SUSINCHAIN
SUSTAINABLE INSECT CHAIN

Safety of insect rearing

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Study background and objectives

Background:

- When upscaling the insect value chain for feed and food in Europe, the safety of insects and derived products should be ensured
- Safe-by-design rather than end-of-pipe approach
- Substrates can contain a variety of microbiological and chemical hazards
- These hazards may (not) accumulate in insect larvae

Objective WP6 Susinchain: Investigate possible accumulation of microbiological and chemical hazards from substrates into insect larvae

Mycotoxin metabolisation experiments

Rationale:

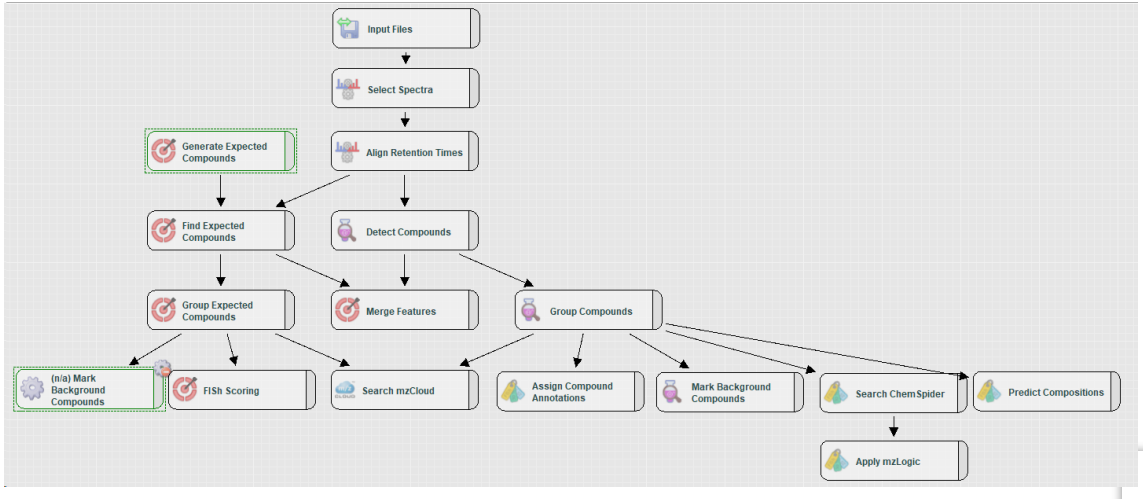
- Mycotoxins seem to be degraded by insects (e.g. Camenzuli et al., 2018)
- Metabolic pathway and toxicity of the compounds formed unknown

