

## IOBC/wprs Bulletin Vol. 33, 2008

Working Group “GMOs in Integrated Plant Production”, Proceedings of the third Working Group meeting “Ecological Impact of Genetically Modified Organisms” at Warsaw (Poland), 23-25 May, 2007. Editors: Jörg Romeis, Michael Meissle & Olivier Sanvido. ISBN 978-92-9067-207-4 [xii + 158 pp.].

Preface .....	i
Contents .....	iii
List of Participants .....	vii

### I. Key notes

#### Exploring the potential of corn borers to develop resistance to Bt-corn in Europe.

*Juan Ferré, Joel González-Cabrera, Yolanda Bel, Baltasar Escriche* ..... 1

**Abstract:** *Bacillus thuringiensis* genes coding for insecticidal proteins (*cry* genes or *Bt* genes) have been transferred to agronomically important crops rendering the so called Bt-crops, which are protected from insect attack. Although no case of field resistance to Bt-crops has been reported so far, the potential of insects to evolve resistance to insecticides is well known, and it is considered to be one of the threats posed to genetically modified insecticidal plants. It is for this reason that the EU funded a project involving 11 groups to evaluate the potential of European corn borer populations to develop resistance to the currently deployed varieties of Bt-corn, the only transgenic insecticidal crop allowed for commercial planting in the EU. The objectives of our group in this project were, among others, to determine the biochemical basis of the mode of action of *B. thuringiensis* toxins in corn borers, and to characterise the *cadherin*-like gene in *O. nubilalis*, a major candidate resistance gene, for the future application to molecular monitoring of resistance alleles.

#### The impacts of novel management on ecosystem dynamics; tales from the UK Farm Scale Evaluations of GMHT crops.

*Alison J. Houghton, David A. Bohan* ..... 7

**Abstract:** Concerns about the possible negative impact of the novel herbicide management associated with GM herbicide-tolerant (GMHT) crops on British farm wildlife led to the establishment of the Farm Scale Evaluations (FSEs). This series of field trials evaluated wildlife changes by comparing the wildlife of a GMHT crop, with its associated herbicide management, against a conventional variety and current ‘best practice’ herbicide management. Using a half-field design, in some 65 fields per GMHT crop distributed across the arable growing areas of Great Britain, the abundance, biomass and diversity of weed plant and invertebrate species or taxa were assessed.

The results showed that there were marked changes in some groups of weed plants and invertebrates with GMHT management. A basic assumption for the trials was that there were no direct effects of GMHT herbicide management on the invertebrates, and that any effects on invertebrates were caused by changes in the weed plants due to differences in herbicide management. Although the experimental approach taken was scientifically rigorous, the repeated testing of the null hypothesis for each species or taxa was bound to generate significant effects just by chance. What was not clear was whether all the observed changes indicated a broad risk to wildlife in farmland, and were the changes important?

The aim of our research in the Ecosystem Dynamics and Biodiversity group is to understand whether there are better approaches to understanding changes in ecosystems with management, and the risk posed. Using analyses of data from the FSEs, we outline some hypotheses for agro-ecosystem structuring, the impact of management on this, and the wider risks to wildlife of these changes. Specifically, we ask: can we detect changes in the agro-ecosystem with novel management; how are invertebrates linked to plants, and can we observe effects of novel

management on these links; and can we extrapolate to biodiversity groups of social importance, such as birds, but which are difficult to measure at the field-scale?

#### Integrating insect-resistant GM crops in pest management systems.

Steven E. Naranjo ..... 15

**Abstract:** In 2006, 102 million hectares of GM crops were produced globally. GM cotton and maize with insect resistance were grown on 12.1 and 20.1 million hectares in 9 and 13 countries, respectively with these crops collectively representing about 32% of all GM crops grown in 2006. These insect resistant GM crops produce various Cry toxins from *Bacillus thuringiensis* (*Bt*) and provide for highly selective and effective control of lepidopteran and coleopteran pests, primarily bollworms, borers and root-worms, which are the most damaging pests of cotton and maize worldwide. It is estimated that between 1996 and 2005 the deployment of *Bt* cotton and maize has reduced the volume of insecticide active ingredient used for pest control by 94.5 and 7.0 million kg and increased farm income through reduced costs and improved yields by US\$7.51 and 2.37 billion, respectively. For cotton and maize pests susceptible to *Bt* toxins, these GM crops are an extremely successful form of host plant resistance, one of many pest management tactics that can be integrated in pest management systems. Reductions in insecticide use through adoption of *Bt* crops have broadened opportunities for biological control of all cotton and maize pests but most other pest management tactics have remained largely unchanged or modified only slightly in *Bt* crops. Many studies have clearly demonstrated enhanced natural enemy abundance in *Bt* crops compared with conventional crops subject to broad-spectrum chemical insecticides. A few studies also have focused on understanding the functional contribution of this natural enemy conservation. In both systems, several non-target pests have become more problematic in *Bt* crop fields in some countries largely due to reductions in insecticide use for target pests. Changes in IPM practice, enhanced biological control and the emergence of nontarget pests are further illustrated by examples from the *Bt* cotton system.

