Scientific Program and Abstracts
related to genetic modification of IXth ECE[1]

"Genetically Modified Plants – Effects on Insects"
symposium of
IXth European Congress of Entomology

August 26, 2010
Budapest, Europa Congress Center

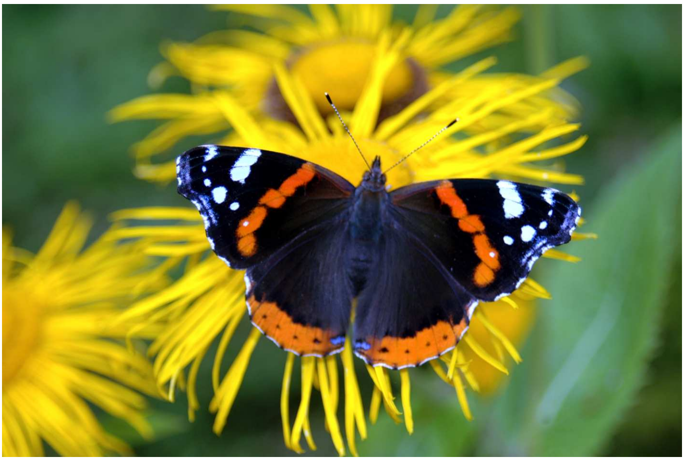
Organizers: Béla Darvas & Andreas Lang

Inachis io eggs – photo: Béla Darvas & Éva Lauber©

[1] lectures of T. Miller, and A. Székács & B. Darvas were introduced in "Biorational Control of Arthropod Pests" symposium (August 23, organized by I. Isaaya, A. R. Horowitz & A. Székács)

Chairs: Gábor Bakonyi, Wolfgang Büchs, Béla Darvas, Isaac Ishaya, A. Rami Horowitz, Andreas Lang, Gábor L. Lövei, Mathias Otto, András Székács and Claudia Zwahlen

Corn pollination – photo: Béla Darvas©



Vanessa atalanta – photo: László Peregovits©

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Abstracts of "Genetically Modified Plants – Effects on Insects" symposium of IXth European Congress of Entomology (2010) – B. Darvas, A. Hilbeck, A. Lang and G. L. Lövei Eds

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Assessing the effects of Cry toxins on ladybird beetles: a case study with *Adalia bipunctata* (Poster)

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For two common insecticidal Cry proteins expressed in commercialized *Bt*-transgenic maize varieties, i.e. Cry1Ab and Cry3Bb1, laboratory and field studies have generally revealed no detrimental effects on several non-target ladybird beetles (Coleoptera: Coccinellidae). However, a recent study by Schmidt *et al.* (2009, *Arch. Environ. Contam. Toxicol.*, 56, 221-228) claimed toxicity of purified Cry1Ab and Cry3Bb1 to 1st instar larvae of the two-spotted ladybird beetle *Adalia bipunctata*. We therefore conducted tritrophic studies to test whether an effect on *A. bipunctata* could be detected under more realistic routes of exposure to these two Cry proteins. Using spider mites as carriers, 1st and 2nd instar *A. bipunctata* were exposed to biologically active, *Bt* maize-expressed Cry1Ab and Cry3Bb1 proteins (events *MON 810* and *MON 88017*, respectively). Ingestion of either of these two Cry proteins by *A. bipunctata* did not affect larval mortality, body mass and development time. Similar results were observed in subsequent direct feeding studies where *A. bipunctata* larvae were fed with either of the two purified Cry proteins throughout their complete immature development at a concentration ten-times higher than that measured in the *Bt* maize fed spider mites. Our experiments clearly show that *A. bipunctata* larvae are not adversely affected after the ingestion of Cry1Ab or Cry3Bb1. The results will be discussed in the context of previous non-target studies conducted with ladybird beetles.

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The potential of transgenic legumes in storage pest management: assessing the impact on bruchid parasitoids (Poster)

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Genetically modified (GM) legumes expressing an alpha amylase inhibitor (αAI-1) from *Phaseolus vulgaris* are protected from the attack of major bruchid pests. However, some bruchid species remain unaffected by this inhibitor. Therefore, the combination of plant resistant factors, like αAI-1, together with biological control agents, especially parasitoids, may be a powerful and sustainable method to control such storage pests. However, the broad range of activity of αAI-1 and its possible transfer through the food-chain necessitate a detailed analysis of the possible impacts on natural enemies. A prerequisite for bruchid natural enemies to be potentially affected by αAI-1 is the presence of α-amylases. Hence, this enzyme activity was first characterized in different bruchid parasitoids based on the *in vitro* characteristics of complete insect extracts. Larval and female extracts of all species were able to hydrolyze the specific substrate potato starch, although a higher activity was observed in the latter. Moreover, all parasitoids were highly susceptible to the specific inhibitors acarbose and wheat αAI. Taken all together, these results reveal that bruchid parasitoids rely on α-amylase activity for carbohydrate digestion. In the second step of our assessment *in vitro* inhibition studies demonstrated that larval and female were highly susceptible to αAI-1, suggesting that they could be harmed by the GM legumes in case they were highly exposed to the inhibitor through their host. Hence, the subsequent steps of the assessment will include tritrophic experiments using GM chickpea and cowpea seeds expressing αAI-1, bruchids and parasitoids. Furthermore, the presence of the inhibitor at the third trophic level will also be investigated.

