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Managing weeds in herbicide-tolerant GM maize for biological control enhancement.

Ramon Albajes, Belén Lumbierres, Xavier Pons 1-8

Abstract: Deployment of transgenic herbicide-tolerant maize that allows post-emergence treatment with broad-spectrum herbicides may lead to changes in the composition and abundance of weed flora. The consequences of these changes on maize arthropods and particularly on insect pest natural enemies are studied in this work. Weeds, insect herbivores and their natural enemies were monitored in maize plots treated twice with glyphosate (V4 and V8) in comparison with plots treated once with conventional pre-emergence herbicides. Plots were sampled by visual observation, pitfall and yellow sticky traps during two consecutive years (2007 and 2008). In spite of the significant differences recorded in weed abundance between the two herbicide treatments, there were very few significant differences in the arthropod groups monitored, in contrast with results of a previous study comparing plots with two glyphosate treatments (as in the present work) and with no herbicide treatment in order to identify the most responsive arthropod to weed abundance alteration. It seems that when maize weed abundance is not drastically altered, populations of arthropod herbivores and natural enemies are not greatly affected. However, more studies are needed to determine the potential impacts of modifying herbicide use on arthropods and particularly on conservation biological pest control.

Staphylinids (Coleoptera: Staphylinidae) in genetically modified maize ecosystems: species densities and trophic interactions.

Adalbert Balog, Ágnes Szénási, Dóra Szekeres, József Kiss 9-15

Abstract: In this paper we present results on rove beetles (species, guilds, densities) from a three-year field experiment conducted in Hungary with Bt maize (MON810, Cry1Ab) and its corresponding near isogenic variety. According to our results there were no significant differences in density for species belonging to the non-aphidophagous predator and parasitoid guilds; however the aphidophagous guild showed differences between the two maize varieties in some years. The abundance of aphidophagous staphylinids did not correlate with the total annual and monthly *Rhopalosiphum padi* density (its prey) in the same year but higher aphid density in one year may have influenced the larval development of rove beetles in that year influencing beetle densities in the following year.

Reduction of damage caused by *Ostrinia nubilalis* Hbn. in south-eastern Poland in 2007 through the cultivation of transgenic maize varieties.

Paweł K. Bereś..... 17-21

Abstract: The objective of the study carried out in 2007 was to evaluate the susceptibility of selected varieties of Bt-transgenic maize (DKC3421YG and Bacilla; transformation event MON 810) and their conventional types without the Cry1Ab protein (DKC3420 and Clarica) to damage caused by the European corn borer (*Ostrinia nubilalis* Hbn.) under the conditions of south-eastern Poland. The study confirmed the high resistance of the Bt maize varieties to damage caused by the caterpillars of this moth. The use of Bt varieties allowed the average reduction of damaged plants in location A (Podkarpackie voivodeship) by 95.2% and damaged cobs by 98.1%. In

location B (Lubelskie voivodeship) the number of transgenic plants damaged by caterpillars was reduced by 99.4% and that of cobs by 98.9%. In addition, the level of plant damage was lower: for Bt plants, i.e. the plants hosted significantly lower numbers of caterpillars, and a lower number of holes gnawed by them was observed in comparison to the corresponding non-transformed control maize varieties.

A perspective on problem formulation and exposure assessment of transgenic crops.

Keri L. Carstens, Katrina Hayter, Raymond J. Layton.....23-30

Abstract: Risk assessment is a science-based decision making process. When risk assessment follows an established framework, it allows for transparency, predictability, and consistency in the regulatory process. Problem formulation is a critical first stage in the risk assessment process; it involves 1) the characterization of the transgenic plant and receiving environment, 2) definition of harm, 3) identification of potential exposure pathways or potential harm, and 4) establishment of assessment endpoints to evaluate the potential for harm based on the plant's characteristics. Risk to non-target organisms can be defined as the co-occurrence of hazard (or toxicity) and exposure. Exposure characterization has often been overlooked in the literature regarding the risk assessment of transgenic plants, with many recent publications focusing only on the hazard portion of the risk assessment equation. Exposure assessment informs the risk assessment and assists in the determination of which types of non-target organisms should be tested in hazard characterization. The purpose of risk assessment is to provide a framework for efficient decision-making, rather than to generally increase scientific knowledge; therefore data collection for risk assessment must be directed toward answering specific questions identified in the problem formulation stage.

