

Formation of a non-toxic metabolite of diacetoxyscirpenol by cooking of *Fusarium* contaminated potatoes



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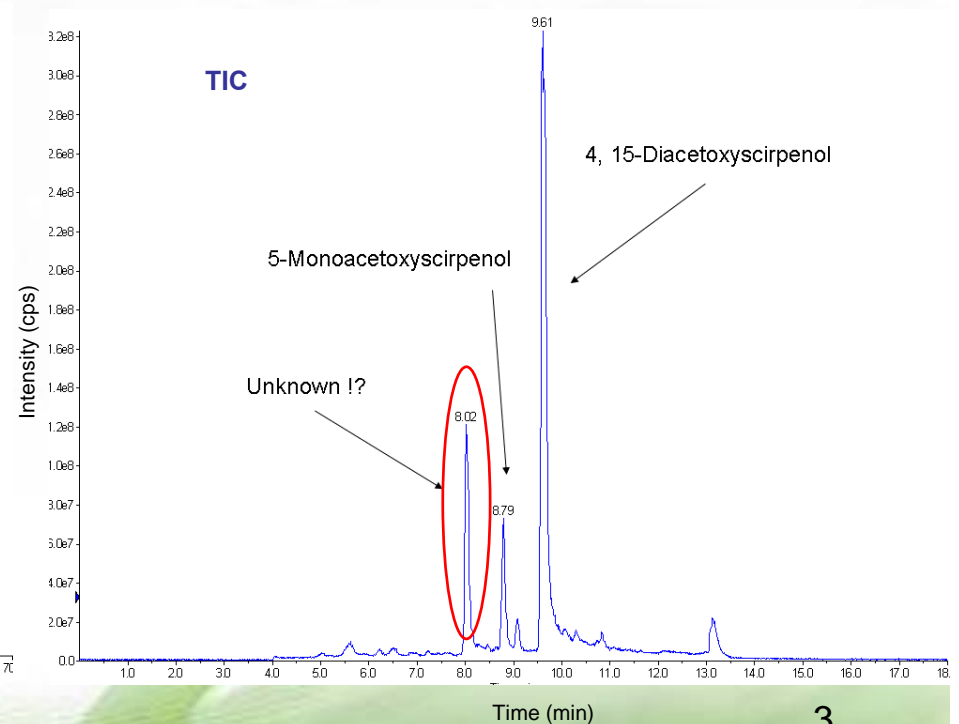
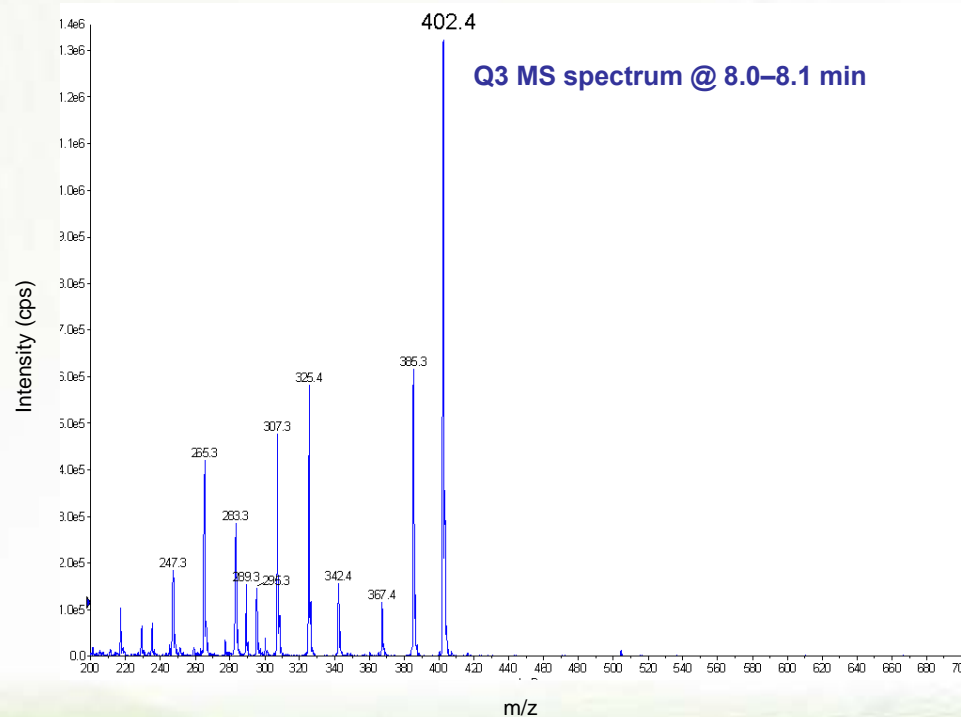
13. MOLD-Meeting
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Introduction

