Non-target studies with plant material – lessons learned from Bt maize and *Daphnia magna*

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Abstract

Previous studies using plant material as test substance reported adverse effects of genetically engineered maize that produces insecticidal Cry proteins from *Bacillus thuringiensis* (Bt) on the water flea *Daphnia magna*. One challenge of such studies is to separate effects of the introduced Cry protein from plant background effects or random effects. One option to address this challenge is to use the Bt trait in different plant backgrounds. Another option is to use different food materials with varying Cry protein concentrations from the same plant.

We illustrate this with a study that investigated effects of flour, leaves, or pollen from stacked *Bt* maize that produced six *Bt* proteins (SmartStax) in two plant backgrounds on life table parameters of *D. magna*. Furthermore, the natural range of variation in the response of *D. magna* was estimated with five conventional maize lines with different properties.

We suggest that effects derived from the insecticidal proteins should be consistent and should correspond to the concentrations in the different lines and tissues. Otherwise, effect are likely plant-related or artefacts. Low nutritional quality of the food material for *D. magna* is likely to increase the probability of artefacts. In any case, several experimental repetitions are recommended, the plant material should be produced under comparable environmental conditions, and the non-Bt controls should be the nearest relatives to the used Bt lines. To assess the biological relevance of detected differences between a particular genetically engineered (GE) plant and a non-GE control, data from multiple unrelated conventional varieties are valuable. Such data allow the definition of a range of natural variation, while assuming that the conventional lines pose no environmental harm. This can be done by discussing historical data and/or by including additional conventional lines in the experiments.

Key words: aquatic organisms, environmental risk assessment, food ecology, *in-planta* assays, non-target effects