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Contents

Abstract: Field trials may be required to assess risks of genetically modified (GM) crops for non-target arthropods (NTAs). In this work we summarize some published data and conclusions of 20 field trials conducted in Spain from 1998 to 2010 to assess risks of *Bacillus thuringiensis* (Bt) and herbicide-tolerant (HT) maize. Most abundant herbivores, predators, parasitoids and detritivores observed in visual sampling and caught in pitfall and yellow sticky traps were statistically analyzed with conventional ANOVA and also with meta-analysis. Main conclusions are: (i) In general, no effects of different Bt traits on non-target arthropods in maize were found in the field, (ii) The former conclusions were confirmed by a meta-analysis approach of the field data that allowed improving drastically statistical power and, therefore, effect detection capacity, (iii) there are a number of representative taxa that should be preferably recorded to achieve an acceptable level of statistical power, (iv) as abundance was the main factor determining effect detection capacity, a minimal value of arthropod abundance may be used to select indicator species for measuring effects of GM maize on NTAs, and (v) other field trial characteristics may reduce considerably the critical value of mean abundance necessary to achieve acceptable levels of effect detection capacity. Some of these conclusions could be reached by theoretical considerations and simulation studies but the fact that they are validated by real field data enhances their value for practical guidance on how environmental risk assessment (ERA) studies may be conducted.

Abstract: As striking advances have been made in the last years to produce a range of biofortified GM crops with an increased level of nutrients, new approaches for environmental risk assessment on non-target arthropods have to be developed. In particular we focus on a new multivitamin maize developed at our university (Naqvi *et al*., 2009), producing an increased level of beta carotene, ascorbate and folate. We argue that problem formulation becomes extremely complex for this GM maize both at the plant and arthropod level. First, although the functions of carotenes and other vitamins are relatively well studied in plants, little is known about how biofortified plants modulate the metabolic pathways to increase the production of these compounds and which are their associated trade-offs. Second, studies on vitamins in insect systems are scarce, especially on their movement among trophic levels.

We propose *Zyginidia scutellaris* (Auchenorryncha: Cicadellidae) as an indicator species to assess risks of GM maize to non-target herbivores guided by the use of the best predicted power versus replication relationships from previous field trials. Additionally, we hypothesize that this

species is the base to build an indicator maize trophic chain given that it is the most abundant herbivore in maize fields. To explore the suitability of leafhoppers as indicators we present a literature review on the effects of insect resistant and herbicide tolerant GM crops and non-GM varieties on different leafhopper species. Finally we suggest an ecological risk assessment as the only way to detect the potential cascading effects of multivitamin crops.

Abstract: In a continuous effort to improve the environmental risk assessment of genetically modified crops, the European Food Safety Authority (EFSA) and the Netherlands Commission on Genetic Modification (COGEM) jointly organized an international workshop to review the latest scientific insights related to the assessment of potential adverse effects of Bt crops on non-target organisms (NTOs). The workshop was held on 29 and 30 November 2012 and was attended by parties from academia, national risk assessment bodies, industry, non-governmental organizations, the European Commission and EFSA. The workshop was divided into six sessions which respectively focused on the specificity of Bt proteins, species selection, the design of laboratory studies and field trials, and other challenging aspects related to NTO risk assessment. The closing session identified common views and remaining questions. Here, an overview of the main issues which were presented and discussed during the workshop is given.

Abstract: In Europe, sustainable land use management is a primary environmental protection goal. Compared to conventional crop production aided by chemical insecticides, insect-resistant transgenic crop production can enhance animal and plant biodiversity and thus ecological services such as biological control of pests and diseases, as well as reducing the need for chemical inputs. The objective of this study was to evaluate the impact of insect-resistant transgenic maize hybrids on the abundance of key non-target arthropods. During the 2010 and 2011 seasons, three treatments were compared, one Bt-transgenic maize hybrid targeting *Ostrinia nubilalis*, the corresponding non-Bt control (negative control), and the non-Bt control treated with insecticide (positive control), each with four replications. Two monitoring methods, pitfall and sticky traps, were used to collect non-target arthropods.

