IOBC/wprs Bulletin Vol. 73, 2012

Working Group "GMOs in Integrated Plant Production". Proceedings of the fifth meeting on Ecological Impact of Genetically Modified Organisms (EIGMO) at České Budějovice (Czech Republic), 22 – 25 June, 2011. Editors: Jörg Romeis, Michael Meissle & Fernando Álvarez-Alfageme. ISBN 978-92-9067-250-0 [XII + 134 pp.].

Abstract: Though genetically modified (GM) crops have been rapidly adopted in world agriculture, concern has been expressed about the environmental risks that they may involve. In order to identify and measure the effects on NTOs (non-target organisms) of transgenic traits of crops such as Bt corn, which has been designed to kill target species, a tiered approach has been proposed. When an effect is detected in laboratory or semi-field steps, it is measured in subsequent higher-tier steps with an increasing complexity and realism in field conditions. In some cases this sequential testing scheme is not applicable because potential effects are not measurable in simple laboratory conditions and the testing procedure has to be initiated with field trials. This is the case, for example, when indirect effects are expressed through the food web or when the main expected effect is a consequence of changes in agricultural technology. However, field trials have many disadvantages and, whenever possible, should be conducted after basic data have been obtained in lower steps. After 13 years conducting field trials of Bt and herbicide-tolerant maize in Spain, the authors review the results to discuss how trials aimed at measuring the effects of GM crops on NTOs can be improved. Two main aspects are considered: (i) statistical power, that is, the probability of a null hypothesis (no differences between a transgenic variety and a non-transgenic comparator in the effects on an NTO) being rejected when it is not true; (ii) species or species assemblages that may be used as surrogates for testing effects on NTOs in the field according to the power for each one, calculated in a number of field trials selected among those conducted in the last years. In the selection it was attempted to combine the maximum variation in trial characteristics: multiyear vs. one-year trials, few varieties vs. many varieties, single vs. stacked transgenic traits, and finally plot size. Results are discussed in order to make recommendations on selecting those surrogates with a power above 0.7 to detect 50% or 25% differences between the GM variety and a non-GM comparator. A classification of suitability of non-target arthropods as surrogates for ERA trials according to the number of replications required to reach at least 0.7 power is proposed.

Abstract: Enormous research efforts and huge amount of funds have supported for more than a decade the studies of potential ecological risks of genetically modified organisms all over the World, especially in Europe. This vast amount of effort on this type of research with the first generation of transgenic crops, currently grown over 148 million hectares in the World, have revealed that cultivation of those crops have no negative impact on the environment that exceeds that of conventional agricultural practices. Today, new and more elaborated alternative methods are available for plant and microbial breeding, and their products will sooner or later be submitted for commercialization. These new methods differ substantially from the classical

approaches of genetic engineering. Thus the definition of GMO has to be revisited since some of the products derived may not need to be regarded as GM and thus not assessed based on the GMO regulation.

Abstract: New tools are urgently needed to help control insect pests of economic importance in the fields of both agriculture and human health. Building on the well understood Sterile Insect Technique (SIT), which has been used successfully for agricultural insect pest control for over 50 years, recent advances in insect genetic engineering can now provide novel solutions for effective pest control in both agriculture and public health. SIT uses repeated releases of sterile male insects to mate with a target female population but no progeny survive from these matings, thereby causing the population of the wild insects to decline, hopefully to either below an economic or disease transmission threshold or to local elimination.

Oxitec Ltd, a British SME is pioneering the use of genetic sterility in insect species and has developed strains of insects that express dominant lethal genes and are genetically "sterile", unless supplied with a repressor molecule in the diet. Insect strains have been developed for use in public health (*Aedes aegypti* and *Aedes albopictus*) and agricultural pest control programmes (*Pectinophora gossypiella, Plutella xylostella,* and the Tephritid fruit flies *Bactrocera oleae, Ceratitis capitata,* and *Anastrepha ludens*). Future projects include extending the techniques to tomato leaf miner (*Tuta absoluta*) and the Anopheline mosquito malaria vectors.

The phased testing of GM insects is on-going with several insect strains tested by independent collaborators in a variety of conditions, ranging from the laboratory, semi-field tests and open field releases. The regulatory pathway for these insects will be described, along with need for context specific community engagement. Consideration will also be given as to how these "sterile" insect strains might be used in an Integrated Vector Management (IVM) or Integrated Pest management (IPM) system.