- Fungi of the genus *Fusarium* can produce a number of different mycotoxins that are hazardous to animals and humans
- **Trichothecenes** like 4,15-diacetoxyscirpenol **DAS** and **T-2** toxin are an important class of mycotoxins and act as potent protein synthesis **inhibitors** in eukaryotic organisms (Smith *et al.*, 1975)
- DAS can be produced by a number of *Fusarium* species, including *Gibberella pulicaris* (asexual stage: *Fusarium sambucinum*), which is a major cause worldwide of **dry-rot of potato tubers** (Boyd *et al.*, 1972, Jeffries *et al.*, 1984)
- Susceptibility of potato cultivars towards *F. sambucinum* correlates highly with the concentration of DAS (Ellner 2002)

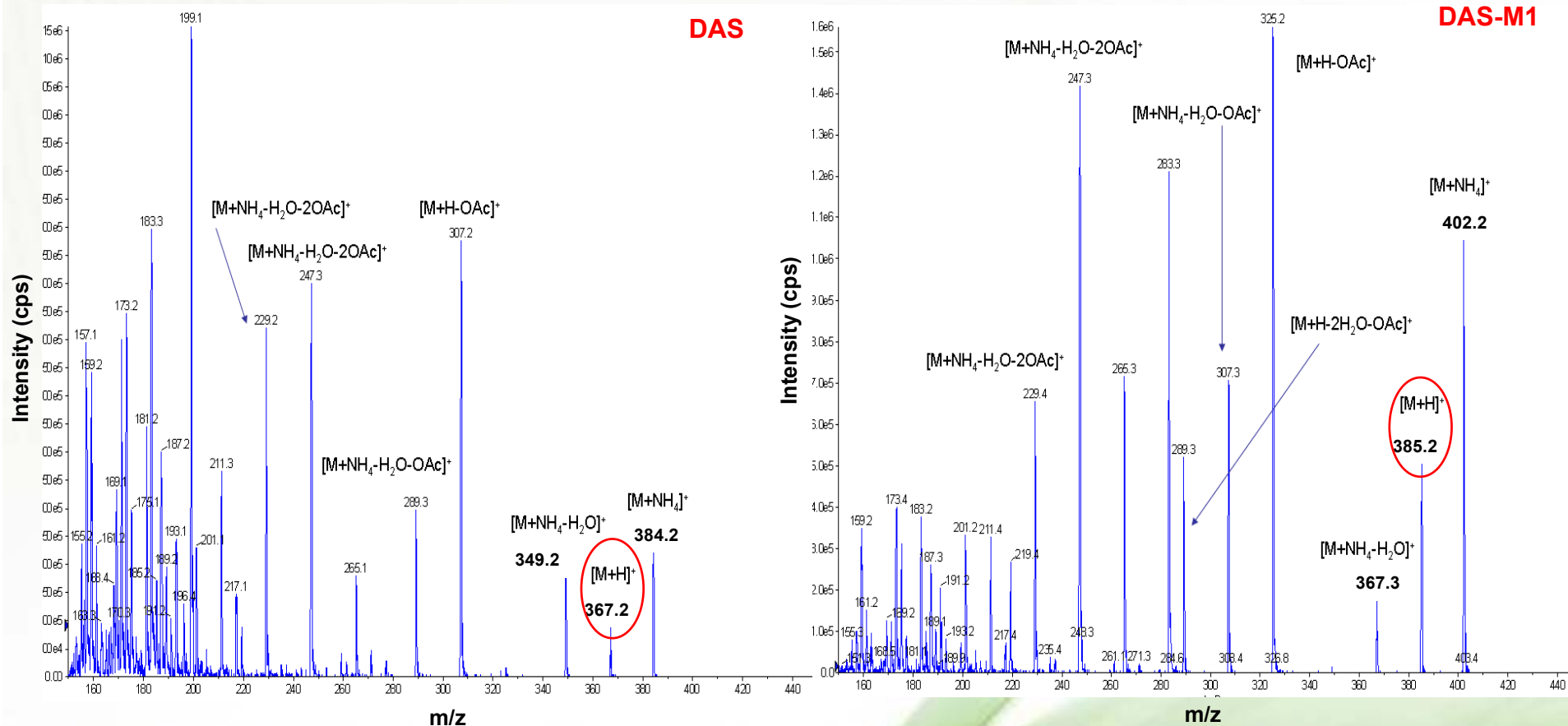
LC-MS experiments of *Fusarium sambucinum* strain MRC514