2 Experiments:

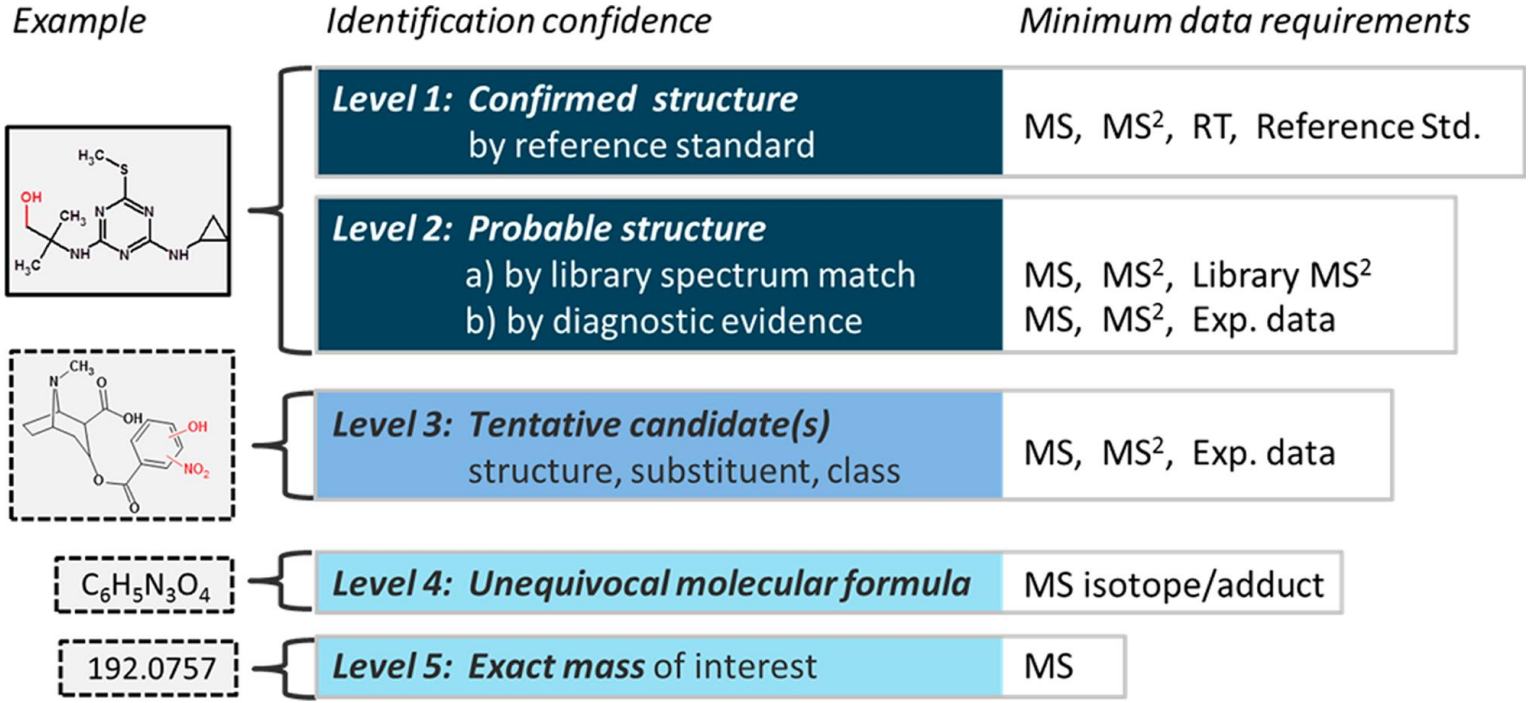
- Lesser mealworm (*Alphitobius diaperinus*):
  - only aflatoxin B1; in 3 life-stages
- Black soldier fly larvae: (*Hermetia illucens*):
  - 4 mycotoxins tested, with Isotope labelled substances

Methodology

- Rearing insects on spiked substrate
- Measure effects survival + growth (weighing)
- Analyses: LC-MS/MS (concentrations) + HRMS (secondary metabolites)
- Mass balance calculations



Methodology



Proposed identification confidence levels in High Resolution Mass Spectrometric analysis. Note: MS2 is intended to also represent any form of MS fragmentation (e.g., MSe, MSn). (source: Schymanski et al. (2014)