Abstract: One of the concerns surrounding the import (for food and feed uses or processing) of genetically modified herbicide tolerant oilseed rape (GMHT OSR) is that, through seed spillage, the herbicide tolerance (HT) trait will escape into agricultural or semi-natural habitats, causing environmental or economic problems. Whether the concerns posed by feral GMHT OSR from seed import spills are scientifically justified is debatable. While OSR has characteristics such as secondary dormancy and small seed size that enable it to persist and be redistributed in the landscape, the presence of ferals is not in itself an environmental or economic problem. Crucially, feral OSR has not become invasive outside cultivated and ruderal habitats, and HT traits are not likely to result in increased invasiveness. Feral GMHT OSR has the potential to introduce HT traits to volunteer weeds in agricultural fields, but would only be amplified if the herbicides to which HT volunteers are tolerant were used routinely in the field. This worst-case scenario is most unlikely, as seed import spills are mostly confined to port areas. Economic concerns revolve around the potential for feral GMHT OSR to contribute to GM admixtures in non-GM crops. Since feral plants derived from cultivation (as distinct from import) occur at too low a frequency to affect the coexistence tolerance threshold of 0.9% in the EU, it can be concluded that feral GMHT plants resulting from seed import spills will have little relevance as a potential source of pollen or seed for GM admixture. This paper concludes that feral OSR in Europe should not be *routinely* managed, and certainly not in semi-natural habitats, as the benefits of such action would not outweigh the negative effects of management.

Abstract: Genetically modified organisms (GMOs) and derived food and feed products are subject to a risk analysis before they can be placed on the market in the European Union. In this risk analysis process, the role of the European Food Safety Authority (EFSA) is to independently evaluate the risk assessment provided by applicants and to provide scientific advice to risk managers on any possible risks of GMOs to human and animal health and the environment. EFSA's scientific advice is elaborated by its GMO Panel with the scientific support of several Working Groups and its GMO Unit. This paper describes EFSA's activities pertaining to the safety assessment of GM animals, with a focus on GM fish, GM insects and GM mammals and birds.

Abstract: In November 2010, the Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) issued a Scientific Opinion (SO) that delivers guidance on the environmental risk assessment (ERA) of genetically modified (GM) plants. Beside the ERA SO, the EFSA GMO Panel developed a separate SO that provides specific guidance for the evaluation of potential adverse effects of GM plants on non-target organisms (NTOs). This paper describes some elements of the ERA SO and NTO SO pertaining to NTO risk assessment, with a focus on: (1) problem formulation; (2) protection goals and limits of concern; (3) intended and unintended effects; (4) species selection for testing purposes; and (5) stacked transformation events. Some scientific comments from EU Member States and stakeholders received during the development of both documents through public consultations are also presented.

Abstract: Global efforts towards the development of genetically modified (GM) arthropods have progressed to a stage where some might possibly be placed on the EU market within the next decade. To facilitate the risk assessment of particular GM arthropod applications the specific risk issues associated with these applications need to be addressed. To inform the development of adequate and comprehensive guidelines by EFSA, a study was carried out to provide background information on the possibilities and state of development of GM arthropods, the potential adverse effects associated with applications relevant for release in the EU and the methods to investigate these effects.

The ERA of GM arthropods should consider various issues regarding the genetic modification and the method used for modification, as well as the purpose of the GM arthropod application. In that respect the specifics of potential applications, e.g. control of a target species by population suppression or applications designed for population replacements need to be acknowledged during assessment. Potential risks also depend on the respective species, which is modified and the receiving environment for releases. Issues concerning gene flow and its consequences, effects on target and non-target organisms, management practices and human health need to be considered in a specific way. It is recommended to follow a case-by-case approach for the ERA of GM arthropods to identify the specific risk issues. Key parameters for the assessment of risk issues were proposed and described and available methods for assessment were evaluated.