## II. Presentations

#### Impact of glyphosate use on arthropods in transgenic herbicide-tolerant maize; preliminary results from studies in Spain.

Ramon Albajes, Matilde Eizaguirre, Daniel Casado, Meritxell Pérez, Carmen López, Belén Lumbierres, Xavier Pons ..... 23

**Abstract:** In 2006, a 4-year farm-scale study on potential impacts of glyphosate-tolerant maize on maize arthropods was initiated in Spain with the sponsorship of the Spanish Ministry of Environment. A four-block complete randomised design with 0.5 ha elementary plots and two treatments (treated with glyphosate twice and untreated) was used. Plots were sampled 7 times during the season with visual inspections, pitfall traps and yellow sticky traps. As expected, weeds in untreated plots were more abundant and different in species composition in comparison with treated plots. On-plant countings revealed that the prevalent herbivores—leafhoppers and aphids— and predatory groups -*Orius* spp., spiders and trombidids- were significantly more abundant in treated plots whereas the remaining groups of predators were not significantly affected. Leafhoppers were also more abundant on the yellow sticky traps located in treated plots but differences were lower than in on-plant countings. Yellow sticky traps recorded more mymarids (as observed in visual countings) in treated plots but fewer braconids and ichneumonids; chalcidids showed no differences. In contrast with the results of visual samplings, there were significantly more *Orius* spp., staphylinids and thrips (both predatory and herbivore ones) on yellow traps placed in untreated than in treated plots. Among soil dwelling predators, spiders and carabids were caught significantly more in pitfall traps of untreated plots, whereas

rove beetle and earwig numbers showed no difference between the two types of plot. Catches of elongate collembola were higher in untreated plots but the differences were not significant.

#### Preventing spread of *Ostrinia nubilalis* Hbn. by cultivation of Bt transgenic maize – First field experiments in southeastern Poland

*Paweł K. Bereś, Robert Gabarkiewicz* ..... 31

**Abstract:** During the last ten years, the area under maize cultivation has been steadily increasing from the traditional southern region to the central or even northern regions of Poland. The main pest species of maize is the European corn borer (*Ostrinia nubilalis* Hbn.). In the southern, warmer parts of Poland, the pest damaged 1 – 25, locally even 70% of the plants between 1957 and 1994. Recently, 60 – 80%, are damaged and some fields are completely destroyed. Recently, serious infestation level have also been reported from central regions where 10 – 15% are infested on the range boundary of *O. nubilalis* and there are predictions that the pest will soon cover the whole country.

The staff of the Rzeszów Experimental Station has carried out long term observations and experiments on abundance, biology, economic importance and control methods (including biological control) of maize pests, including *O. nubilalis*. In the 2005 and 2006 growing season the project has included observations on Bt transgenic cultivars and their parent non-Bt cultivars grown in three locations in the vicinity of Rzeszów.

Tested Bt maize cultivars demonstrated high resistance to the European corn borer. The reduction of plants damage was equal to 97.8 – 100% in comparison to the non-Bt plants. At the same time other infestation symptoms affecting yield quality and quantity (e.g. broken stem below the cob and damaged cob base by larval feeding) were also reduced for Bt plants. However, a low damage level due to the sporadic establishment of *O. nubilalis* larvae in maize stems of Bt cultivars was observed.

When looking at economical (cost of equipment) and technical problems with chemical control of stem borers (needs for high wheel tractors and sprayers) and at the relatively unpredictable efficacy of biological control using *Trichogramma* spp. we believe that the cultivation of Bt maize varieties may reduce and delay the presently observed expansion of the European corn borer, which currently brings intolerable yield losses of farmers in Poland.

