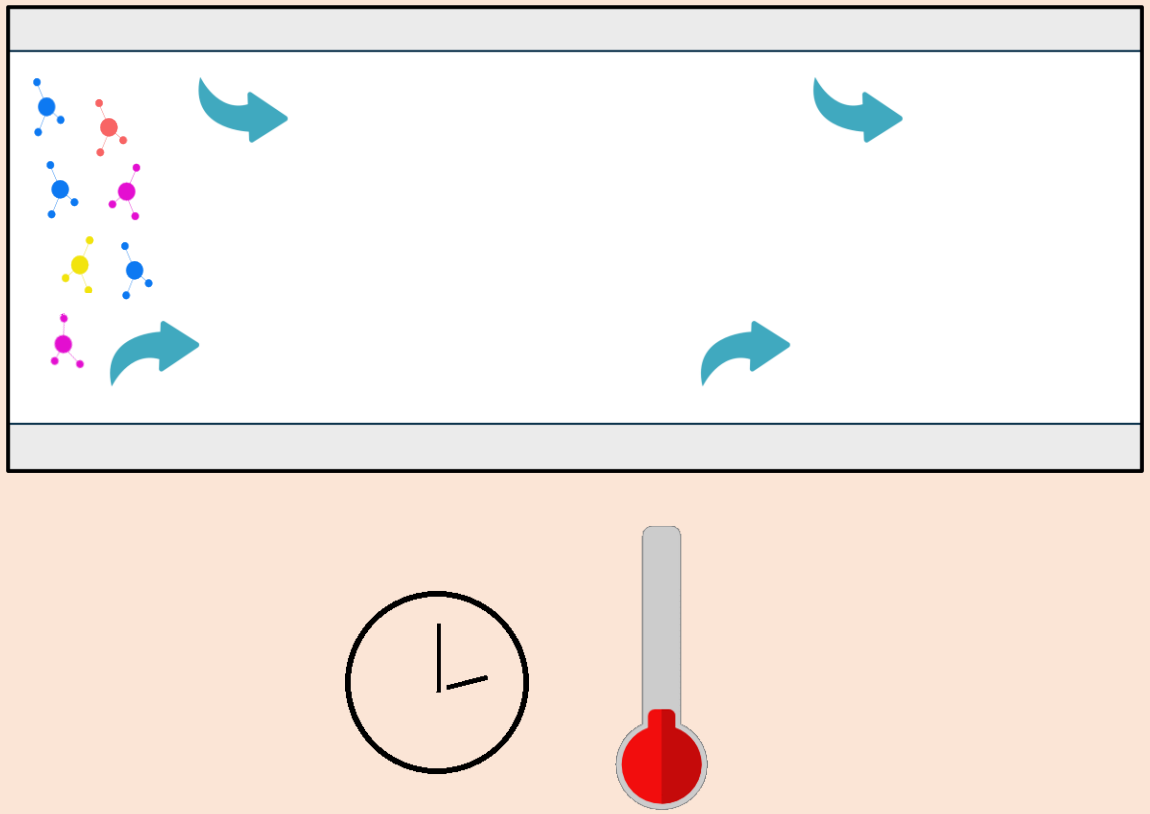
SPME (Solid phase micro-extraction)