Abstract: Historically, the European corn borer (ECB), *Ostrinia nubilalis,* has been the most widespread insect pest of maize in the U.S.; damage was typically estimated at more than $1billion per year. Due to the difficulty in monitoring for the pest, or timing insecticide applications, most growers acquiesced and accepted annual yield losses. Beginning in 1996, transgenic maize, engineered to express insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) became one of the most widely adopted technologies in U.S. agriculture. In 2010, Bt maize was planted on more than 22.2 million ha, constituting 63% of the U.S. crop. Using statistical analysis of per capita growth rate estimates (for 3 states; 1996-2009), it was determined that areawide suppression of ECB was significantly associated with Bt maize use (Hutchison et al. 2010). Cumulative net revenues over 14 years, for 5 major maize producing states (Illinois, Iowa, Nebraska, Minnesota and Wisconsin) were conservatively estimated at $6.9 billion (U.S.) for both the Bt maize and non-Bt maize hectares. However, ~$4.3 billion, or ca. 62% of the total benefit accumulated on the *non*-Bt maize hectares. These value estimates are conservative in that they do not include additional savings that may have accrued as a result of less disease pressure (stalk or ear fungal infections) or savings due to less insecticide use. Given the fact that the same target pest species occurs throughout much of the European maize producing countries, the possibility of similar areawide economic benefits are discussed.

Abstract: Laboratory bioassays were conducted to assess the impact of pollen from the genetically modified corn (*Zea mays*) event Mon810 on larvae of the Small Tortoiseshell (*Aglais urticae*), a non-target butterfly species. Plants with the event Mon810 express the Cry1Ab protein from *Bacillus thuringiensis* in all tissues and are resistant against the European corn borer. Third instar larvae of *A. urticae* were fed with corn pollen deposited on leaves of its host plant (stinging nettle) in no-choice tests (I) once in a single dose experiment (1,000 Bt-pollen grains per cm^2) and (II) over two and a half days to assess impacts of prolonged exposure. Additionally, a (III) single dose experiment with 9,000 pollen grains per cm^2 was performed. Larval mortality, development, weight gain and feeding activity were observed. No statistically significant differences in mortality, developmental time and weight gain in comparison to control groups were detected either in the single dose or the multiple dose experiment. However there was a significantly lower feeding activity in the Bt-treatment groups in both the single and the multiple dose experiments. Based on literature data on expected environmental concentrations of corn pollen a negligible risk for *A. urticae* larvae is determined in this study.

Abstract: Extracellular serine proteases are produced by a wide range of microbial and fungal pathogens of agricultural plants. For example, they are involved in potato blight and wilting caused by *Phytophthora infestans* and *Fusarium oxysporum*, respectively. To improve potato resistance to the pathogens, we prepared plants containing a gene derived from the wax moth *Galleria mellonella* and expressing the silk protease inhibitor 2 (SPI2). SPI2 is secreted by the silk glands and inhibits microbial peptidases such as subtilisin and proteinase K. The synthetic *SPI2* gene (modified to match the codon usage in potato) was fused with *mGFP-5* to facilitate detection of the transgene and its protein product. A construct of the fusion gene with plant regulatory elements (promoter *35S* and terminator *OCS*) was prepared in a plasmid and inserted as a restriction fragment EcoRI – HindIII into the binary vector pRD400. The final construct was introduced into *Agrobacterium tumefaciens* (strain GV2260 with plasmid pGV2260). Leaf discs

of potato cultivar Velox, which exhibits medium resistance to *Phytophthora infestans*, were transformed with *A. tumefaciens*. Transgenic plants were regenerated in the presence of kanamycin antibiotics and the content of *SPI2* DNA was confirmed in the plantlets by PCR. Polyclonal antibodies were raised commercially against the natural SPI2 purified from the extract of *G. mellonella* silk using the RP HPLC. Western blotting was used to verify antibody reaction with a protein of expected size in the extracts of transformed potato plants. The presence of the fusion protein in resistant plants was confirmed with additional analytical methods. Indirect ELISA was employed for the quantification of SPI2. The results showed up to 3 fold increase of protease inhibition in transformed plants compared to the control background level. Potato plants expressing the *SPI2* gene were tested for resistance to late blight either *in vitro* (isolates of *Phytophthora* were collected from infected potato plants in the latter half of growing season) or in field trials (actual isolates of *Phytophthora*). The results revealed heterogeneity among transgenic lines, i.e. some lines were more resistant and others were more susceptible than the non-transgenic plants.