Arthropods caught in pitfall traps differed significantly between years. Carabids and collembolans were more abundant in 2010 than in 2011. In contrast, staphylinids and millipedes were more abundant in 2011 than in 2010. Arthropod abundance based on captures on sticky traps also differed significantly between the 2010 and 2011 seasons. Aphids and parasitoid wasps (chalcidids, ichneumonids, and braconids) as well as predators (lacewings, dance flies and spiders) were more abundant in 2011 than in 2010. In contrast, thrips, leafhoppers, mirids, and the beneficial insects *Aeolothrips intermedius*, syrphids, and *Propylea* spp. were more abundant in 2010 than in 2011. Most arthropods were recorded at the VT growth stage (fully visible tassel). Overall, we observed stronger variability between study years than among treatments. Furthermore, our study results indicate that biodiversity is greater in untreated Bt-transgenic maize compared to non-Bt maize treated with conventional insecticides. Thus, transgenic maize should be considered for sustainable land use management.

Abstract: Bt cotton was introduced in Burkina Faso (West Africa) in 2008, while countries that adopted Bt cotton earlier have been facing increasing pressure by sucking pests. We conducted a three year comparative study in Bt and non-Bt cotton fields of South-West Burkina Faso to assess

hemipteran populations and to detect potential insect outbreaks as observed in other countries such as China and USA. Hemipteran insect sampling was carried out three times per year and insect abundance was evaluated on the base of families. The study was complemented with a three year damage assessment on leaves and bolls and a boll production analysis in 2010. Cicadellidae (Typhlocibinae), Pyrrhocoridae, Pentatomidae, Coreidae and Miridae had the highest incidence in cotton fields. The dominant trend in their population dynamics shows higher insect frequencies in Bt cotton and an increase over time in both cultivars (Bt or non-Bt). Leaf and boll damages were significantly higher in Bt cotton than in non-Bt cotton. Our study suggests different and additive possible causes of the hemipteran outbreak that should further be investigated in depth: 1 – the reduction of insecticide spray numbers in Bt-cotton fields, 2 – the loss of genetically inherited resistance (leaf hairiness), 3 – the decreasing efficacy of insect control programs in non-Bt cotton and (particularly) 4 – the emergence of insecticide resistance in some hemipteran populations, especially in non-Bt fields. The authors also draw the attention of the Burkinabe cotton sector stakeholders on the importance of a quick implementation of IPM measures to guarantee the sustainability of Bt-cotton cultivation in Burkina Faso.

Abstract: Recent controversies over peer-reviewed papers on potential impacts of genetically modified organisms underline the necessity for an explicit, transparent and unbiased reviewing of published results. Evidence synthesis approaches represent powerful tools to collect, evaluate and summarize such research results in a transparent, reproducible and unbiased manner. The EU-funded project GRACE (GMO Risk Assessment and Communication of Evidence) aims to explore, adapt, apply, and promote these tools to enhance accountability in decision-making by providing policy makers with comprehensive, science-based information on potential impacts (scenarios) of genetically modified plants and their derived products. Furthermore, GRACE will establish an open-access database in order to support the review process, to mirror the evidence synthesis, to assist the dissemination of results and conclusions, as well as to act, in more general terms, as an information resource on biosafety of genetically modified plants.

Abstract: The only genetically modified (GM) maize planted in Europe is Bt maize resistant to lepidopteran stemborers, cultivated on a large scale in Spain. Maize expressing herbicide tolerance and insect resistance traits singly or stacked will predictably also be cropped in the future. EC legislation demands that an environmental risk assessment (ERA) for GM crops be carried out prior to release into the environment and that they be monitored after release by the implementation of post market environmental monitoring (PMEM) to detect possible adverse effects unanticipated in the ERA. Butterflies are often used for monitoring because they are sensitive to environmental changes, they are relatively easy to see and identify in most cases they are socially valuable. For PMEM of GM maize butterflies are particularly meaningful because they belong to the same taxonomic group as the target insect in the case of maize expressing Bt toxins and they feed on weeds that could be affected by the herbicide regime of HT maize. There is little information regarding butterfly species that can be exposed to the hazard of those two types of GM traits and which could be used for PMEM in southern European countries. This study addresses this knowledge gap and, after a bibliographic search and a year of field sampling, identifies some potential indicator species.