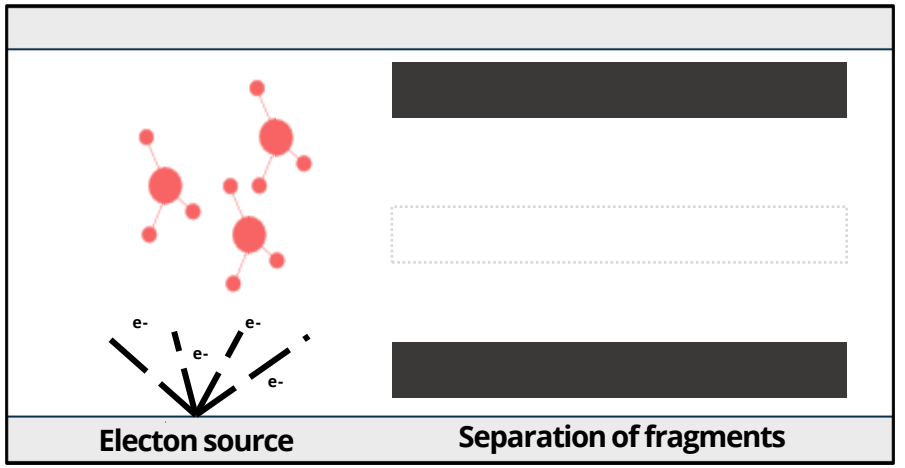


Injector → desorption of the VOCs trapped

Method of analysis

SEPARATION & DETECTION



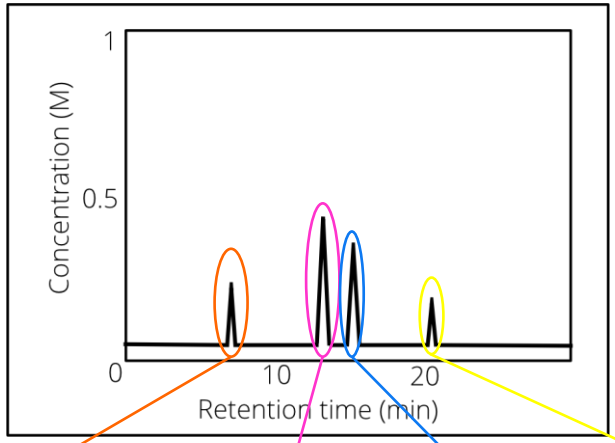


Gas chromatography → separation of the VOCs

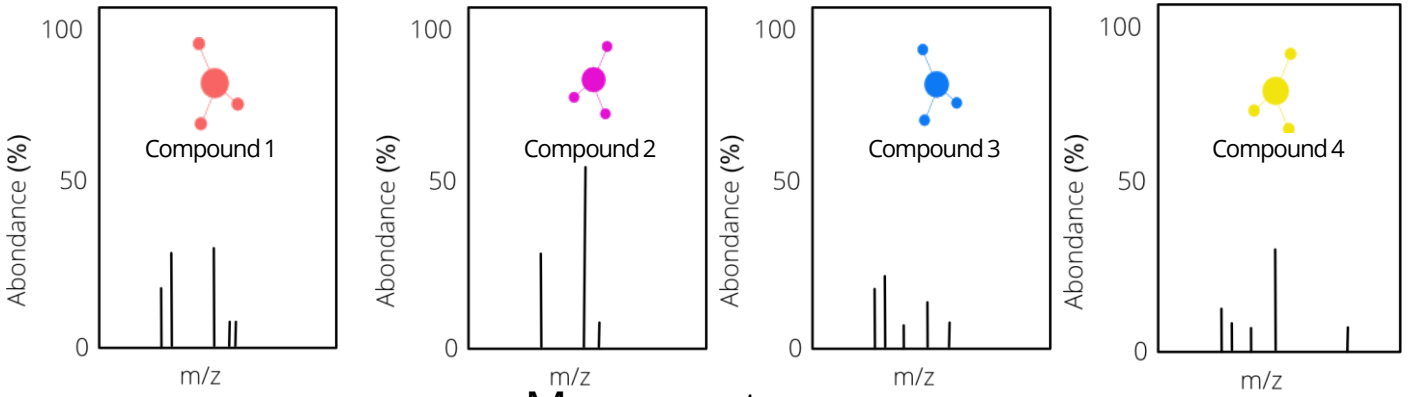
Mass spectrometry → Detection of the VOCs