- QTrap (AB Sciex) with a Heated Nebulizer source (APCI)
- Characterization of the metabolites from *Fusarium sambucinum* MRC514 (wt) in liquid culture (2 week, 20°C)
- Q3 full-scans (positive mode, m/z 200-700)
- Chromatographic separation (Agilent 1100 HPLC, RP-C18 column)
- Linear gradient from 20% aqueous MeOH to 97%, containing 5 mM NH_4OAc



LC-MS/MS experiments

- The enhanced product ion scan mode (EPI) was used to acquire MS/MS spectra between **150** and **450** amu



LC-HR-MS experiments

- The elemental formula of DAS-M1 was confirmed by **high resolution** mass spectrometry using a **LTQ-Orbitrap** (internal mass calibration)

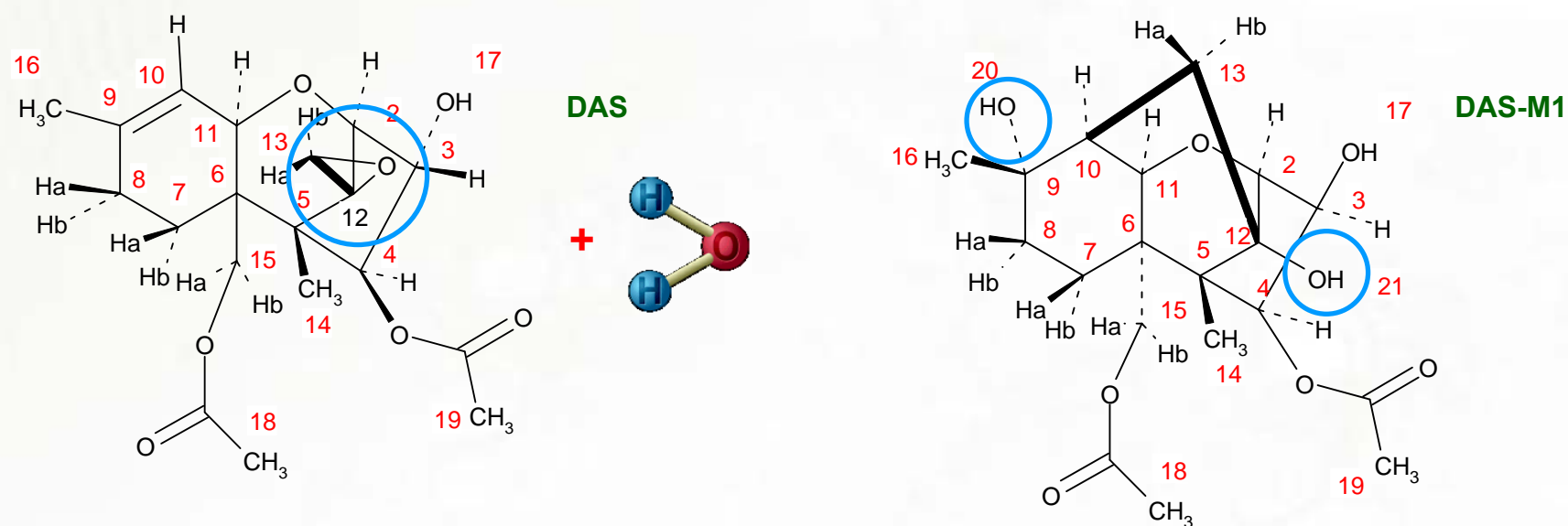


metabolite	sum formula	m/z [M+NH ₄] ⁺ Theoretical	m/z [M+NH ₄] ⁺ measured	Δm (ppm)	m/z [M+Na] ⁺ theoretical	m/z [M+Na] ⁺ measured	Δm (ppm)
DAS-M1	C ₁₉ H ₂₇ O ₈	402.2122	402.2124	+ 0.5	407.1676	407.1677	+ 0.1