Absence of Cry1Ab resistance in a Spanish *Ostrinia nubilalis* population from an infested greenhouse.

Cristina M. Crava, Yolanda Bel, Juan Ferré, Baltasar Escriche.....31-36

Abstract: Transgenic corn expressing *Bacillus thuringiensis* (Bt) toxin Cry1Ab has been planted in Spain to control corn borers as *Ostrinia nubilalis* (Hübner), since 1998. Indeed, 79 thousand hectares have been planted to Bt-maize in different Spanish areas in 2008. The high selective pressure may produce the development of resistance in populations of the target pest, decreasing the effectiveness of the transgenic crop. The selection may be even higher in neighbour crops in which *O. nubilalis* is a pest and where it is controlled by conventional Bt-spray products containing Cry1Ab.

A sample of *O. nubilalis* was collected from a greenhouse in south-eastern Spain that was suffering high levels of infestation after repeated treatments with different Bt products. Insects were brought and reared in our laboratory and their susceptibility to activated Cry1Ab toxin, Cry1Ab protoxin and the Bt standard HD-1-S-2005 product was tested. As a susceptible control, insects from France kept in the laboratory for more than 10 years without exposure to Bt were used. The "effective growth inhibition" was recorded seven days after treatment and accounted for both dead larvae and larvae which not passed the first instar. PROBIT analyses of the data revealed no significant different response between the strains to activated Cry1Ab toxin and to HD-1-S-2005. Cry1Ab protoxin showed 7-fold lower activity in the laboratory strain when compared to the field strain. These data suggest an absence of a relevant shift in the resistance to Cry1Ab in the insects from the field strain as compared to the laboratory one, and point to a deficient Bt product application in the greenhouses.

EFSA's activities on the environmental risk assessment of GM plants.

Yann Devos, Sylvie Mestdagh, Karine Lheureux.....37-42

Abstract: The European Food Safety Authority (EFSA) plays a central role in the risk assessment of genetically modified (GM) plants in the European Union by providing (1) independent science-based advice on the safety of GM plants and derived food and feed products, and (2) risk assessment guidance to assist applicants in the preparation and presentation of their GM plant market authorisation applications. The EFSA's scientific panel on genetically modified

organisms (GMO Panel) has taken several initiatives to consider the latest experience gained, as well as technological progress and scientific developments made in the field of the risk assessment of GM plants and derived food and feed products. In this respect, the EFSA GMO Panel is currently in the process of revising the environmental sections of its guidance document for the risk assessment of GM plants and derived food and feed products.

Environmental impact of herbicide regimes used with genetically modified herbicide-resistant maize.

Yann Devos, Mathias Cougnon, Sofie Vergucht, Robert Bulcke, Geert Haesaert, Walter Steurbaut, Dirk Reheul.....43-48

Abstract: With the potential advent of genetically modified herbicide-resistant (GMHR) crops in the EU, changes in patterns of herbicide use are predicted. Broad-spectrum, non-selective herbicides used with GMHR crops are expected to substitute for a set of currently used herbicides, which might alter the agro-environmental footprint from crop production. To test this hypothesis, the environmental impact of various herbicide regimes currently used with non-GMHR maize in Belgium was calculated and compared with that of possible herbicide regimes applied in GMHR maize. Impacts on human health and the environment were calculated via the pesticide occupational and environmental risk (POCER) indicator. Results showed that the environmental impact of herbicide regimes solely relying on the active ingredients glyphosate (GLY) or glufosinate-ammonium (GLU) is lower than that of herbicide regimes used in non-GMHR maize. This beneficial environmental impact is reduced or counterbalanced depending upon the use of other herbicides in association with GLY or GLU in GMHR maize.

Effects of Bt maize on non-target lepidopteran pests.