Method of analysis

RESULTS & SEMI-QUANTIFICATION

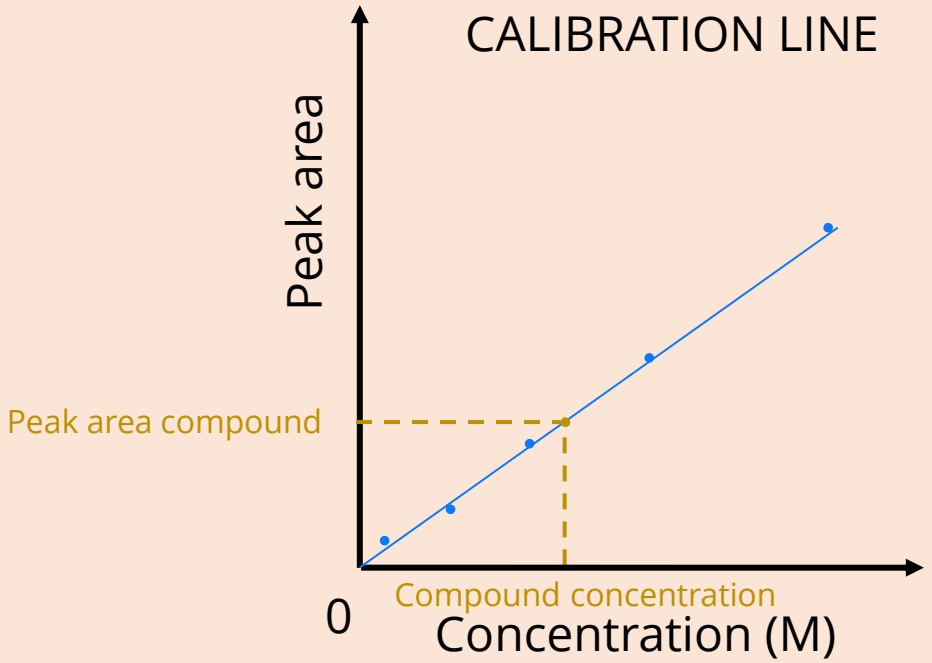


Chromatogram



Mass spectrum

Identification



Semi-quantification

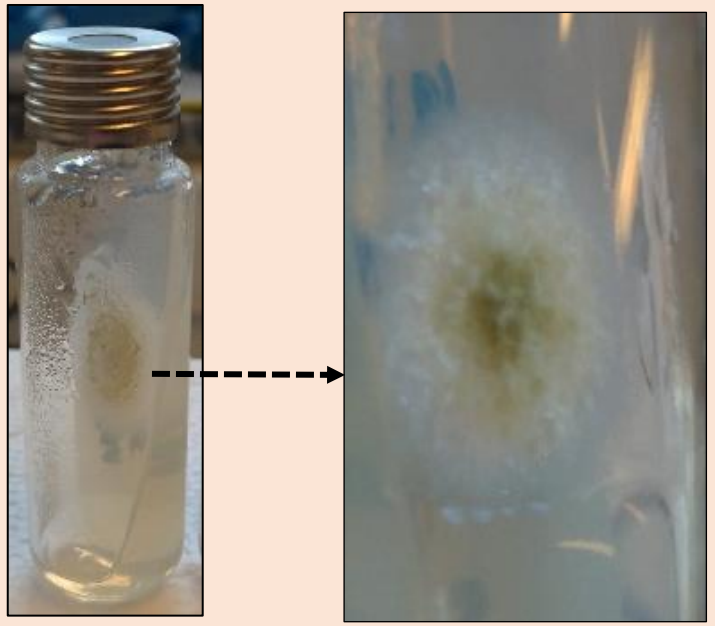
Fungal volatile organic compounds, can be used to develop aflatoxin-specific sensors?

Published work - Josselin et al. Toxins 2021

Material & Method

- ITEM 8088: non-toxigenic strain
- ITEM 8111 (toxigenic strain – aflatoxin producer)
- ITEM 8111* (non-toxigenic strain – non aflatoxin producer)

Study of the volatile organic compounds and the aflatoxin B1 on different days (3, 5, 7 and 9 days after inoculation)