Sum formula \longleftrightarrow DAS + H₂O

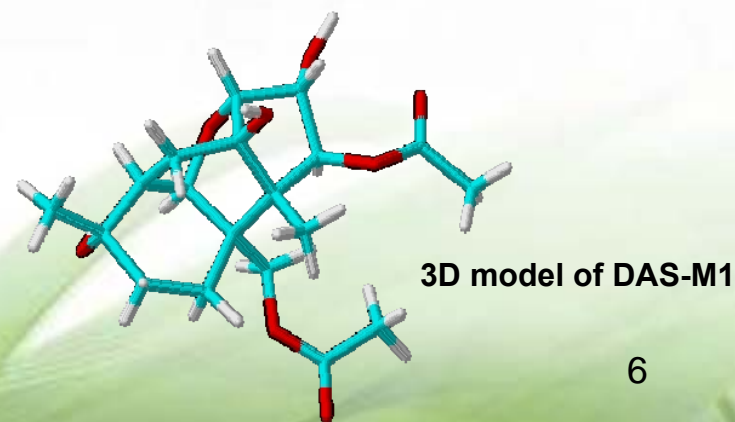
NMR characterization

^1H and ^{13}C NMR measurements revealed the following structure for DAS-M1



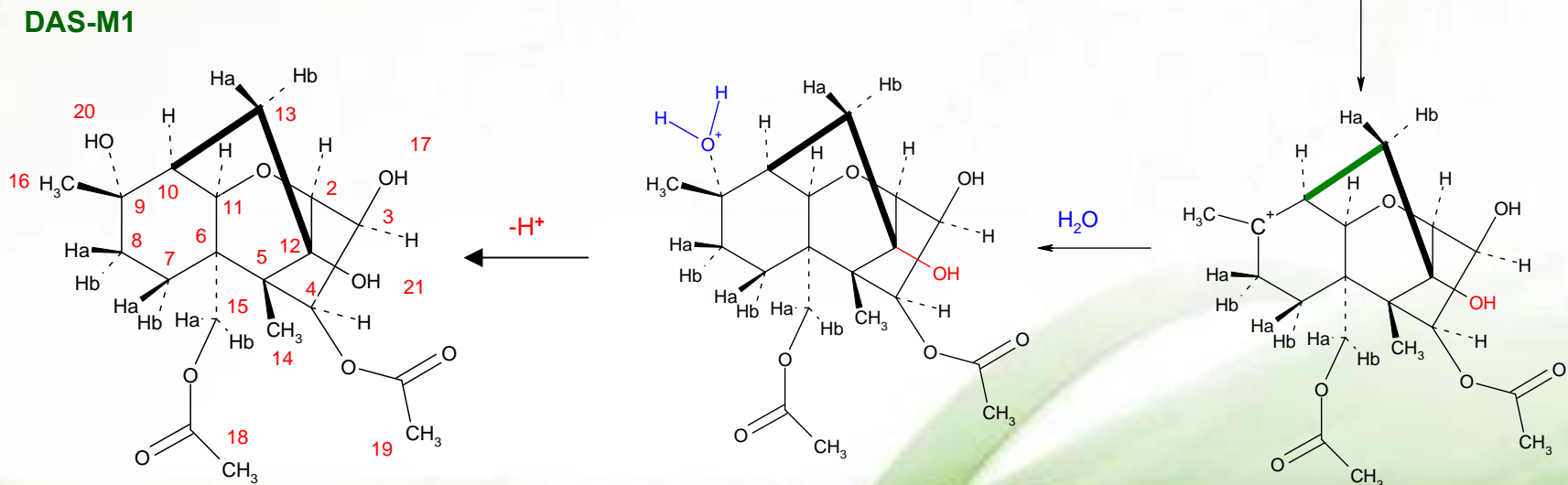
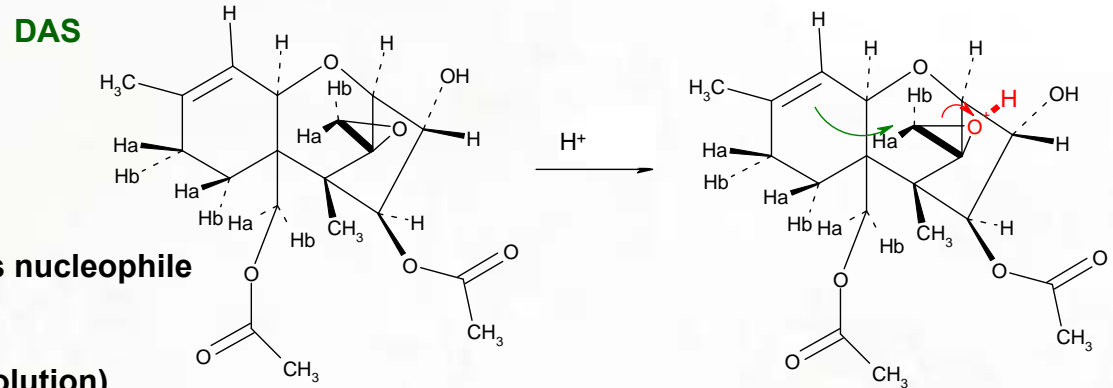
According to NMR data from DAS-M1:

- epoxide disappeared
- two additional hydroxy groups



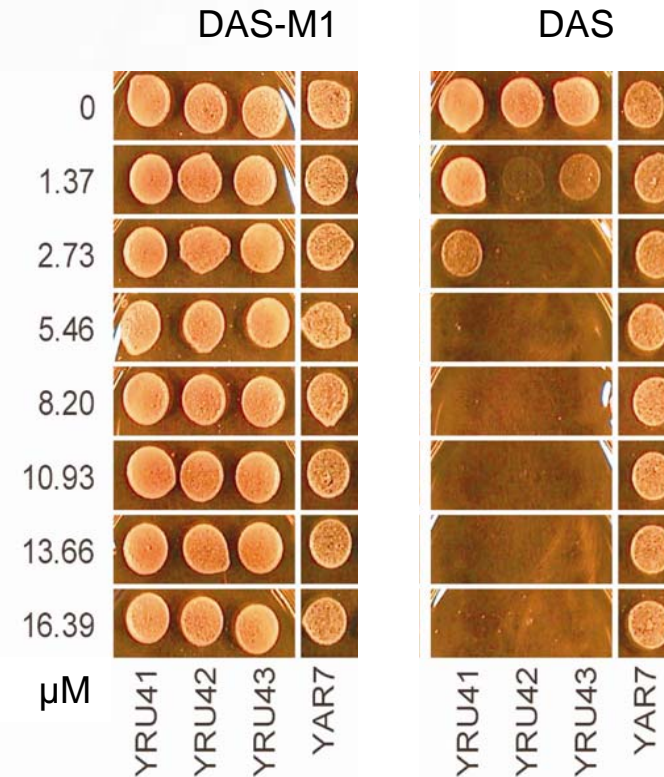
Possible pathway for the conversion of DAS to DAS-M1

- Catalyzed by **protonation**
- Intramolecular attack by the double bond as nucleophile
- Formation of a tertiary carbonium ion
- Addition of water
- Stable metabolite (**not** reversed to DAS in solution)