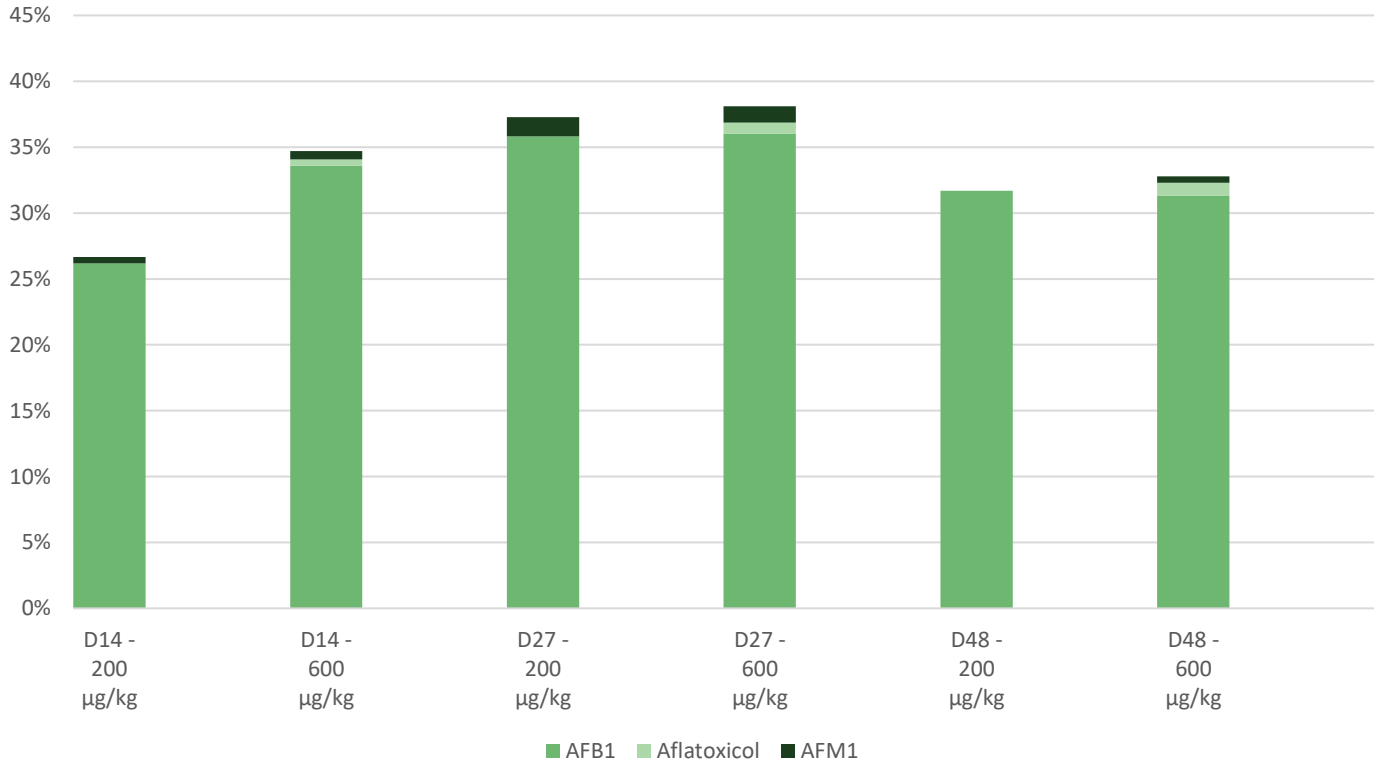
Mycotoxin experiment 1: LMW – methodology

- Substrate spiked with aflatoxin B1 at 2 different concentrations (200 and 600 µg/kg) + control
- Experiment at Protifarm with LMW, with 3 insect phases: larvae (D15), prepupae (D28), beetles (D49)
- Chemical analyses: substrate, larvae and residual materials
  - LC-MS/MS for all known metabolites, and high resolution mass spectrometry (HR-MS) analyses for possible unknown metabolites
  - Mass balance evaluation

LMW – First results

- No differences growth/survival of LMW, every life stage
- Known metabolites (Aflatoxin B1, M1, P1, Q1, and aflatoxicol):
  - **Larvae** (all 3 stages x 2 concentrations): all <LOQ
  - **Frass**: aflatoxicol + AFM1 were slightly >LOQ for 600 µg/kg. AFB1 was ~0.6 of feed concentration.
- Secondary metabolites:
  - Larvae (all 3 stages, concentrations): no minor metabolites identified
  - Frass: tentative identification of additional +O and +H2SO4 metabolites