#### Baseline susceptibility of *Helicoverpa armigera* (Hübner) to Bt toxins Cry1Ac and Cry2Ab2 in West Africa.

*Thierry Brevault, Patrick Prudent, Maurice Vaissayre*..... 37

**Abstract:** The susceptibility of African populations of the bollworm *Helicoverpa armigera* (Hübner) to Bt toxins is still unknown, and nothing has been published regarding resistance management measures required as a follow-up to the introduction of second-generation Bt cottons in Africa. CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) is currently building a model to help local authorities to develop a strategy for a sustainable use of Bt cotton in small farming systems. A key input factor for the model is the efficacy of Bt toxins against naïve populations of the bollworm. The purpose of the present study was to determine the susceptibility of bollworm strains collected in various cotton growing areas of Western and Central Africa to Cry1Ac and Cry2Ab as well as to their association in a 1/1 ratio. As expected according to their mode of action, Bt toxins had detrimental effects on *H. armigera* larvae, both in terms of mortality and larval growth. Despite some methodological difficulties in evaluating larval mortality following Bt toxin ingestion by *H. armigera* larvae, the LC<sub>50</sub> as well as the GI<sub>50</sub> values obtained in West Africa were quite similar to data published elsewhere in the Old World.

#### Direct effects of *Galanthus nivalis* agglutinin (GNA) and avidin on the ladybird beetle *Coccinella septempunctata*.

*Mukesh K. Dhillon, Nora C. Lawo, H.C. Sharma, Jörg Romeis* ..... 43

**Abstract:** Genes encoding *Galanthus nivalis* agglutinin (GNA) and avidin have been incorporated in several crops to enhance their resistance to a range of insect pests. The ladybird beetle, *Coccinella septempunctata* (L.) is an important predator of aphids and other soft-bodied

insects in different crops. Thus, *C. septempunctata* is likely to ingest insecticidal proteins expressed by genetically modified (GM) plants either directly by feeding on pollen or through its prey. We have conducted two experiments to test the direct effects of GNA and avidin on a range of life-table parameters of *C. septempunctata*. The insecticidal proteins were provided dissolved in a 2M sucrose solution at a concentration of 1% (weight per volume). In the first experiment, neonate *C. septempunctata* larvae were fed either a pure sucrose solution (control) or a sucrose solution containing GNA or avidin. Every alternate day, predator larvae were fed exclusively with aphid prey. Ingestion of avidin resulted in a reduced *C. septempunctata* larval survival and adult emergence compared to the control group. It appeared that the predator larvae were more sensitive to GNA, expressed in a 100% mortality. In the second experiment first instar predator larvae were fed exclusively and continuously with pure sucrose solution (control) or sucrose solution containing GNA or avidin. While GNA reduced larval longevity significantly, avidin consumption caused no effect compared to the control. The results indicate that both GNA and avidin pose a hazard for *C. septempunctata* larvae under high-dose exposure conditions. To assess the risk that GNA- or avidin-expressing GM plants would pose to *C. septempunctata*, additional tests under more realistic exposure conditions would need to be conducted.

#### Validation of some techniques used in the evaluation of GM plant effects on tri-trophic interactions.

*Julia Górecka, Zbigniew T. Dąbrowski, Monika Godzina, Karolina Kubis..... 51*

**Abstract:** Our greenhouse and laboratory experiments initiated in 2005 have included DKc307, a maize cultivar expressing the *cry1Ab* gene (transformation event MON 810; Bt maize) as the GM reference crop and cv. Monumental, its near-isoline, as representatives of the first trophic level and: (a) the Mediterranean flour moth *Ephestia kuehniella* Zell. and its parasitoid - *Venturia canescens*; (b) *Rhopalosiphum padi* L. and its parasitoid *Aphidius colemani* (Viereck) as the second and third trophic level, respectively. Because our preliminary qualitative chemical analysis indicated some level of Cry1Ab toxin in ground DKc307 kernels, we have chosen *E. kuehniella* as phytophagous flour pest and its parasitoid - *Venturia canescens*. No significant differences in larval survival of *E. kuehniella* on Bt maize flour in comparison to the control cultivar was observed, however, the average weight of larvae reared on Bt maize flour was significantly lower. This effect did not affect the level of parasitism by *V. canescens*. In spite of our conclusion that *V. canescens* should not be used as a sensitive indicator of food quality change of its host, the results clearly showed that grain and flour made of Bt maize should be less infested by the moth larvae during their storage and the role of *V. canescens* as a parasitoid should not be disturbed. It was confirmed that the Bt maize did not cause a toxic effect on *R. padi*. In addition, aphids developed higher populations on the transgenic plants (significant only in the winter experiment), both in the winter and summer greenhouse experiments. Higher parasitization by *A. colemani* was observed on Bt maize in the experiments conducted during the winter. This result could not, however, be confirmed during the summer experiments, indicating a seasonal effect on the tri-trophic relations.