In vivo toxicity test

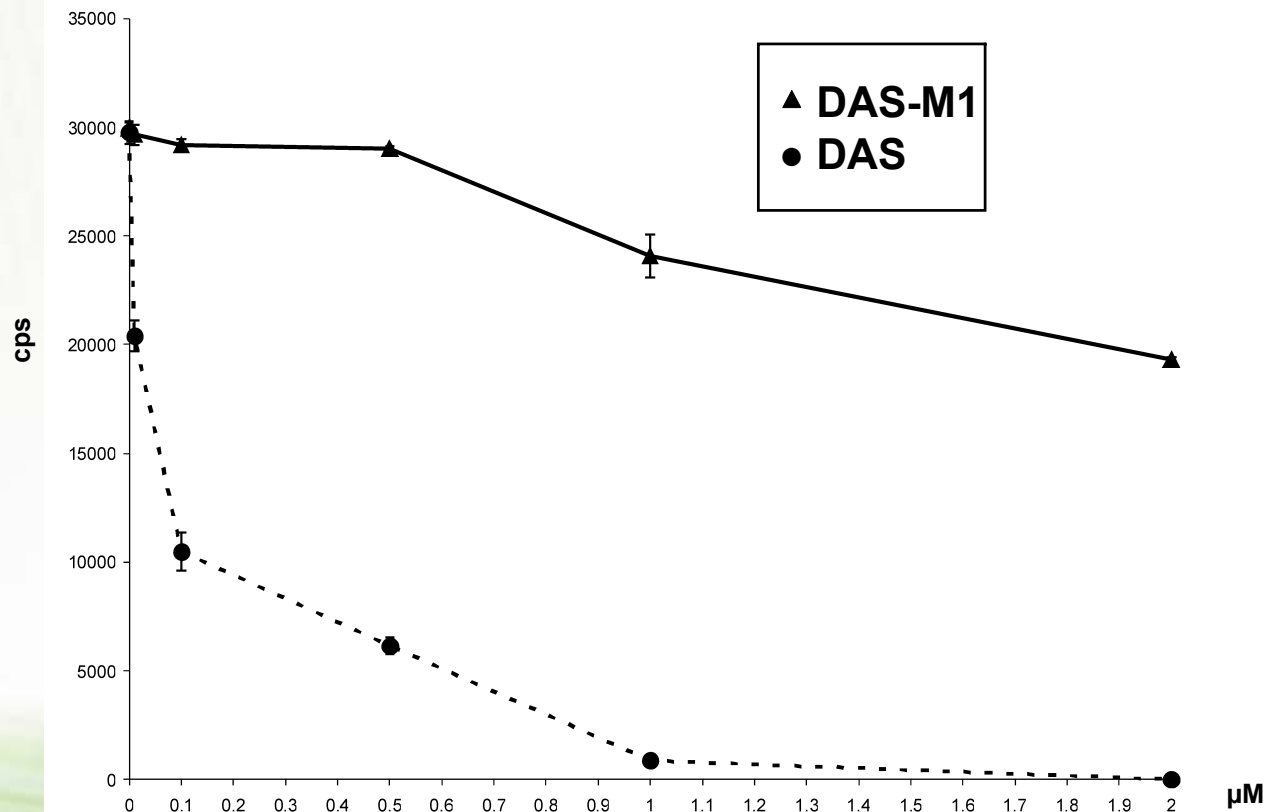
- Dilutions ($OD_{600} = 0.05$) of exponentially growing yeast cells were spotted onto YPD plates supplemented with different concentrations of DAS-M1 or DAS
- YRU41, YRU42 and YRU43 are **hypersensitive** to trichothecene mycotoxins
- YAR7 contains the trichothecene **resistance-conferring** RPL3-W255C allele (Mitterbauer *et al.*, 2004) of RPL3



Plates were incubated at 30°C for two 48 hrs

In vitro toxicity test

- At the cellular level, one of the main toxic effects of trichothecenes is the inhibition of **protein synthesis** via binding to ribosome (Rotter *et al.*, 1996)
- Based on inhibition of protein synthesis using translation of firefly **luciferase** in wheat germ extract (Promega-TNT® **Wheat Germ Extract**)
- Mixed with appropriate amounts of toxin pellets (incubated for 30 minutes at 30°C)



The amount of luciferase activity in each assay was measured in a multichannel-Luminometer (Wallac 1420 VICTOR2™)

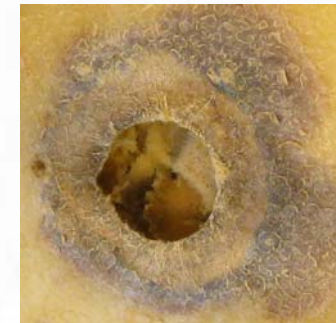
Thermal treatment *F. sambucinum* inoculated potatoes

- 25 potatoes of the cultivar Tosca (similar sizes)
- Inoculated with *Fusarium sambucinum* strain MRC514 wt
- Incubated for 14 days (in Magenta boxes) at 20°C in the dark
- 5 control potatoes were chosen randomly as **controls** (uncooked)

- **Cooking experiments:** 2 x 5 potatoes were cooked in osmosis H₂O for 1 and 4 hours respectively (100°C)