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***Bt*-maize (*MON 810*) effect on the collembolan *Folsomia candida* – some new aspects**

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There is a considerable ongoing debate about the environmental effect of the *Bt*-maize *MON 810*. Results referring to springtails are based on standard laboratory or field tests. Little effort was made using classical ecotoxicological methods such as dose-response relationships or long-term consequences. To make up for this omission, two series of laboratory experiments were performed. In the first experiment, the Cry1Ab toxin content of the maize was different along a gradient. The springtail *F. candida* fed more from, and reproduced better on isogenic than *Bt*-maize. No dose-response was detected. However, the highest concentration tested was only about one-third of the toxin, which is produced by *MON 810* in the field. In the second series of experiments *F. candida* populations were fed continuously on *Bt*-maize for up to 31 months. Collembolan food consumption, reproduction and food preference did not correlate with the duration of *Bt*-maize consumption. Nevertheless, differences of these parameters between populations were significant. Populations which preferred isogenic over *Bt*-maize fed more and reproduced better than those showing no preference.

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Metabolomic responses to herbivory in genetically modified potato

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An important question in risk assessment of genetically modified (GM) crop plants is whether unintended genetic or phenotypic side-effects occur in the plant after modification. Side-effects can be caused by gene disruption or altered gene regulation, and one result may be a change in the production of plant metabolites. Such effects on the metabolome level can have ecological consequences for a plant's interactions with pathogens and herbivores, since many of these interactions are mediated by chemical composition of the plant. In this study, we present results of a metabolomic approach to analyse leaf chemistry in response to herbivory or virus infection in a GM-potato variety, in comparison to its respective non-modified counterpart. The GM-potatoes are modified in their starch metabolism to reduce amylose content in tubers for industrial starch production. Analysis of leaves with ^{1}H-NMR spectroscopy and multivariate data analysis revealed no separation in metabolomic profiles, suggesting that metabolite levels were not changed due to genetic modification. Both varieties showed similar chemical responses to aphid herbivory and virus infection, i.e. a decrease in sugars, an increase of phenolic compounds and a change in glycoalkaloids patterns. We suggest that metabolomic techniques should be an integral part of ecological risk assessment of GM crops.

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Occurrence of Diptera and secondary pests in *Diabrotica*-resistant *Bt*-maize

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Different maize cultivars (*Bt*-maize *MON 88017* toxic to *Diabrotica* beetles, isogenic partner DKC 5143, conventional cultivars Benicia and DK-315) were grown in a field experiment at Schwarzenau, Germany, and the species assemblage of Diptera was analysed during 3 years. Additional feeding trials with maize plant residues were conducted in the laboratory with saprophagous Diptera larvae. The larval hatching rate of saprophagous flies and midges ranged between app. 800 to 1000 ind./m² per season, but were not significantly different between the treatments. Feeding trials with decomposing larvae of *Lycoriella castanescens* (Sciaridae) which were fed with *Bt*- or non-*Bt* maize litter, roots or pollen did not show any significant differences between the four maize cultivars regarding mortality of the larvae, rates and duration of pupation and hatching rates. Even so, all larvae which had fed on *Bt*-maize contained Cry3Bb1-toxin up to 263 ng/g larva. However, the hatching rates of phytophagous larvae of *Oscinella frit* (Chloropidae) were significantly higher in *Bt*-maize. Also, predacious dipteran larvae were significantly more abundant in *Bt*-maize plots in comparison to the controls; this was mainly due to the high number of aphidophagous larvae of *Aphidoletes aphidimyza* (Cecidomyiidae) which reacted to increased aphid abundance. The results indicate that *MON 88017 Bt*-maize may favour secondary pests which are not the target of the Cry3-toxin transferred to the transgenic maize event.