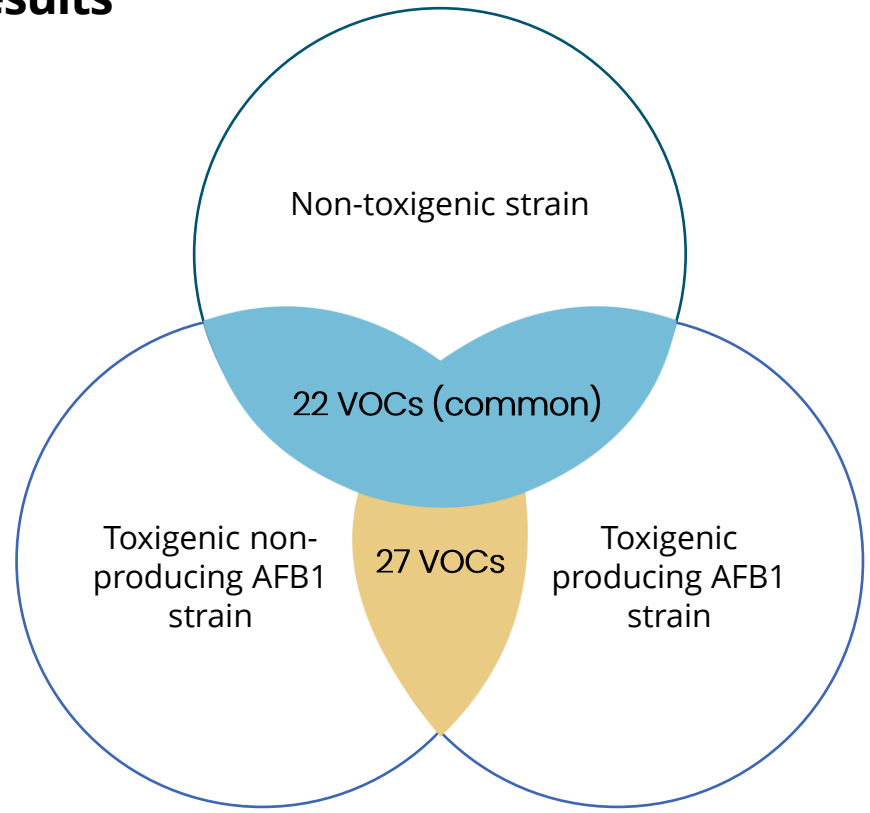


Growth of ITEM 8111 on slanted PDA medium in GCMS vials (photo)

Is there a difference between toxigenic and non-toxigenic strains ?

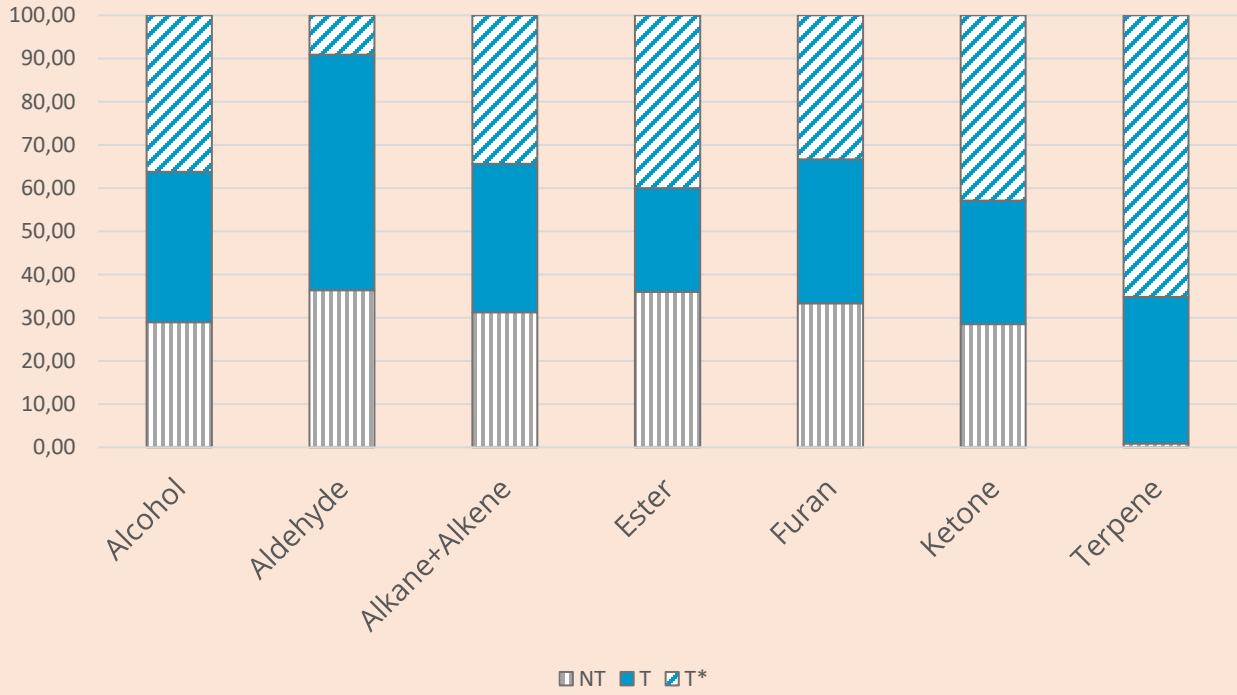
Published work - Josselin et al. Toxins 2021