#### Round robin quantitation of Cry3Bb1 using the qualitative PathoScreen ELISA.

*Hang Thu Nguyen, Heinz Hunfeld, Michael Meissle, Rona Miethling-Graff, Sibylle Pagel-Wieder, Stefan Rauschen, Corinne Zurbruegg, Sabine Eber, Frank Gessler, Jörg Romeis, Christoph C. Tebbe, Wolfgang Nentwig, Johannes A. Jehle..... 59*

**Abstract:** The potential of quantitative detection of Cry3Bb1 protein using a commercially available qualitative enzyme-linked immunosorbent assay (ELISA) kit (PathoScreen) was evaluated based on a round robin test in six different laboratories in Germany and Switzerland. Three standardized sources of purified Cry3Bb1 protein, one sample with a Cry3Bb1 concentration unknown to the experimenters, and two standardized plant samples of the transgenic maize event MON88017 were measured by all laboratories. Different extraction methods and different incubation conditions for the ELISA were used by the different laboratories. The variability of the quantitation of Cry3Bb1 protein was  $\pm 16.9\%$  among assays

in the same laboratory. Among different laboratories, the variability was also observed. Our results indicated that not the ELISA conditions but the extraction methods were the major factors contributing to the variation among the laboratories. In addition, different dilution levels of the antigen also contribute to the variability of quantitation of the Cry3Bb1 protein.

## F2 Screen and field sampling with light trap cages, two methods for a resistance monitoring in Bt crops.

*Heike Engels, Ingolf Schuphan, Sabine Eber* ..... 67

**Abstract:** Large-scale cultivation of Bt crops will exert high selection pressure on the target pest, which may consequently evolve resistance. So far, no resistance of the European corn borer *Ostrinia nubilalis* to Bt crops has been reported. As yet, no anticipatory resistance monitoring plan has been established for Europe.

When resistance alleles are rare, the most efficient method for a resistance monitoring is assumed to be the F<sub>2</sub> screen. This method preserves the genetic variation among isofemale lines and concentrates potential resistance alleles into homozygous genotypes of the F<sub>2</sub> generation. This way it is possible to estimate the frequency of resistance alleles in field populations. In our study 450 isofemale ECB lines, started from 650 females from field populations in four German regions, were screened over three cultivation periods.

Simultaneously a simpler monitoring method has been developed and tested. Target pest insects are thereby attracted to light-trap cages containing insect resistant transgenic plants. Trapped target insects lay their eggs onto these plants and hatching neonates will thus be screened for resistance by feeding on them. For resistance estimates the number of females and egg masses can be quantified in the light-trap cages throughout the season. In a single test trial during one cultivation period in one cage 1670 egg masses and approx. 50,000 neonate larvae were screened. The two methods are compared in terms of their expenses, requirements and suitability for a long-term resistance monitoring during the cultivation of transgenic crops. Whilst the F<sub>2</sub> screen enables to detect resistance development at an early state at low resistance allele frequencies, it is also a very expensive method, both in terms of finances and time. Monitoring by light trap cages, on the other hand, is a less sensitive method, as it can only detect resistance at the homozygous stage. It is, however, a very simple and unexpensive method, which makes it possible to screen large numbers of target pest insects in the field .

## Diversity and seasonal phenology of spiders, ground beetles and rove beetles in conventional and transgenic maize in Central Spain.

*Gema P. Farinós, Marta de la Poza, Pedro Hernández-Crespo, Félix Ortego, Pedro Castañera* ..... 75

**Abstract:** A farm scale trial was performed during three years to compare the phenology and community structure of the three prevalent groups of aboveground arthropods (spiders, ground beetles and rove beetles) that inhabit Bt maize in Central Spain with those of conventional maize, with and without imidacloprid insecticide seed-treatment. The variability in their activity-density patterns was mostly affected by the year, but no detrimental effects could be associated to transgenic plants. No shifts in richness and diversity indices of spiders, ground beetles and rove beetles were found between conventional and transgenic maize crops, but rove beetles richness was reduced in plots with imidacloprid.