Other studies have found that the number of samples needed to detect even large changes in butterfly populations is impracticable. Thus, ways to integrate PMEM into existing monitoring schemes that cover large areas of Europe, such as the butterfly monitoring scheme (BMS), are discussed.

Abstract: As part of the LIFE+ MAN-GMP-ITA project concerning validation of risk management tools for GM plants in protected areas, two years of field trials were carried out in a maize-cropped area within the SCI-SPA Biotopes and environmental restoration areas of Bentivoglio, San Pietro in Casale, Malalbergo e Baricella in Northern Italy.

A first array of experiments was aimed to quantify the drift of maize pollen in the receiving environment. By means of Petri dishes covered with gelatin we confirmed the findings that most maize pollen falls very close to the crop edge. Collections by leaf-print technique were carried out on nettles (*Urtica dioica*) wild growing in maize field margins to evaluate the actual amount of pollen deposited and retained by nettle leaves. Three samplings were performed in the same fields at 80% maize flowering, at 100% maize flowering and at the end of anthesis. Nettles were at a mean distance of 6.2 m from maize and the maximum mean pollen density (2.1 grains cm^{-2}) was observed in the first sample.

Moreover, we checked the phenology of the second brood of peacock butterfly (*Inachis io*) in nettles growing in margins of maize field. Larval colonies were detected both in 2011 and 2012 and a complete temporal overlap with maize flowering was observed. The distribution of larvae was clumped with most nettles having no caterpillars and few plants being highly infested.

Abstract: Maize is among the most important field crops in Europe. The only genetically modified (GM) crop that is cultivated on large scale in Europe is MON810 maize expressing the insecticidal *Bacillus thuringiensis* (Bt) protein Cry1Ab. One important part of the regulatory dossier of new GM crops is the environmental risk assessment, which identifies, characterizes, and assesses potential risks associated with the cultivation of the new crop. For the protection of biodiversity and the functioning of the agro-ecosystem, knowledge on the species present in the receiving environment of the new GM crop facilitates the formation of most relevant risk hypothesis. For European crop fields information on arthropods has been compiled in a publicly available database in a project commissioned by the European Food safety Authority (EFSA). The database contains 5'499 records of 1'679 arthropod species for maize. Most records of the functional group of herbivores are available for Lepidoptera (including the main maize pests *Ostrinia nubilalis* and *Sesamia nonagrioides*), Hemiptera (including aphids) and Coleoptera (including *Diabrotica virgifera virgifera*). Predator records are dominated by Coleoptera (mainly Carabidae, Staphylinidae, and Coccinellidae) and Araneae (mainly Linyphiidae). Decomposing species include Collembola, Coleoptera, Diptera, and Acarina. Parasitoids are mainly composed of Hymenoptera and Diptera (Tachinidae). For the environmental risk assessment of GM crops and other environmental stressors, the database can assist in selecting test species for laboratory studies, higher-tier studies, and post-market environmental monitoring.

Abstract: Epigeic collembolan communities were studied in maize plots at the locality Borovce (N 48°34.831´ E 17° 43.302´) in western Slovakia. The soil type of the area was loamy luvic chernozem. Hybrids included DK440 (conventional) and DKC4442YG (Bt maize, event Mon 810) sown in 10 repetitions each. Plot size was 10 x 10 m and plots were isolated by 5 m wide strips of barley. Pitfall traps were collected on 11 dates from June to October 2012 and the captures were analysed after 7 days of installation. *Entomobrya handschini* (together 549 specimens in all traps of non-Bt maize and 672 specimens in Bt maize), *Entomobrya marginata* (419 and 466), *Lepidocyrtus violaceus* (147 and 161), *Orchesella cincta* (24 and 32), *Xenylla*

welchi (25 and 17), *Orchesella albofasciata* (11 and 9), and *Pseudosinella octopunctata* (3 and 1) were the collembolan species identified in pitfall traps. The results showed that occurrence of springtails did not depend on the maize hybrid. Thus cultivation of genetically modified maize did not influence collembolan populations. The populations of springtails generally increased in periods when the amount of rainfall was higher.