Matilde Eizaguirre, Filipe Madeira, Carmen López49-55

Abstract: Genetically modified (GM) maize with the insecticidal capacity of *Bacillus thuringiensis*, (Bt maize, expressing Cry1Ab) was first authorized in Spain in 1998. Since then its cultivated area has increased year by year to reach 78'000 ha in 2008, representing 21% of the total maize-growing area in Spain. In the study area (Lleida, Catalonia, NE Iberian Peninsula) it represents almost 80% of the total. Bt maize provides an effective control of two key lepidopteran pests, *Sesamia nonagrioides* (Lefèbvre) and *Ostrinia nubilalis* (Hübner). However, in addition to the two corn borers, two other non-target Lepidoptera, *Mythimna unipuncta* (Haworth) and *Helicoverpa armigera* (Hübner), cause occasional but severe damage to maize. Effects of Bt maize on these two Lepidoptera were studied in laboratory and field trials. Some larvae of both species can survive and complete development when feeding on Bt maize. Field evaluations carried out from 2005 to 2008 showed no differences in the number of *H. armigera* larvae per plant between Bt and isogenic varieties in most of the trials. In the laboratory, *M. unipuncta* showed a larval survival of 15%, which is significantly lower than that recorded in isogenic varieties. Additionally, larval development in survivors was significantly longer when they were fed Bt maize. Adults resulting from larvae developed on transgenic maize laid 13% fewer eggs than those resulting from larvae developed on isogenic maize. When they had the choice, neonate *M. unipuncta* larvae preferred first *Sorghum bicolor*, then isogenic maize plants and finally Bt plants for feeding. Recorded differential mortality caused by Bt maize on non-target Lepidoptera in comparison with targeted corn borers may affect the composition and abundance of the Lepidoptera community in maize as a consequence of Bt maize deployment.

Current challenges in environmental risk assessment: The assessment of unintended effects of GM crops on non-target organisms.

Monica Garcia-Alonso.....57-63

Abstract: As part of the regulatory approval process for commercial cultivation of genetically modified crops in the EU applicants have to assess the potential adverse effects that GM crops may have on human and animal health and the environment. This includes an assessment of potential adverse effects on non-target organisms arising from intended and unintended results of the genetic modification. The methodology to be used for the environmental risk assessment of

GM crops to non-target organisms has been a major subject of debate for many years. However, after much research, a conceptual framework based on a tiered approach is now widely accepted by risk assessors, regulators and the scientific community. This methodology works well when the assessment is aimed at establishing the risk associated with intended effects of the genetic modification or unintended effects that have been identified during the comparative assessment. There the transformed plant is grown alongside its conventional counterpart and a number of plant characteristics are measured and compared. However, the regulatory process in the EU now also considers the requirement to assess the risk of potential unintended effects that have not been identified during the comparative assessment. This represents a major challenge for risk assessors in that there is no clear basis in which to set testable hypothesis. This paper discusses some of the problems encountered by risk assessors when trying to fulfill this regulatory requirement and explores ways forward.

A faunistic database as a tool for identification and selection of potential non-target arthropod species for regulatory risk assessment of GM maize.

Simon Knecht, Jörg Romeis, Louise A. Malone, Marco P. Candolfi, Monica Garcia-Alonso, Oxana Habušťová, Joseph E. Huesing, József Kiss, Wolfgang Nentwig, Xavier Pons, Stefan Rauschen, Ágnes Szénási, Franz Bigler65-69

Abstract: In order to assess the similarities in arthropod species composition and abundance in maize fields in different geographic areas of Europe, we are compiling a database of species found and their ecological functions in five selected European maize producing countries (Czech Republic, France, Germany, Hungary and Spain). Countries were selected to represent both the extent of maize production as well as relevant ecological zones in which maize is grown. The database contains information on the taxonomy, distribution, abundance and ecological function(s) of each species. By comparing the species representing particular ecological functions and/or taxonomic groups in each country, we will determine the extent of faunal similarities or differences among the countries studied and identify those species that could supply the most widely applicable data for non-target arthropod risk assessment of GM maize. This database will have broad utility in the EU and is designed to allow for inclusion of other world geographies in future.

Involving public sector research in regulations and international negotiations on biotechnology.