Preliminary mass balance LMW



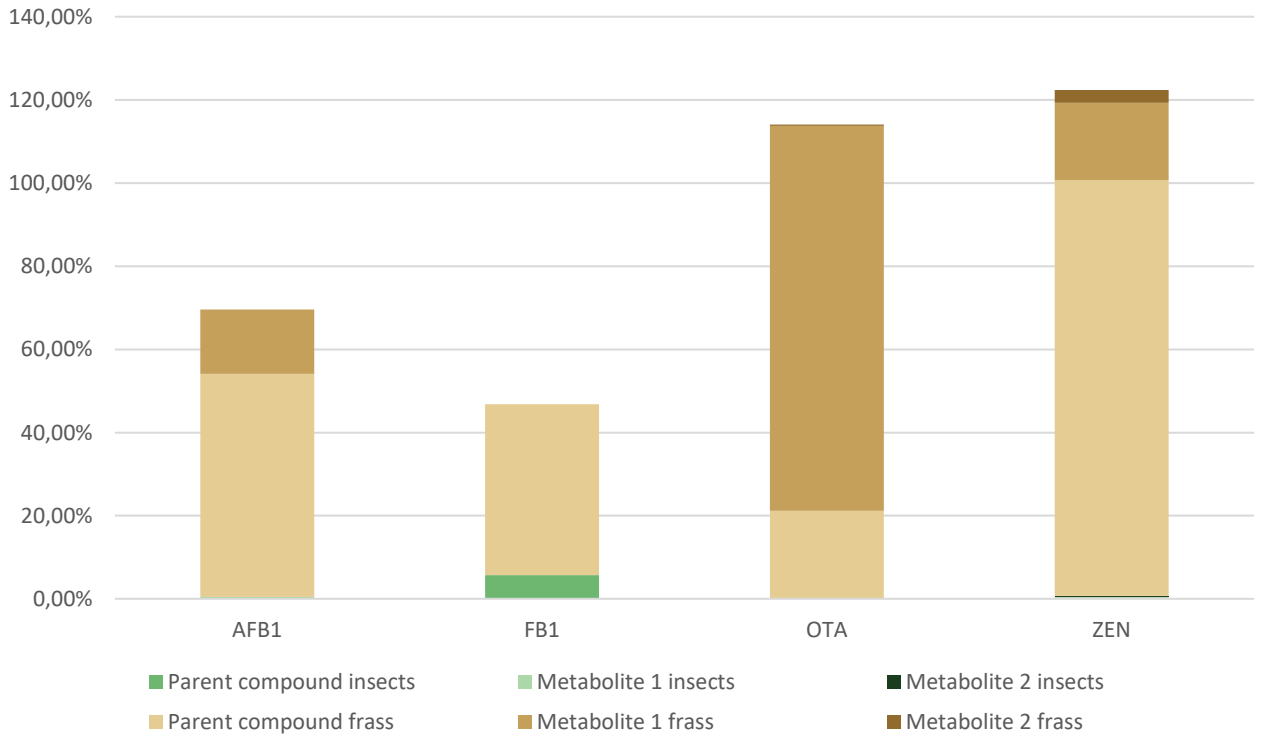
Mycotoxin experiment 2: BSFL – Methodology

- Mycotoxins: AFB1, Fumonisin 1 (FB1), ochratoxin A (OTA), Zearalenone (ZEN)
- Spiked in isotope labelled form + non-labelled forms
- Chemical analyses substrate, larvae and residual materials (mass balance)
  - Secondary metabolites can be determined by comparing isotope labelled samples vs. not labelled
  - LC-MS/MS and HR-MS
- Experiment performed at Bestico: larvae D7-14

BSFL – preliminary results

- No significant effects yield + survival
- **Larvae**:
  - All 4 parent compounds quantified, but low concentrations
  - Bio-accumulation mycotoxins very low (~0.01), only FB1 around 0.4-0.5.
  - Known metabolites found: AFP1, Otα, α-ZEN, β-ZEN.
- **Frass**:
  - Higher concentrations parent compounds than in larvae
  - Known metabolites: same as larvae + OTB.
  - Certain secondary ZEN metabolites, but unclear which ones

Preliminary mass balance BSFL



Discussion

- No significant effects survival + growth for LMW and BSFL, in line with previous studies
- Absent (LMW) or very low (BSFL) concentrations of mycotoxins in larvae
- Some differences metabolites found between LC-MS/MS and HRMS: HRMS lower sensitivity?
- Mass balance still incomplete: toxicity study *in vitro?*

Conclusions and take home messages

- With the emerging insect value chain, safety needs to be addressed on safety-by-design approach
- Needs case specific focus, per insect x substrate x hazard
- Possible break-down mechanisms of contaminants by insects; these can provide great opportunities, but first need further investigation

People involved

- WFSR: Nathan Meijer, Rosalie Nijssen, Ed Boers, and others
- Bestico: Lisa Zoet
- Protifarm; Marlou Bosch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