Results



VOCs emitted only by one of the three strains and compounds common to two or three strains - venny 2.1.0

Distribution of the relative proportions of each family of molecules

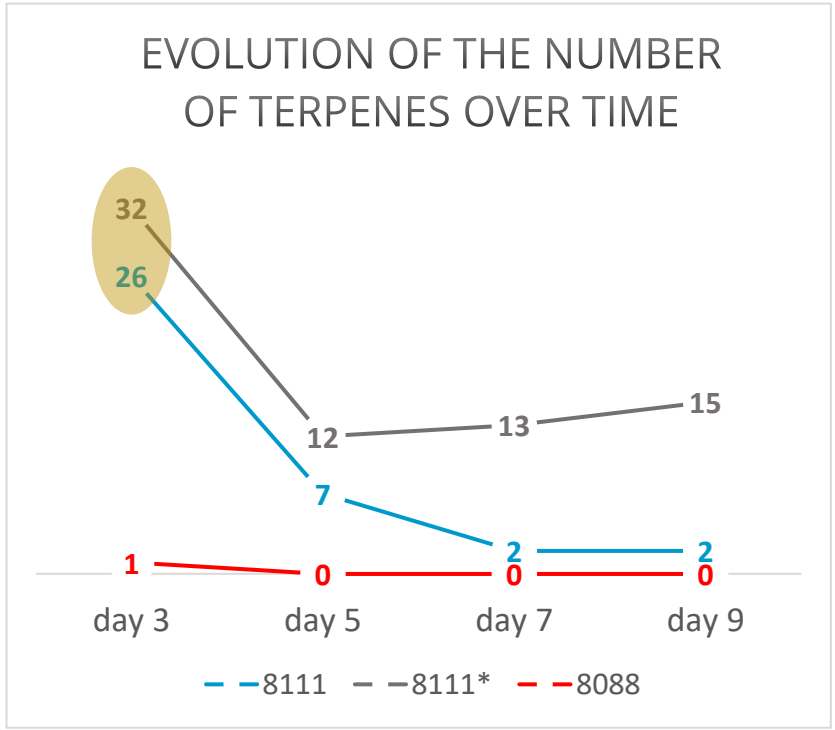


NT= ITEM 8088, T= ITEM 8111, T=ITEM 8111* no producing*

→ Difference mainly related to the terpene family

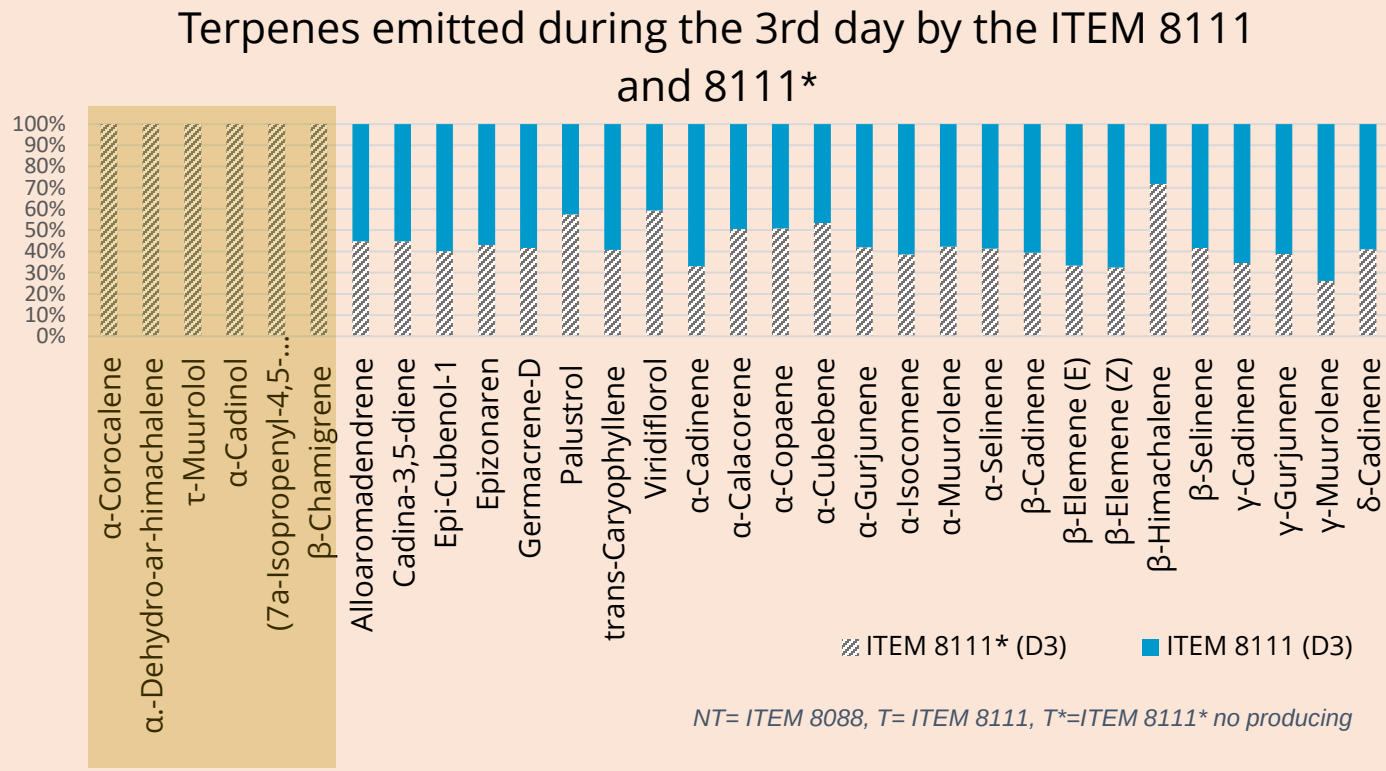
Is there a difference between toxigenic producing or not aflatoxin B1 ?