Zuzana Kulichova, Piet van der Meer71-73

Abstract: Thousands of research projects in modern biotechnology have been and are carried out in public sector research institutes worldwide to help strengthening sustainable production of food, feed, fiber and fuel. As activities involving biotechnology expand, harmonization of domestic regulations with international agreements, such as the Cartagena Protocol on Biosafety, becomes increasingly important. International regulations and agreements define which role public sector research institutes can play in addressing agricultural challenges. For a long time the public sector has not been represented during international negotiations, which resulted in the confirmation of the misperception that biotechnology is only the domain of big handful multinational companies. The Public Research and Regulation Initiative (PRRI) was established in 2004 with the objective to offer a forum for public sector scientists to be informed about and involved in international discussion about biosafety.

Modelling of minimal distances from GM oilseed rape in Lithuania.

Algimantas Paulauskas, Milda Jodinskienė, Jana Radzijeuskaja, Eugenija Kupcinskiene, Danius Lygis, Ozeraitienė Danutė, Skuodienė Regina75-84

Abstract: The study aimed to evaluate distances required for oilseed rape to prevent outcrosses between different cultivars and also between neighboring weeds of Brassicaceae. Evaluation of possible outcrosses of traditional cultivars growing in close proximity is a good model to estimate the probability of gene flow from GM plants. Modelling was made by field experiments,

combining them with molecular analysis and pollen study. Five cultivars ('Maskot', 'Sw Savan', 'Heros', 'Ural', 'Landmark') of spring oilseed rape (*Brassica napus* L.) were grown in experimental plots in Lithuania. The number of weeds was registered in plots established parallel to the different cultivars. Plastid SSR primers MF1, MF2, MF3, MF4 were used for the molecular experiments. Amplification was successful with all used primers; two SSR loci used in our study were polymorphic after amplification with primers MF2 and MF7. Results of oilseed rape genetic diversity showed a significant genetic variation among individuals and different cultivars. Estimations were done concerning dispersion of pollens of oilseed rape by bees. The amount (as mass proportion, estimated in %) of oilseed rape pollens was 66 and 92 % in two bee hives placed near *B. napus* cultivars at the beginning of the study period (July 12) and decreased to 24 and 49 % until July 28 in the two hives, respectively. Examined cultivars significantly differed in respect to the concentration of N, Ca, Mg, and S on the pollen surface. Our preliminary data suggest the possibility to use pollen surface element analyses for the identification of specific cultivars.

Occurrence and field densities of Coccinellidae in the maize herb layer: Implications for environmental risk assessment.

Stefan Rauschen, Frank Schaarschmidt, Achim Gathmann 85-90

Abstract: One aspect of the Environmental Risk Assessment (ERA) of genetically modified plants is the potential ecological impact on the receiving environment. Plants with genes from *Bacillus thuringiensis* (*Bt*) that produce proteins with entomotoxic properties need to be assessed for their potential effects on non-target organisms (NTO), especially beneficials.

One important group of NTO are the ladybird beetles (Coleoptera: Coccinellidae), as they serve important biological control functions. Their exposure to the Cry proteins from *Bt*-plants depends on their consumption of exposed prey and plant materials such as pollen. *Bt*-plants expressing Cry3 proteins directed against the Western corn rootworm *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) especially pose a potential hazard to beneficial Coleoptera.

To assess the suitability of higher tier test systems (i.e. semi-field and field experiments) within the ERA, we analysed data from two 3-year, field-scale experiments with *Bt*-maize varieties containing the transformation events MON810 and MON88017. We present data on the densities of Coccinellidae in maize and calculated confidence intervals (CI) suitable for a test of equivalence between the *Bt*-maize varieties and their respective near-isogenic lines. We also report the results of a Monte Carlo simulation study assessing the probability that 90% CI are included in pre-specified margins of irrelevant change of mean abundances. The results show the limits of addressing questions regarding the non-target impact of *Bt*-maize on Coccinellidae in field experiments, given their low densities and the large natural variability.

Interplay of arbuscular mycorrhizal fungi with transgenic and non-transgenic wheat.