## Changes in biochemistry of cucumber carrying the thaumatin II gene: relevance to herbivores.

*Małgorzata Kiełkiewicz, Janina Gajc-Wolska, Maria Szwacka, Stefan Malepszy* ..... 79

**Abstract:** The study evaluates the consequence of the thaumatin II gene insertion for nutritional suitability of GM-cucumber plants to insect/mite herbivores. Biochemical analyses revealed that among transgenic cucumber lines the constitutive level of leaf and fruit biochemical components differs. Therefore, the nutritional value of leaves and fruits of transformed and non-transformed cucumber lines was determined by calculating the ratio of primary (glucose, fructose, soluble protein) to secondary (methanol soluble phenolics; lignin) and secondary to primary compounds.

Changes in the mutual proportions between primary and secondary metabolites seem to be relevant to insect/mite herbivore development as well as to consumers other than arthropods.

#### Belowground volatile emission of Bt maize after induction of plant defence.

*Michael Meissle, Ivan Hiltbold, Ted C. J. Turlings, Jörg Romeis* ..... 85

**Abstract:** Roots of maize plants attacked by larvae of the Western corn rootworm (*Diabrotica virgifera virgifera*) commonly emit  $\beta$ -caryophyllene. This volatile sesquiterpene has been shown to attract entomopathogenic nematodes, potential biological control agents against this serious pest. Transgenic maize expressing coleopteran-specific insecticidal Cry3Bb1 protein from *Bacillus thuringiensis* (*Bt*) is another available control option. To improve efficacy of *Bt* maize and reduce the probability of resistance development, a combination of both methods could lead to a more sustainable system, as *Bt* maize kills or deters neonate larvae and nematodes would be capable of infecting older larvae surviving the Cry3Bb1 exposure. In the present study, we examined the emission of  $\beta$ -caryophyllene of two different *Bt* lines and their corresponding non-transformed near isolines after induction by a bacterial elicitor or by *D. v. virgifera* larvae. All maize lines were similarly capable of emitting  $\beta$ -caryophyllene after roots were incubated with coronatine, although variation was high. Emission after feeding by *D. v. virgifera* larvae was lower and less reliable compared with coronatine. The two *Bt* maize/control lines showed rather low  $\beta$ -caryophyllene levels compared to a highly attractive control line. Whether or not the emission levels in the varieties tested would be enough for successful nematode attraction and consequently effective biological control remains to be shown.

#### Assessment of possible non-target impacts of the novel Bt-maize event MON88017 resistant against the Western Corn Rootworm *Diabrotica virgifera virgifera* (LeConte).

*Stefan Rauschen, Ingolf Schuphan, Sabine Eber* ..... 93

**Abstract:** The Western Corn Rootworm (WCR) *Diabrotica virgifera virgifera* (LeConte) is regarded as a major threat to European maize cultivation since its introduction to the Balkans in the early 1990s. So far it has spread very rapidly, reaching economically significant levels in Serbia, Croatia and Hungary.

The novel Bt-maize event MON88017, which expresses the coleopteran-specific protein Cry3Bb1, is resistant to WCR. We investigated possible non-target effects of this maize variety under field conditions. The occurrence and abundance of non-target arthropods, e.g. herbivores, pollen feeders, and generalist predators, was assessed in a 4 hectare field experiment with MON88017, its near isogenic line DKC5143 and the two conventional hybrids DK315 and Benicia. The four maize lines were planted in a randomized plot design with eight replicates each. Several sampling methods were tested for monitoring the abundance of non-target species.

Two arthropod species were selected for detailed studies, based on their prevalence and density in the field, and their exposure to Cry3Bb1 as documented by ELISA tests: the cicadellid leafhopper *Zyginidia scutellaris* (Herrich-Schäffer) and the mirid bug *Trigonotylus caelestialium* (Kirkaldy). Results from two cultivation periods did not show significant differences between the densities of both species in plots planted with Bt-maize and the near isogenic line. Significant differences exist, however, between these two hybrid lines and the two conventional hybrids. Analyses of the abundance data of *T. caelestialium* in terms of data gathered on the soil characteristics of individual plots showed no obvious influence of particular soil parameters. For *Z. scutellaris* the recorded densities varied strongly for different sampling methods and sampling dates.