Published work - Josselin et al. Toxins 2021



Aflatoxin concentrations of the ITEM 8111 strain range from 0.07 to 2.3 µg/kg.

→ Day 3 has the greatest abundance of terpenes

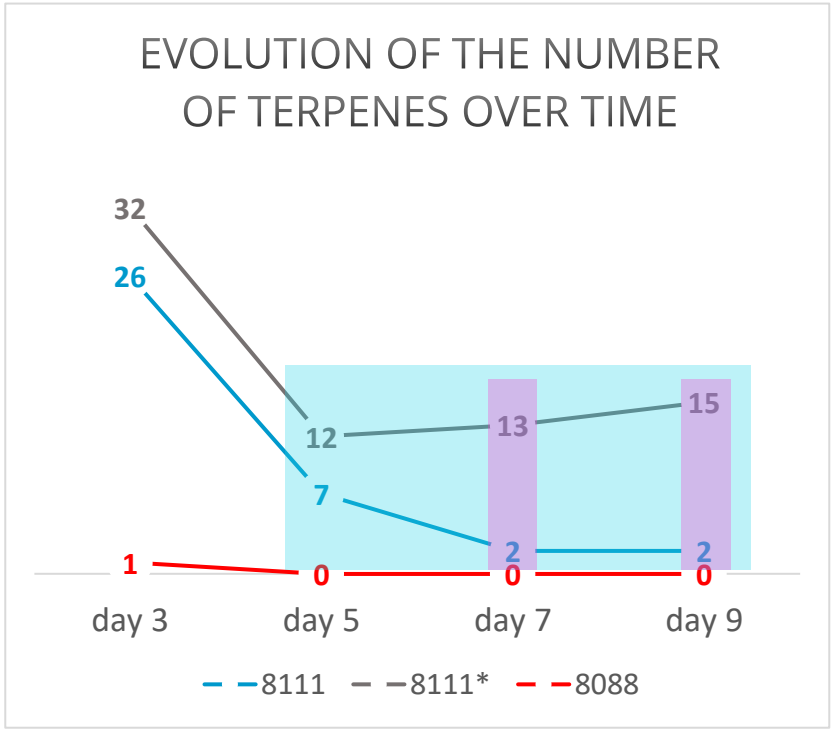


NT= ITEM 8088, T= ITEM 8111, T=ITEM 8111* no producing*

→Only 6 terpenes produced in the absence of aflatoxin B1 production

Is there a difference between toxigenic producing or not aflatoxin B1 ?