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Relationships of *Helicoverpa armigera, Ostrinia nubilalis* and *Fusarium verticillioides* on *MON 810* maize

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Ostrinia nubilalis is a stem/cob pest on maize of low economical significance in the Pannonian Region. Efficient parasitisation is provided by dipteran and hymenopteran species. *Helicoverpa armigera* is presently a more important cob pest in Hungary. Several insecticides are registered for maize, but farmers do not use them. *MON 810* maize was developed against *O. nubilalis* and advertised to indirectly decrease *Fusarium* infestation. During 2009 extremely high cob damage occurred in Júlia-major (Hungary). Investigating near 4000 cobs on five different non-GM plots, 85-95% of damage originated from *H. armigera* larvae; *O. nubilalis* contributed to this damage only in a single plot. *F. verticillioides* infection appeared only in a smaller part (25-35%) of damaged cobs. *H. armigera* and *O. nubilalis* larvae feeding on *F. verticillioides* mycelia can distribute its conidia with faecal pellets.

MON 810 had 100% effectivity against *O. nubilalis* on stem, and 90-95% against cob damage. The Cry1Ab toxin content of silk/young seeds is lower than leave/stem of *MON 810*. *Fusarium*-infected *MON 810* cobs were drastically decreased, and it can occur only after *O. nubilalis* larval damage. *H. armigera* larva cannot tolerate *Fusarium* infected food and tries to move out from the cob. In a further infestation it use the side of cob, but cannot reach seeds thought 8-12 husks. Cob damage (on top) at a high level (40-60%) resulted in only 4-9% decrease in yield and some fusariotoxin content in seeds.

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A new method for *in-situ* measurement of *Bt*-maize pollen deposition on host-plant leaves

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Maize is wind pollinated and produces huge amounts of pollen. In consequence, the Cry toxins, if expressed in the pollen, will be distributed by the wind in the surrounding vegetation where they may be consumed by NTOs. Non-target Lepidoptera larvae, which may be affected by *Bt*-pollen on their host plants, can serve as an example. Although some information is available to estimate the amount of pollen on host plants, recorded data are based on indirect measurements such as shaking or washing off pollen, or removing pollen with adhesive tapes. These methods often lack precision and do not allow to gain specific information such as spatial and temporal accumulation of pollen on the leaves.

Here, we present a new method for recording in-situ the amount and the distribution of *Bt*-maize pollen on host plant leaves. The method is based on the use of a mobile, small digital microscope (DinoLite Pro, including DinoCapture software) which can be used in combination with a laptop-computer in the field. During experiments in 2009 and 2010, the method was evaluated. Maize pollen could be correctly identified and pollen densities as well as the spatial heterogeneity of maize pollen was recorded on maize and different lepidopteran host plants (*Centaurea scabiosa*, *Rumex* spp., *Succina pratensis* and *Urtica dioica*) growing adjacent to maize fields. First data will be presented and analyzed for their implications regarding the exposure assessment of non-target Lepidoptera.

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An overview about the effects of transgenic *Bacillus thuringiensis* maize on non-target Lepidoptera

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One of the major applications of transgenic crops in agriculture are the *Bacillus thuringiensis* (*Bt*) plants, in particular *Bt*-maizes which produce insecticidal Cry toxins that target specific orders such as the Lepidoptera or Coleoptera. We reviewed publications that reported on the direct toxic effects of *Bt*-maize and/or Cry toxins of current *Bt*-maize events on larvae of non-target Lepidoptera. In total, 20 peer-reviewed publications were identified, of which 16 papers contributed laboratory-based data and seven field-based data. An adverse effect on caterpillars was recorded in 52% of all laboratory-based and in 21% of all field-based observations. The variables most often studied and having the highest occurrence of effects were larval survival, body mass and developmental time. Overall, 11 lepidopteran species were tested. The majority of the studies originated from the USA, whereas other species and other parts of the world were widely neglected. The papers we reviewed indicated a potential hazard for Lepidoptera that are exposed to and feed on lepidopteran-specific *Bt*-maize pollen. Even so, hazard characterization appears yet incomplete due to the still limited numbers of publications and concurrent lack of knowledge, the restriction of data to only a few species, the over-representation of North American species, and the identified limitations of both laboratory and field experiments.

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Protected lepidopteran larvae and Cry1Ab toxin exposure by *Bt*-maize pollen in the Pannonian Region

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A significant portion of the drifting pollen is deposited at field perimeters. The critical zone for *MON 810* pollen (~100 ng Cry1Ab toxin/g dry pollen) is 6-10 meters for rare and protected species. For other events (*SYN-Ev176*, *DAS-1507*) producing more Cry1 toxin in pollen new critical zone analysis is needed.

