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http://sites.google.com/site/ngriffith1/corndam3-large.JPG

Fig.1: Maize detritus washed into stream



Photo by Keith Weller  
http://commons.wikimedia.org/wiki/File:Ostrinia\_nubilalis.jpg

Fig.2: European corn borer, *Ostrinia nubilalis*

## Introduction

Transgenic plants are used since the nineties and are cultivated worldwide. The amount of area under cultivation increases every year. Bt maize (*Zea mays*) is genetically modified to produce the toxin Cry1Ab of *Bacillus thuringiensis* against the lepidopteran European corn borer (*Ostrinia nubilalis*, Fig. 2). By wind drift or run-off, chopped maize material can enter surface waters (Chambers *et al.*, 2010; Fig. 1). The Bt protein in submerged plant material is often regarded as very labile, due to rapid decaying processes. Thus, exposure and release of the Bt protein seems to be negligible. However, up to now the degradation of the Cry1Ab

protein in decaying submerged corn material is insufficiently investigated. Therefore a fate study by use of decaying plant material in small microcosms was conducted to answer the following questions:

- How fast does the Bt protein Cry1Ab in corn leaves disappear under aquatic conditions (half life)?
- Are there differences in leaf components (N, C<sub>org</sub>, cellulose, lignin, polyphenols) between Bt maize and Non-Bt maize during decomposition in water?

## Methods

### Test design & sampling

- Leaf discs (2 cm ø, without leaf-vein) of Bt maize PAN 6Q-321B and its isoline maize PAN 6Q-121 were used
- Leaf discs were exposed for 23 days in stream water (5 L, aerated)
- Mean water temperature: 17,5°C (±1°C)
- Light-dark cycle: 12:12h
- pH, O<sub>2</sub>, and electrical conductivity were measured daily
- Samples were frozen at -20°C and freeze-dried after

### Analyses

- Bt protein Cry1Ab: ELISA-test (Hilbeck, Ecostrat)
- Lignin and cellulose: according Van Soest (1963) and modified acc. Gessner (2005)
- Phenols: according Bärlocher and Graça (2005)
- Carbon and nitrogen content: Elementar Vario el III analyzer (Hanau, Germany) acc. DIN-ISO-10694 (1995)

### Statistics

- Mann Whitney U-test (PRISM, V. 4)

## Results

During the investigation period of 23 days, the Bt protein concentration decreased with a half life of 3.4 days (C.I. 95%: 2.1-8.6; r<sup>2</sup>: 0.65; Fig. 3).

No significant differences were detected for the concentration of total protein between Bt maize and Non-Bt maize (U-test, p ≤ 0.05).

Leaf components like cellulose, polyphenols, lignin, dry weight, ash, and carbon showed also no significant differences between Bt maize and Non-Bt maize (U-test, p ≤ 0.05).

The carbon content was constant during the study (data not shown).

The nitrogen content differed significantly between Bt maize and Non-Bt maize at the beginning. During the experiment nitrogen increased in both maize types (U-test, p ≤ 0.05; Fig. 4). Accordingly the C:N ratio decreased (Fig. 5).

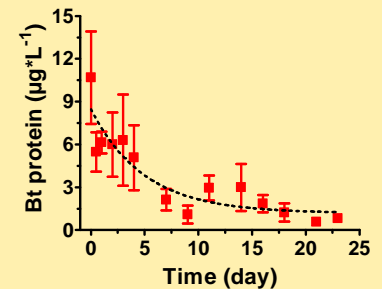


Fig. 3: Mean degradation rate of the Bt protein in maize leaves (n=4, SD)

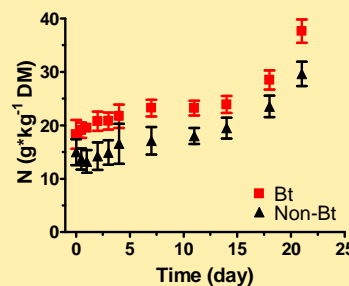


Fig. 4: Nitrogen content per dry matter in a 21 day trend (n=4, SD)

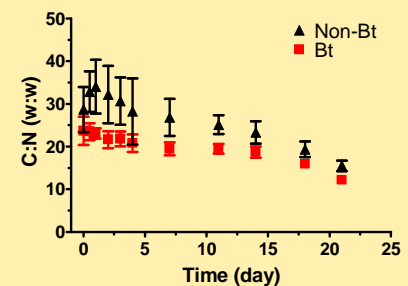


Fig. 5: C:N - ratio in a 21 day trend (n=4, SD)

## Discussion & Conclusion

The increasing N content in decaying Bt and Non-Bt leaves may result from the proliferating biofilm, growing on the maize litter. The observed C:N ratios (Fig. 5) are in the same order of magnitude as published by Griffiths *et al.* (2009) for different maize hybrids. Here, in both maize types the shift of the C:N ratios is also driven by increasing N levels (Fig. 4).

The study at hand showed that the Bt protein was quantifiable even after 23 days in decaying and submerged maize leaves. The calculated

half life of the Bt protein reached 3.4 days. In contrast to that, Griffiths *et al.* (2009) published a half life of less than 1 hour. After 70 days Griffiths *et al.* detected 20 % of the initial content, whereas in this study 5-8 % were quantified after 21-23 days. Both studies reveal that an exposure of Bt protein in the aquatic environment is given to some extent. Consequently, further research activities should focus on potential harm to stream organisms as proposed by Rosi-Marshall *et al.* (2007).

### Acknowledgement

We would like to thank Bonny Alischer and Ina Schmieding for chemical analyses and their assistance during sampling. We thank Stefan Loth for the technical support. We were also indebted to the working group of Angelika Hilbeck (ETH Zürich; Ecostrat), Gabriele Weiß (Ecostrat), and Gert Dudel (TU Dresden) for their cooperation and expertise.

### References

- Chambers, C.P., Whiles, M.R., Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Griffiths, N.A., *et al.* (2010). Responses of stream macro-invertebrates to Bt maize leaf detritus. *Ecological Applications*, 20(7), 1949–1960. Eco Soc America.
- Graça, M.A.S., Bärlocher, F., & Gessner, M.O. (2005). *Methods to Study Litter Decomposition - A Practical Guide* (pp. 1-314). Springer
- Griffiths, Natalie A., Tank, Jennifer L., Royer, Todd V., Rosi-Marshall, Emma J., Whiles, Matt R., Chambers, Catherine P., *et al.* (2009). Rapid decomposition of maize detritus in agricultural headwater streams. *Ecological applications* : a publication of the Ecological Society of America, 19(1), 133-142
- Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Whiles, M.R., Evans-White, M., Chambers, C., *et al.* (2007). Toxins in transgenic crop byproducts may affect headwater stream ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, 104(41), 16204-8.
- Van Soest, P.J. (1963). Use of detergents in the analyses of fibrous feeds - II. A rapid method for the determination of fiber and lignin. *J. Assn. Offic. Agr. Chem.*, 46, 829-835.