Published work - Josselin et al. Toxins 2021



Aflatoxin concentrations of the ITEM 8111 strain range from 0.07 to 2.3 µg/kg.

→ Day 3 has the greatest abundance of terpenes

→ Persistence of terpenes during the days when no aflatoxin B1 is produced

<u>Constant emission for toxigenic strains:</u>

- Epizonaren
- δ-Cadinene

<u>Persistent emission for the non-AFB1 producing toxigenic strain</u>

- γ-Gurjunene
- γ-Cadinene
- β-Selinene
- β-Himachalene

Is there a difference between toxigenic/non-toxigenic and toxigenic producing or not aflatoxin B1 ?

Published work - Josselin et al. Toxins 2021

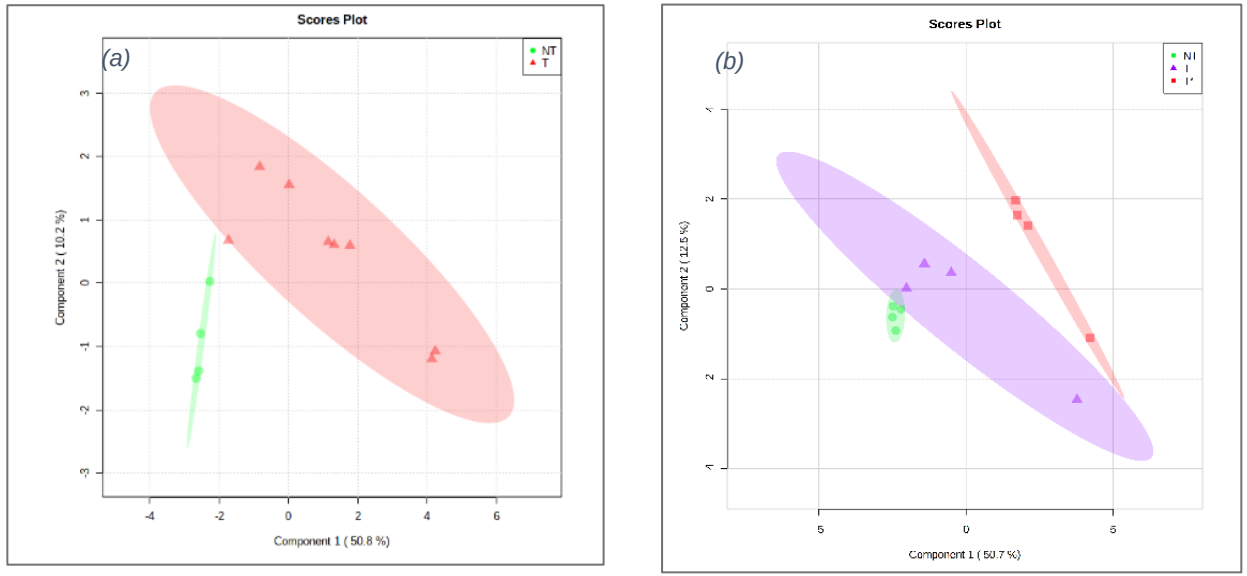


Figure 5: PLSDA applied on the data (a) of the toxigenic (T-Δ) and non-toxigenic (NT-o) strains, (b) of the aflatoxin producing (T-Δ), the non-producing (T-□) and non-toxigenic (NT-o).*

PLS-DA cross validation details:

Measure	1 comps	2 comps	3 comps	4 comps
Accuracy	0.66667	0.58333	0.66667	0.66667
R2	0.65163	0.93144	0.97249	0.99146
Q2	0.35946	0.59931	0.62235	0.61309

Toxigenic **Non-toxigenic**

→Epizonaren, δ-cadinene, germacrene-D, β-himachalene, γ-cadinene, β-selinene, γ-gurjunene, α-isocomene, α –cadinene

→ Ethyl 2-methylbutyrate and heptane

→ Styrene, β-selinene and γ-gurjunene

→ Terpenes only for the non-AFB1 strain (7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol, α.-dehydro-ar-himachalene, α-corocalene, α-cadinol, β-chamigrene, τ-muurolol

AFB1 producer **Non AFB1 producer**

Semi-quantification of volatile organic compounds of interest emitted by *Aspergillus flavus*