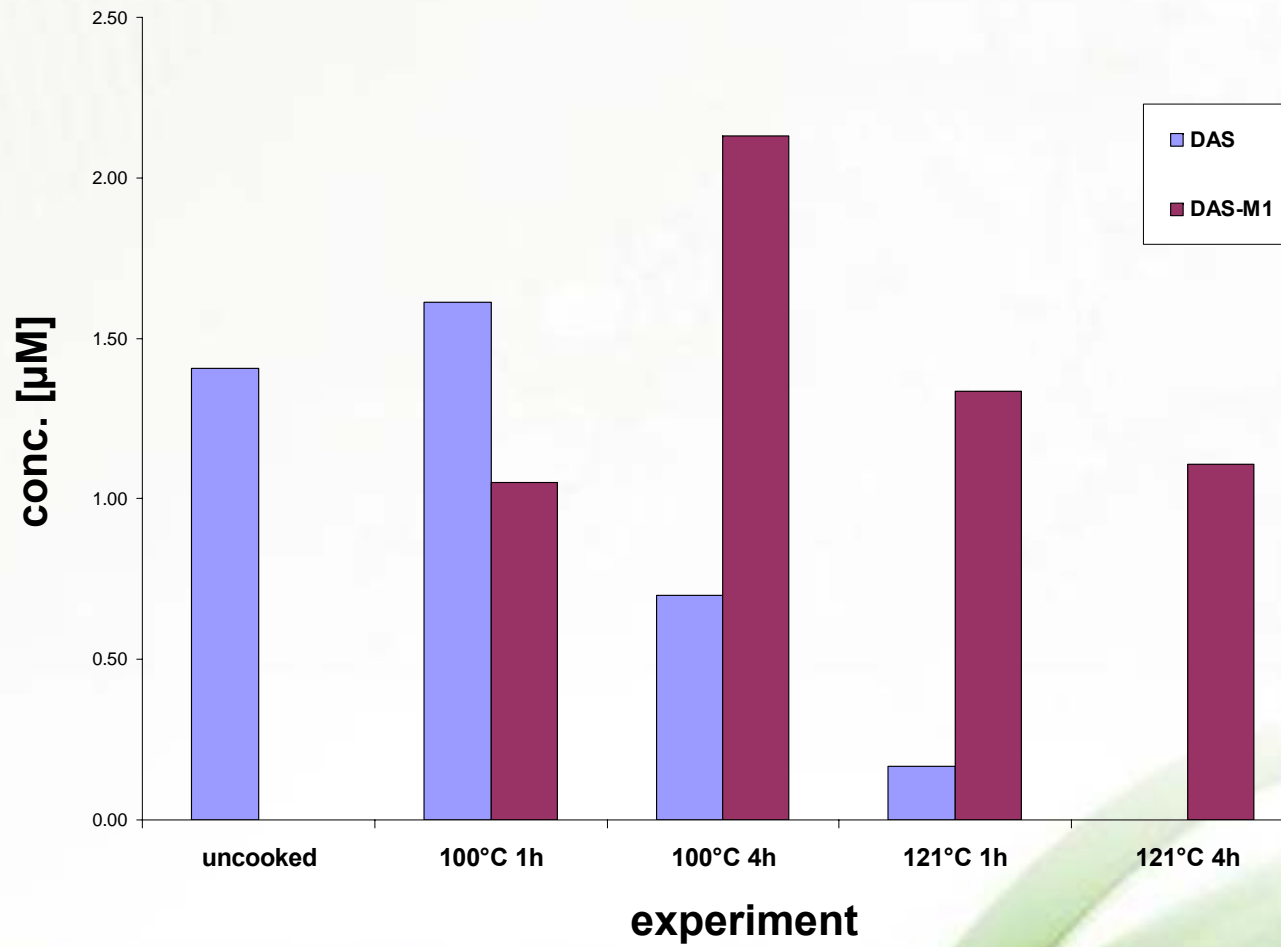
- **Pressure cooking experiments:** 2 x 5 potatoes, autoclaved for 1 and 4 hours (4 x 1h) respectively (121°C, 2 bars)

The concentration of DAS and DAS-M1 was determined by LC-MS/MS



14 days post-inoculation
with MRC514 wt

Thermal treatment *F. sambucinum* inoculated potatoes



Conclusion

- An unknown metabolite was detected in the liquid cultures of *F. sambucinum* MRC514
- The sum formula of this novel metabolite (termed **DAS-M1**) was confirmed as **C₁₉H₂₇O₈** by high resolution mass spectrometry
- DAS-M1 converted from DAS by addition of **H₂O** under **thermal treatment**
- NMR analysis of DAS-M1 confirmed the loss of the **epoxide-group**
- *In vivo* and *in vitro* toxicity tests have shown **reduced toxicity** of DAS-M1 compared to DAS (approx. 100fold)
- DAS in **potatoes** can be reduced by cooking (approx. 90% reduction at 121°C for 1h)

ACKNOWLEDGEMENTS

FWF

Der Wissenschaftsfonds

**THANK YOU FOR
YOUR ATTENTION!**