#### Application of environmental risk assessments of pest resistant crops in different environments.

*Jeremy B. Sweet* ..... 101

**Abstract:** Studying effects of pest and disease resistant crops on sensitive non-target organisms (NTOs) is a complex process and involves studies of life history traits of NTOs, such as reproduction, feeding biology and distribution, toxicity to different developmental stages and related features such as the regional distribution of GM pest resistant (PR) non-GMPR crops,

other alternative host plants and their distribution in the landscape, in order to determine the exposure of the NTO. This paper proposes risk assessment methods that consider different receiving environments when trying to assess impacts on sensitive NTOs.

#### Ground beetles (Coleoptera: Carabidae) in transgenic herbicide tolerant maize hybrids: Impact of the transgenic crop or the weed control practice?

*Dóra Szekeres, Ferenc Kádár, Zita Dorner*..... 105

**Abstract:** The cultivation of genetically modified herbicide tolerant (GMHT) maize cultivars and the change in weed control practice by using GMHT crops may have effects on carabid beetles. It is important to know whether the genetical modification, the herbicide regime or a combination of both are responsible for potential effects on carabids. In a field experiment, carabid assemblages from non-glyphosate (commercially available registered herbicide used in maize) treated GMHT maize hybrids with or without insecticidal properties are compared with those of glyphosate treated GMHT ones. Pitfall trapping was conducted during four different growth stages of maize in 2006. Altogether 12335 individuals of 44 carabid species were captured during four weeks. *Pseudoophonus rufipes*, *Poecilus sericeus*, *Dolichus halensis*, *Calathus ambiguus* and *Pseudoophonus calceatus* were the most common species in all treatments. The abundance of carabids was significantly higher on non-glyphosate treated maize plots, especially in the second part of the growing season. Recorded weed density was significantly higher in non-glyphosate treated maize plots. We found that the weed control practice applied in GMHT maize is likely to be different from conventional maize crops, which has an impact on the activity density of ground beetles.

#### Reduction of mycotoxin threats to mammals and birds through the cultivation of Bt maize cultivars in Poland.

*Agata Tekiela, Robert Gabarkiewicz*..... 111

**Abstract:** The aim of this work was to study *Fusarium* spp. occurrence and mycotoxins content in grain of four non-transgenic maize cultivars and their genetically modified (Bt) counterparts in the years 2005 and 2006 in the southern part of Poland.

*Fusarium* infestation on corn cobs as well as mycotoxin content in grain was evaluated in the four non-transgenic varieties DKC 3420 (FAO 250), PR39F58 (FAO 270), PR39D81 (FAO 260), PR38F70 (FAO 260), and in their genetically modified counterparts DKC 3421YG, PR39F56, PR39D82, PR38F71. The field trials were located in the Podkarpackie and in the Wielkopolskie provinces in the southern part of Poland. In both years, our field experiments on selected MON 810 maize cultivars showed substantially reduced cob infestations by larvae of *Ostrinia nubilalis* Hbn. securing a higher yield and lower ear rots infection of kernels, confirming similar results obtained in other countries growing Bt insect-resistant cultivars.

The chemical analysis of mycotoxin contamination originating from *Fusarium* in kernels of Bt maize cultivars grown in southern Poland showed only traces.

#### Can plants produced from callus culture be used as near-isogenic standards in comparative analyses of transgenic potato clones?

*Ramona Thieme, Helmut Griess, Thomas Thieme*..... 117

**Abstract:** For the analysis of the possible impact of transgenic plants on other organisms the choice of a standard plant is essential. In a study of the induction and analysis of somaclonal variation in the agronomic traits of plants from *in vitro* callus cultures of stem explants, about 13,000 independently regenerated plants (defined as somaclones) of five cultivars and two potato breeding clones were used. The somaclones were planted in a greenhouse and the tubers grown on in the field. During this process the plants were subjected to the multistage selection procedure commonly used in potato breeding. That is, after transferring the *in vitro* plants to a greenhouse and the first field generation, the weaker plants and those with abnormal leaf shape, colour and poor tuber development, were discarded. Therefore, depending on the genotype only 8 - 22 % of all the somaclones were selected for the second field generation. The haulm growth, maturity type (length of vegetation period) and yield in terms of tuber number, size, shape, eye depth, starch content and starch yield of the somaclones, grown over a total five years and

involving three field generations, were assessed and compared with that of the donor genotypes. The frequencies of similar, negatively and positively different somaclones were determined, which characterized the proportion of variants relative to the total number of somaclones produced *in vitro* and transferred to a greenhouse. In addition, the frequencies of somaclones with deviations among second generation field-grown clones were calculated.