Published work - Josselin et al. Toxins 2021

Compound	ITEM 8111	ITEM 8111*
α-Cadinene	0.432	0.277
α-Cadinol	-	0.175
α-Isocomene	0.950	0.720
α-Muurolene	0.282	0.209
α-Selinene	1.817	1.565
β-Chamigrene	-	0.370
β-Elemene	8.897	5.181
β-Himachalene	0.737	2.590
δ-Cadinene	6.042	7.874
γ-Gurjunene	2.615	1.895
γ-Muurolene	0.769	0.381
τ-Muurolol	-	0.105
Aromadendrene	0.205	0.255
Epi-cuben-1-ol	0.311	0.360
Epizonaren	7.128	5.948
Germacrene-D	1.132	0.996
Styrene	261.75	29.8×10^6
2-Methylbutan-1-ol	2.223	0.888
3-Methylbutan-1-ol	0.934	0.440

Table of the quantified molecules

Fungal volatile organic compounds, can be used to develop aflatoxin-specific sensors?

Published work - Josselin et al. Toxins 2021

Fungal contamination

3-Methylbutan-1-ol

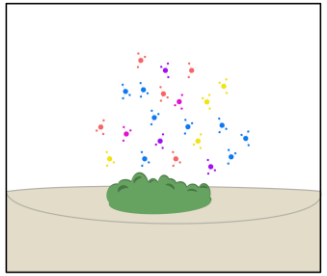
2-Methylbutanal

A. flavus contamination

δ-Cadinene

γ-Gurjunene

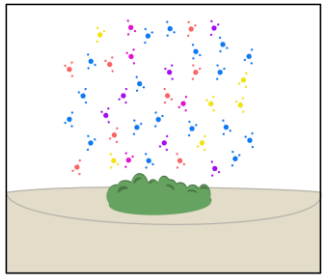
Non-toxigenic strain



Day 3

A single terpene is emitted during the 3rd day and is then no longer detected.

Toxigenic strain

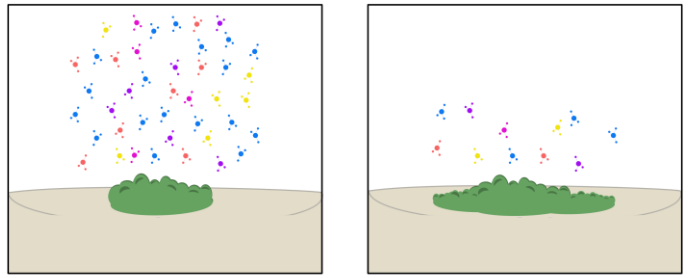


Day 3

Two terpenes are emitted overtime.

Epizonaren

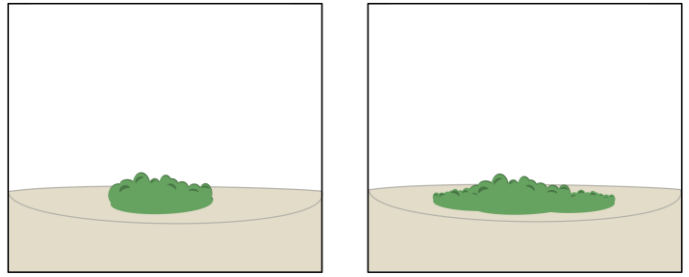
Toxigenic strain = production of aflatoxin



Day 3 Day 9

On day 3, terpene emission is higher and then decreases with time during the production of aflatoxin

Toxigenic strain = no production of aflatoxin



Day 3 Day 9

Terpenes are emitted constantly overtime.

Terpenes are specifically emitted in the absence of aflatoxin prodution.

γ-Gurjunene
γ-Cadinene
β-Selinene
β-Himachalene

τ-Muurolol
β-Chamigrene
α-Corocalene

Conclusion

Summary and perspectives

Is there a difference between a toxigenic and a non-toxigenic strain?

Yes
The main difference is related to the emission of terpenes.

Is there a difference in toxigenic strains when AFB1 is present or absent?

Yes
Terpene emission are constant over time in the absence of AFB1.

Perspectives

- ✓ Biological model (Maize)
- ✓ Increase the number

→ link between terpene production and the absence of AFB1

THANKS FOR YOUR ATTENTION.

Laurie Josselin
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