Abstract: Weed management in genetically modified herbicide tolerant (GMHT) crops may change the weed composition and arthropod (specifically non-target arthropod) species spectrum, densities and impact higher trophic layers as well. Integrated Pest Management aims at maintaining biological diversity, and ecological functions (e.g. natural control mechanisms) in agro-ecosystems. Changes however do not necessarily result in adverse effects. Therefore the magnitude and direction of changes outlined above needs to be understood and studied. HT maize hybrids (including stacked events of lepidopteran and coleopteran resistance), were surveyed for botanical and arthropod diversities in maize hybrid treated with glyphosate or with conventional herbicides. Our null hypothesis was that there is difference between the effects of glyphosate and non-glyphosate treatment on above diversities. HT maize plots were treated twice with glyphosate or once with non-glyphosate herbicides in 2007 and 2008. These plots were established and arranged in a randomized complete block design with four replications. Weed species and their area coverage were estimated on 3x1 m on each plot in the same growth maize stages in both years. Arthropods were sampled at four dates by visual observation, pitfall, and yellow sticky traps. Total weed coverage was significantly lower in glyphosate treated plots compared to plots with conventional herbicide treatment. Weed composition was also altered by herbicide treatments. The Rényi diversity profile showed higher weed diversity in glyphosate treated plots in most cases. Indirect effects of altered weed composition and coverage did not result in significant changes of abundance of most invertebrate groups.

Abstract: Genetically modified maize with the insecticidal capacity of *Bacillus thuringiensis* (expressing Cry1Ab) represents almost 80% of the total maize sown in the study area (Lleida, Catalonia, NE Iberian Peninsula). Bt corn is highly effective for controlling maize borers but 2 other non-target Lepidoptera, *Mythimna unipuncta* (Haworth) and *Helicoverpa armigera* (Hübner), cause occasional but severe damage to maize in the study area. Previous studies carried out in the laboratory and the field on the effect of Bt maize on these 2 Lepidoptera demonstrated that some larvae of the 2 species can survive and complete their development when feeding on Bt maize. In this study the suitability of a diet containing lyophilized maize leaves for trials conducted to evaluate the susceptibility of *H. armigera* larvae to Bt maize varieties was demonstrated. Bt toxin produced a quite steady cumulative mortality in the different larval instars of *H. armigera*. The Bt protein was well detected in the lyophilized Bt leaves and in the diet prepared with lyophilized leaves. In the *H. armigera* larvae the protein was detected in the

content of the peritrophic membrane, in the midgut and in the hemolymph of the larvae fed on a Bt diet. Once ingested, the majority of the toxin was degraded inside the peritrophic membrane and the growth rate of these larvae was lower than that of larvae fed on a non-Bt diet. These may be some of the reasons for the low susceptibility of *H. armigera* larvae to the Bt toxin.

Abstract: We present the results from a field experiment in Borovce, Slovakia, during 2008 and 2009 with eight maize hybrids. Three genetically modified hybrids expressing *Bacillus thuringiensis* insecticidal proteins (MON 8903, MON 88017, and MON 8903 × MON 88017) were included. Coccinellidae abundance was assessed using yellow sticky traps (Pherocone AM). A synecological analysis to reveal the role of each species was conducted, taking into account the abundance, constancy, dominance and ecological significance index. The species with the greatest ecological significance (W_5) was *Propylea quatuordecimpunctata*. *Psyllobora viginti-duopunctata* was considered as accompanying species (W_3) in 2008 and as accessory species (W_2) in 2009. *Coccinella septempunctata* was considered as accessory species (W_2), and *Adalia bipunctata* as accidental species (W_1) in 2008 and accessory species in 2009. Finally, *Chilocorus bipustulatus* and *Cynegetis impunctata*, collected only in 2008, were accidental species (W_1). There were no significant differences in the structure and quantity of Coccinellidae among the maize hybrids.

Abstract: We present the results from a field experiment in Borovce, Slovakia, during 2008 and 2009 with eight maize hybrids including 3 genetically modified ones expressing *Bacillus thuringiensis* insecticidal proteins (MON 8903, MON 88017, and MON 8903 × MON 88017). The Neuroptera abundance was assessed using yellow sticky traps (Pherocone AM). A synecological analysis taking into consideration abundance, constancy, dominance and ecological significance index was conducted. Regarding dominance, in 2008, 3 species (*Chrysoperla carnea*, *Osmylus fulvicephalus* and *Hemerobius humulinus*) were eudominant species, and *Drepanepteryx phalaenoides* was a dominant species. In 2009, *C. carnea*, and *O. fulvicephalus* were eudominant species, while *D. phalaenoides* became a subdominant species. The ecological significance index, which is most important as it takes into consideration both constancy and dominance, revealed that in 2009, all 3 observed species (*C. carnea*, *O. fulvicephalus,* and *D. phalaenoides*) were edifying species. In 2008, *O. fulvicephalus* and *H. humulinus* were dominant species, *D. phalaenoides* was accompanying species and *C. carnea* an accessory species. No significant differences in the structure and quantity of Neuroptera species was observed among the different hybrids.