79 - 94 % of the somaclones were different and discarded in the greenhouse generation because of their abnormal appearance. In the second field generation, depending on genotype and trait, 69 - 98 % of the somaclones were indistinguishable from the donor genotype. Up to 20 %, 28 % and 31 % of the somaclones differed in terms of haulm growth, length of the vegetation period and tuber yield per plant, respectively.

Plants of potato cultivars regenerated from callus culture showed differences in agronomic traits. Besides the similar plants there were those that differed in foliage, maturity type, yield and tuber characteristics. Somaclonal variation may affect the phenotypes of potato clones recovered from callus culture, even in the absence of genetic transformation. Thus, clones produced via callus culture cannot be used as near-isogenic standards in comparative analyses of transgenic potato clones.

#### A method for selecting non-target organisms for testing the biosafety of GM plants.

*Jacqui H. Todd, Padmaja Ramankutty, Louise A. Malone* ..... 123

**Abstract:** An essential requirement for GM plant risk assessment is the determination of impacts on non-target invertebrates. However, the potential list of non-target invertebrates in any agro-ecosystem is vast, and only a sub-set of these can be tested. We aimed to develop a rational, repeatable method for selecting non-target species for testing the biosafety of GM plants. Protocols for GM biosafety testing suggest a variety of non-target organism selection criteria, all of which are encompassed by the following five questions: could this crop pose a hazard to this organism?; will this organism be exposed to the hazard?; will there be impacts on ecosystems if this organism is affected?; do people value this organism?; can we perform tests with this organism? We combined these criteria using the following equation to give each candidate species a numerical score corresponding to its suitability as a test organism:

$$\text{Species score} = \frac{H \times E}{R} \times (S + V + T)$$

where: H = hazard; E = exposure; R = resilience (ability of organism to mitigate the effects of the hazard); S = status of species in ecosystem (species biomass + number of food web links + special ecosystem function); V = anthropocentric value (economic, social, cultural, ethical concerns); T = ease with which tests may be performed. To test the model, we used the example of a hypothetical Bt-pine forest in New Zealand and compiled a database of biological and ecological information on 80 randomly selected invertebrate species found in NZ pine plantations. This was combined with information about a GM Bt-pine plant. The database was composed of answers to 100 questions for each species, and each answer was given a score out of 10. The database was then interrogated to derive values for each of the model's parameters (H, E, R, S, V, T) for each species. Final species scores were generated using the model, resulting in a ranked list of non-target species with the most suitable candidate for biosafety testing at the top.

#### Impact of transgenic Bt corn on European corn borer (*Ostrinia nubilalis* Hübner) in Lower Silesia, Poland. Preliminary results.

*J.P. Twardowski, M. Hurej, L. Kordas* ..... 129

**Abstract:** The aim of this preliminary study performed in Lower Silesia, Poland, was to determine the impact of transgenic Bt corn (MON 810) on its target pest, the larvae of the European corn borer (ECB), as compared to a non-transgenic isolate control. Abundance of the pest as well as non-target organisms (data not included here) was monitored once a week, from the beginning of moth flight until the end of the corn growing season. At the end of the season, 100 plants from each treatment were taken to the laboratory for further analysis. Apparently higher level of damage caused by ECB larvae was recorded on conventional corn stalks and cobs when compared to transgenic Bt-corn. The differences were noticed throughout the whole growing season. Positive effects of the transgenic cultivar in comparison to non-Bt corn on

different parameters of plant damage caused by ECB larvae were confirmed by the laboratory analysis.

#### Non-target organism risk assessment in Bt crops.

**Zigfridas Vaituzis**..... 133

**Abstract:** This is a concise summary of the USEPA GM plant (PIP) non-target organism risk assessment process used for the last 12 years with citations of the supporting documentation. The summary is meant to frame the issues in a “Regulatory Risk Assessment Science” context since the hazard/risk issue is frequently overlooked when viewed from a pure toxicity perspective. It should be useful to anyone who may want to articulate how the environmental risk assessments were performed on the GM crops currently registered by the USEPA, and to understand that reports of adverse effects to non-target organisms *per se* do not automatically imply risk under field use conditions. To minimize data requirements and avoid unnecessary tests, the EPA risk assessments are structured such that risk is determined first from estimates of hazard under “worst-case” exposure conditions. A lack of adverse effects under these conditions provides sufficient confidence that there is no risk and no further data would be needed. Such screening tests conducted early in an investigation are broad in scope but relatively simple in design, and can be used to demonstrate acceptable risk in most circumstances. When screening studies conducted in a laboratory setting suggest potentially unacceptable risk, additional studies are designed to assess risk under more realistic field exposure conditions. These later tests are more complex than earlier screening studies. Use of this “tiered” testing framework saves valuable time and resources by organizing the studies in a manner that eliminates unnecessary lines of investigation. The initial, lower tier, high dose screening studies also allow tighter control over experimental variables and exposure conditions, resulting in a greater ability to produce statistically reliable results at relatively low cost.