Abstract: Within the IOBC-WPRS working group "GMOs in Integrated Plant Production", a special activity on "Non-target risk assessment and regulation" was running from 2005 to 2013. Within this activity a group of experts has developed and published a tiered non-target risk assessment approach for insect-resistant GM crops (2008), proposed criteria for selecting test species for early-tier laboratory studies (2013), and provided guidance and recommendations on the experimental design for early-tier laboratory studies (2011). In this paper the work of this expert group is summarized.

Abstract: Storage pests cause economic losses to stored grain worldwide. The traditional synthetic chemicals that have been used for their control are increasingly removed from the market because of increased regulatory constraints and insect resistance. The use of biopesticides based on *Bacillus thuringiensis* (*Bt*) spores offers a safe alternative but some storage pests show low sensitivity to any natural Cry toxin. Several recombinant proteins with N-/C-terminal and binding domain modifications of Cry3Aa were prepared in search for a derivative active on *Tenebrio molitor* and *Tribolium castaneum* (Coleoptera: Tenebrionidae). Test compounds were added to the standard larval diet and larval mortality was recorded in seven-day intervals for eight weeks of exposure. Probit analysis was used to estimate lethal effects of four doses of Cry3Aa and its derivatives and the LC_{50} was calculated. Slightly enhanced effects of some Cry3Aa derivatives in comparison with natural Cry3Aa toxin were detected.

Abstract: Previous research has reported on the presence of *Bt* Cry toxins in herbivores and the subsequent effect on predators in tritrophic study systems. Further investigation has revealed that effects observed on the predator in *Bt* treatments were likely due to a nutritional deficiency caused by the suboptimal, *Bt*-sensitive prey (indirect effects) when predators have no alternative prey choices. Therefore, the question arises whether predators prefer optimal prey that is not affected by the Cry toxin uptake when given a choice. To answer this question, choice and no-choice experiments were performed using the generalist predator, *Harmonia axyridis*, feeding upon the fall armyworm, *Spodoptera frugiperda*, a major insect pest of maize. *Harmonia axyridis* larvae were fed with 1-5 day-old *S. frugiperda* larvae fed *Bt* or non-*Bt* maize expressing Cry1Ab. *Spodoptera frugiperda* larvae are sensitive to Cry1Ab; larvae feeding on *Bt* maize are smaller and development is delayed. In choice-experiments, *H. axyridis* ate more *Bt*-suppressed *S. frugiperda* larvae than larvae of the same age that had fed on non-*Bt* maize. *Harmonia axyridis* was not likely choosing prey based on whether it had fed *Bt* or non-*Bt* maize, but rather on the size of the larvae. These results may have implications for insect resistance management (IRM) for *Bt* crops.

Larvae of pests harboring various levels of resistance against plant-expressed *Bt* toxins could be smaller than the sensitive larvae developing on a non-*Bt* crop. No differences between *H. axyridis* fed on *Bt* or non-*Bt*-fed *S. frugiperda* were observed in length of development and body weight. If *Bt*-fed *S. frugiperda* larvae are more likely to be preyed upon by *H. axyridis*, it is possible that such larvae, having experienced *Bt* selection, may be preferentially removed from local field populations. This scenario is now more likely to be played out under field conditions in the U.S. where the "refuge in the bag" approach, consisting of seed blends of *Bt* and non-*Bt* hybrid seed, randomly mixed within commercial fields (e.g., blends of 5 or 10% non-*Bt* seed placed in each *Bt* seed bag). The frequency of this phenomenon should be studied further under field conditions.

No abstract