Daphnia magna as an indicator for aquatic species in the non-target risk assessment of genetically modified maize

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Background & objective

- Environmental risk assessments for genetically engineered maize producing insecticidal proteins from *Bacillus thuringiensis* (Bt) include non-target studies with aquatic species.
- Maize pollen and detritus can enter streams and expose aquatic species.
- *Daphnia magna* is commonly used as a surrogate species for testing.
- No standardized test protocols for plant material containing insecticidal proteins with oral activity exist.
- **This study aims to separate non-target effects of the Bt proteins from effects of the plant background**

Maize material as food for *D. magna*

- *D.* magna can survive, grow, and reproduce on maize pollen, flour, and leaf powder (Fig. 1).
- Performance on maize material is poorer than on green algae, indicating nutritional stress.

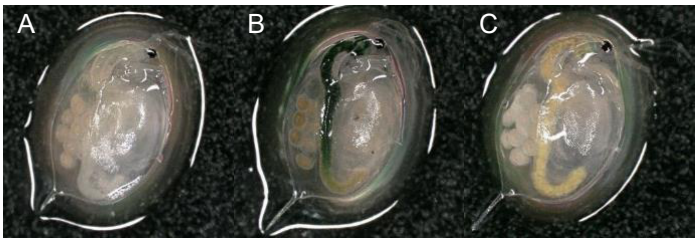

Fig. 1: *D. magna* ingested maize A) flour, B) leaf powder, and C) pollen

Natural range of variation among maize varieties [1]

- Reproductive parameters show much variability with highest to lowest ratios between 1.4 and 2.5 for total offspring (Fig. 2).
- Growth parameters (development time, weight) are less variable.

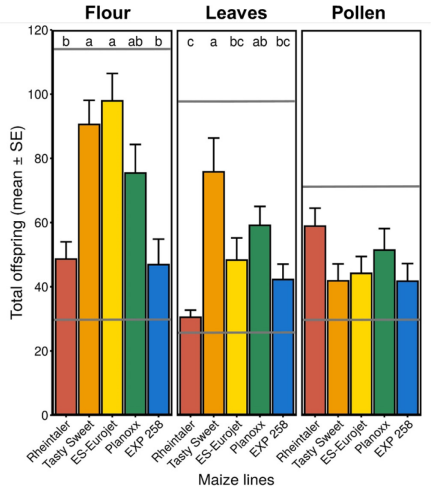

Fig. 2: Total offspring of *D. magna* fed with flour, leaves, or pollen from different maize varieties. Grey lines indicate natural range of variation based on 95% confidence intervals of highest and lowest values

Effects of stacked Bt maize on *D. magna* [2]

- Adverse effects observed for Bt maize flour, originating from different production fields and years, but not for leaves or pollen, produced from plants grown concurrently in the glasshouse (Fig. 3).
- Leaves contained 8-10× more Bt protein than flour
- Most values within the natural range of variation

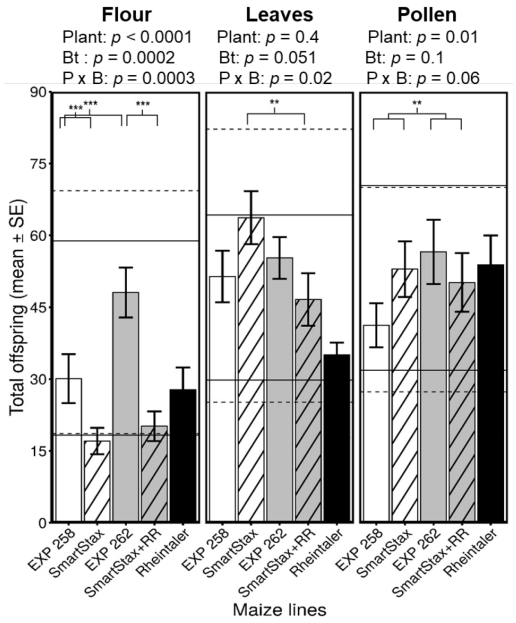

Fig. 3: Total offspring of *D. magna* fed with flour, leaves, or pollen from two SmartStax Bt maize hybrids, the nearest non-Bt counterparts, or the Swiss landrace Rheintaler. Solid and dashed lines indicate the in-study [2] and the external [1] range of variation, respectively.

Conclusions & implications for risk assessment

- Plant background effects influence the results of non-target arthropod studies
- Effects of Bt proteins can be separated from effects of plant background by using
  - the same Bt trait in several plant backgrounds
  - different plant materials containing Bt proteins
- Establishing the natural range of variation helps to judge on the biological relevance of observed effects

References:

[1] Chen Y, Romeis J, Meissle M, 2021. Performance of *Daphnia magna* on flour, leaves, and pollen from different maize lines: Implications for risk assessment of genetically engineered crops. Ecotox Environ Safety 212: 111967. https://doi.org/10.1016/j.ecoenv.2021.111967

[2] Chen Y, Romeis J, Meissle M, 2021. Addressing the challenges of non-target feeding studies with genetically engineered plant material – stacked Bt maize and *Daphnia magna*. Ecotox Environ Safety 225: 112721. https://doi.org/10.1016/j.ecoenv.2021.112721

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