#### Transgenic *Escherichia coli* co-expressing cry1Ca and cry1Ac: toxicity and synergy against three agricultural pests.

**Arieh Zaritsky, Eitan Ben-Dov**..... 139

**Abstract:** The genes *cry1Ac* and *cry1Ca* from *Bacillus thuringiensis* subsps. *kurstaki* HD-73 and *aizawai* 4J4, respectively, encoding  $\delta$ -endotoxins against lepidopteran larvae, were isolated, cloned and expressed in *Escherichia coli*, separately and together, under control of the early T7, *P<sub>AI</sub>* inducible promoter. Toxicities were examined against larvae of three major agricultural pests: *Pectinophora gossypiella*, *Helicoverpa armigera* and *Spodoptera littoralis*. The clone expressing *cry1Ac* (pBt-1A) was the most toxic to *P. gossypiella* (LC<sub>50</sub> of 0.27×10<sup>8</sup> cells g<sup>-1</sup>). Clone pBt-1CA expressing both genes displayed the highest toxicity (LC<sub>50</sub> of 0.12×10<sup>8</sup> cells ml<sup>-1</sup>) against *S. littoralis*, with a synergy factor of 164 between them.

#### Assessing the effects of Bt-maize pollen on *Typhlodromus pyri* (Acari: Phytoseiidae).

**Rostislav Zemek, Zuzana Vávrová**..... 145

**Abstract:** Transgenic maize carrying the lepidopteran-active *cry1Ab* gene from *Bacillus thuringiensis kurstaki* proved to be effective in the control of stem borers. Since the Cry toxin is also expressed in maize pollen it could potentially be harmful for pollen-feeding non-target arthropods. We assessed the impact of MON 810 maize on the performance of *Typhlodromus pyri* Scheuten, an omnivorous predatory mite which is considered important for biological control of many phytophagous mites. Its ability to utilize a wide range of food including pollen of various plant and tree species has an important role for its success at low prey density. Various parameters including longevity, developmental time and fecundity were measured in laboratory experiments when predatory mites were offered Bt or non-Bt maize pollen as a food source. The obtained results revealed that predatory mites survived, developed and reproduced well on Bt maize pollen and no significant differences compared to mites reared on non-Bt maize pollen were found. We can thus conclude that pollen from Bt maize has no detrimental effect on *T. pyri*.

### III. Report from a special WG activity

#### Non-target arthropod risk assessment of insect-resistant GM crops.

*Jörg Romeis* ..... 149

**Abstract:** Transgenic insecticidal crops have the potential to pose risks to non-target organisms. These risks need to be addressed as part of the environmental risk assessment that precedes the commercialization of any novel transgenic crop. An international initiative has been launched to develop a scientifically-sound, generic, and pragmatic approach to assess the risks to terrestrial non-target arthropods. The basis for this work is the widely-established and effective tiered testing approach from regulatory toxicology. The basic principles of this approach are described. These may provide guidance to countries that are currently developing their own non-target risk assessment guidelines and help to harmonize regulatory requirements in different regions.

#### Special activity: Non-target risk assessment and regulation. Protocol of the discussion.

*Elisabeth Schulte* ..... 157

The *Non-target risk assessment and regulation* initiative has been meeting regularly as part of the scientific IOBC/WPRS working group meetings on “GMOs in Integrated Plant Production” since 2005.

This year’s update presentation took place during the 3rd meeting in Warsaw, Poland from 23-25 May 2007. After an introduction by Jörg Romeis, coordinator of the initiative, Alan Raybould (Syngenta, UK), Franz Bigler (Agroscope ART, Switzerland) and Joe Huesing (Monsanto, USA) presented the developed non-target risk assessment approach. The presentations were then opened up for discussion by the 80 or more delegates. The moderator was Dr. Sabine Ebert, scientist and project leader (RWTH Aachen University, Institute of Environmental Research, Germany). For details on the presented approach, see Romeis (this volume) and Romeis *et al.* (2008; Nature Biotechnology 26, 203-208)