Yi Song Wilson, Andres Wiemken, Thomas Boller 91-95

Abstract: This study aims to assess whether genes transferred to wheat (*Triticum aestivum* L.), directed against specific pathogenic fungi, may also influence the beneficial arbuscular mycorrhizal fungi (AMF). The results of our field experiment show: first, the level of fertilization used according to current farming practices leads to a significant reduction of mycorrhizal colonization, both in non-transgenic and transgenic wheat lines; second, the destruction of plants in certain experimental field plots by vandals also significantly affected mycorrhiza formation of the remaining healthy plants. Third, although these results must still be confirmed using more samples from other plots, the differences of root colonization between transgenic and non-transgenic plants do not seem to be significant.

Ground beetles (Col., Carabidae) in Bt-maize – preliminary results from the first large scale field experiment in Poland.

Jacek P. Twardowski, Paweł Bereś, Michał Hurej, Zdzisław Klukowski 97-102

Abstract: The ground beetle fauna was studied at two experimental field sites in Poland. The aim of this study was to determine the long term impact of Bt maize on non-target organisms in comparison to conventional maize. For this purpose, Bt maize (DKC 3421 YG) expressing the

Cry1Ab toxin and the respective isogenic non-Bt variety (DKC 3420) were cultivated under identical conditions. For comparison, two non-Bt cultivars sprayed with a lambda-cyhalothrin were also included. Population density of surface-active invertebrates was monitored using pitfall traps (4 per plot). In the first year of the study, no significant differences between Bt maize and the conventional treatments were detected.

Assessment of Bt maize effects on non-target arthropods in field studies using the evaluation approach of “good ecological state”.

Claudia Wendt, Bernd Freier, Christa Volkmar, Markus Schorling, Katrin Wieacker

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Abstract: In the context of GMO safety research, the occurrence of non-target arthropods were monitored in maize fields and evaluated in order to detect possible effects of Bt maize using the “good ecological state” approach. These studies were performed in half-fields of Bt maize (MON 810) (BT) and conventionally cultivated maize (CV) planted in the Oderbruch region (Brandenburg, Germany), an infestation area of the European corn borer (*Ostrinia nubilalis*), in the years 2000-2007. Non-target arthropod taxa and densities were determined by counting insects on plants (5 sampling points/half- field) and pitfall traps (6 sampling points/half-field). In most cases, taxa had to be pooled to higher taxonomic units (indicator groups), e. g. Thysanoptera and aphid predators (predator units), due to low density or difficulty in identifying species. Carabids and spiders in pitfall traps were determined to the species level but also pooled for statistical analysis. Using five (visual counting) or six (pitfall trapping) sampling points per CV and BT half-field, we tested for density variation within and between the respective half-field within and between the respective years. The approach utilises baseline values to calculate “corridors of good ecological state”, defined as a range delimited by the 10% and 90% quantiles of densities in the CV half-fields during the last 5 years (2003-2007). Corridors were defined for each indicator group, e. g., for Thysanoptera and carabids, 2.9 to 12.6 individuals per stem and 23.2 to 60.4 individuals per trap and week, respectively. If significant differences between CV and BT half-fields are found in connection with CV or BT values outside the corridor limits, these cases should be subjected to particularly thorough evaluation. This approach proved to be inappropriate for arthropods with extreme abundance variation, e.g. aphids. Its utilisation as a tool for a „case-specific monitoring“ of effects of Bt maize on non-target arthropods is being discussed.

Consideration of the case-specific monitoring of genetically modified potato and appropriate monitoring endpoints.

Angelika Ziegler, Ralf Wilhelm 111-117

Abstract: A study on the feasibility and the choice of criteria for developing a general outline for a case-specific monitoring regime for transgenic potato has been performed. Possible impacts of genetic modifications on non-target organisms are assessed, considering the direct or indirect effects of metabolic alterations of the GM plants. Criteria for an appropriate risk assessment and recommendations for case-specific monitoring are suggested based on a review of the scientific literature on crop-organism interactions. First, we identify relevant pathways of plant-organism interactions through nutrients, toxins, or mechanisms of attraction and defence. Second, we consider the need for case-specific monitoring, taking into account variability of existing potato varieties and environmental conditions.