Abstract: In order to determine the effect of aphid (*Myzus persicae*) feeding and infection with potato leafroll virus (PLRV) on the metabolism of potato (*Solanum tuberosum*) plants, two sequential trials were designed and carried out, followed by a molecular-chemical characterization of the tissue (leaves) of the plants using Pyrolysis-Field Ionisation Mass Spectrometry (Py-FIMS). Thus the comparison of the samples is based on the application of pattern-recognition techniques in combination with multivariate statistics. The *m/z*-signals of highest discriminative power indicate the molecular-chemical diversity. In the first experiment we tested the effects of aphid feeding and virus infection. The discriminant analysis resulted in a complete separation of the treatments. The second experiment provided evidence of the effects of grafting and transgenes, but there were fewer significant differences in the *m/z*-signals than in the

first experiment. As a consequence, separation of the sample sets by only one factor was impossible. The *m/z*-signals of highest discriminating power were evaluated and verified and the majority were tentatively assigned to marker signals of isoprenoids, which are associated with plant defence and aphid and/or virus modulated metabolism.

Abstract: The expression of the insecticidal toxin Cry3Bb1 in maize MON 88017 can curb spreading of the Western corn rootworm (WCR, *Diabrotica virgifera virgifera*), and the tolerance of this maize to glyphosate can facilitate weed management. To examine the general environmental impact of this GM maize, it was planted along with a non-GM but otherwise nearly isogenic cultivar (treated or not treated with the insecticide Dursban 10G) and with two reference cultivars. Effects on non-target organisms were studied in each treatment on 5 plots of 0.5ha each. The content of Cry3Bb1 measured in plant tissues by ELISA fluctuated during season and depended on the weather condition and the growth phase of maize. The diversity and abundance of plant-dwelling arthropods (plant sucking arthropods, predators, and parasitoids) and epigeic arthropods (spiders, ground and rove beetles) were monitored three times and five times during the season, respectively. Neither the abundance nor the species composition, were affected by the transgenic plant. The data indicate that MON 88017 has no effect on the non-target arthropod fauna.

Abstract: The long-term effects on the fecundity and fertility of *Coccinella septempunctata* preying on *Rhopalosiphum padi* living on maize containing genetic event 59122 (Herculex® RW *Bt* maize, *DAS-59122-7*), its isogenic counterpart (*PR36D79*) and three other commercial Pioneer hybrids (*37M34, 37N01* and *36V52*) were characterised during and following pollen shed. The concentration of the Cry34Ab1 and Cry35Ab1 binary toxin was analysed by commercial enzyme-linked immunosorbent assays (ELISAs). Concentrations of Cry34Ab1 and Cry35Ab1 in R1 phenological stage green leaf blades were 81.1 ± 17.7 and 75.1 ± 11.9ng/mg lyophilised dry weight, respectively. Pollen contained 47.4 ± 12.3ng Cry34Ab1/mg fresh weight, whereas concentrations of Cry35Ab1 were below the limit of detection of the ELISA (< 0.12ng/mg). Maize plants in the V14-R1 phase (8 from each variety) under mesh cages (full plant isolator) were pre-infested with approximately 500 aphids at least one week prior to placing 20 newly emerged L1 *C. septempunctata* larvae onto each plant. Larval to adult development was monitored every 3-4 days. Remaining within the isolators, the adults were allowed to prey on *R. padi* feeding on the respective maize variety and to copulate under semi-field conditions for two more weeks after adult emergence. In mid-September (2010) entire plants with isolators were transferred into the laboratory, where sexing and weighing of the ladybirds was performed. Additionally, in the laboratory under a long photoperiod (L:D 16:8, 25°C) females were paired with two males under a cob isolator. Under each isolator, a cob and adjacent ear leaves fixed in a water flask were infested with *R. padi*. *C. septempunctata* mating groups were monitored and the frequency of egg laying was recorded daily. Results from this study demonstrate that for all

treatment groups, the sex ratio was 45:55 female/male, and the female average weight (45.3 ± 6.2mg) was not significantly different across varieties. Additionally, no significant differences were observed during the tested period in either fecundity or fertility across varieties (the *DAS-59122-7* treatment group averaged 25 eggs/female and 56% fertility; the isogenic or commercial hybrid treatment groups averaged 24-34 egg/female and 58-77% fertility).