The food plants of 213 protected Lepidoptera species in the Pannonian Region were compared with plant surveys (weeds, bushes and trees) nearby the Hungarian cornfields. Results were focused on the pollen shedding period to find the affected plant – insect relationships. Leaf structures and the deposited pollen densities were evaluated. Plants with wide, horizontal and hairy leaf surface retain deposited pollen for a longer period. Larvae of *Aglais urticae*, *Inachis io*, *Polygonia c-album*, *Vanessa atalanta* feeding on *Urtica dioica*; *Argynnis niobe*, *Brenthis ino*, *Euplagia quadripunctaria*, *Pandoriana pandora*, *Spialia sertorius* feeding on *Rubus caesius*; and *Acherontia atropos* feeding on *Datura stramonium* may be affected principally in the Pannonian Region. Choosing models from them is necessary for European authorization. Cry1 toxin sensitivity of larvae (depending on species), Cry1 toxin content of pollen and pollen yield of a GM-cultivar are important parameters of this topic. *I. io* is a suitable model for perimeter of maize fields.

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A review of laboratory data on the impact of transgenic plants on natural enemies indicates non-random effects

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We analysed the peer-reviewed literature on the impact of genetically modified plants on arthropod natural enemies in laboratory experiments. Significantly more non-neutral responses were found than expected at random, indicating that Cry toxins and proteinase inhibitors have non-neutral effects on natural enemies that . There is a continued bias towards a few predators, especially the green lacewing, *Chrysoperla carnea*, which may be more sensitive to GM insecticidal plants (17% of the quantified parameters significantly negative) than other predators (11% significantly negative). Parasitoids were more susceptible than predators, with fewer positive (18%, significant and non-significant positive effects combined) than negative effects (66%, significant + non-significant). Proteinase inhibitors produced significantly less neutral effects (27% on predators, 29% on parasitoids) compared to Cry1A/Cry2A (35% on predators, 26% on parasitoids) or Cry3A/B (43% on predators, 31% on parasitoids). GM plants can have a positive effect on natural enemies (5% of responses significantly positive), although significant negative (21%) effects were more common. Data exist on 48 natural enemy species, still far from adequate to predict the effect of GM-plants on natural enemies. In spite of this, it is clear that the impact of transgenic plants on beneficial arthropods cannot be assessed on laboratory experiments alone.

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Genetically modified insect as a tool for biorational control

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Use of transgenic insects was thought to be an ideal way to improve the Sterile Insect Technique either by modifying or replacing sterilization by radiation with conditional lethal genes, genetic sexing strains producing all males, genes that confer vector incompetence or by using fail-safe genetic markers that could unequivocally distinguish released insects from wild types. The latter would eliminate the need for imposing expensive quarantine restrictions that sometimes interfere with commerce. Non-PCR methods of identification incur a 6% - 17% error rate in the pink bollworm project. The main SIT projects around the world are each in various stages of development. Progress will be summarized. Regulatory delay is caused mostly by reluctance on the part of scientists, not public perceptions of the technology.

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Grasshoppers and butterflies as biodiversity indicators in a GM-plant monitoring program – an Austrian case study

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Biodiversity is a key parameter in an ecological monitoring of GM-plants which is mandatory according to the Directive 2001/18/EC. The program ‘Biodiversity Nature Safety’ (BINATS) was developed and implemented on 100 representative test areas (625x625 m) – which include fields and adjacent landscape elements – in the Austrian agrarian region. Vascular plants and landscape structures already have become standard elements of biodiversity monitoring programs in cultural landscapes. In addition to these indicators Orthoptera and Lepidoptera were selected as representative indicators based on an *a priori* cost-benefit-calculation and tested for their applicability in the field.

In total, 53 different grasshopper and 41 butterfly species were registered across the test areas. In general, habitat diversity, landscape patch shape complexity and share in grassland were positively, and land use intensity as well as temperature negatively correlated with species numbers. Only in case of butterflies the relationship between summer temperatures and diversity was hump-shaped.

BINATS provides a standardised design for future inventories of biodiversity in the agrarian region into which additional animal indicators and their particular inventory needs can easily be integrated if needed. Baseline data are now available as reference for detecting and assigning unintended biodiversity effects of GMP cropping as well as of other changes in agricultural practices by means of regular monitoring.

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A full life cycle test as tool for the evaluation of potential effects of genetically modified plants on the ground beetle *Poecilus cupreus* (Poster)

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The cultivation of genetically modified maize varieties expressing Cry proteins may pose a risk for beneficial non-target organisms. The stepwise approach of an environmental risk assessment (ERA) starts with laboratory bioassays looking for potential effects of the expressed Cry proteins. For this purpose, the establishment of a standardized test procedure with the capability of reproducible results is needed.

Ground beetles (Carabidae) are common epigeal predators in the maize field, representing an important agent of integrated pest management, and are therefore in the focus of research. The breeding of the carabid *Poecilus cupreus* (L.) is well described because this species is used in the ERA of pesticides.

The test procedure used in pesticide evaluation had to be adapted for the testing of the Cry proteins. The newly developed test design and first results of the bioassay with more than 600 carabid larvae are presented. The comparison of an approach with Cry3Bb1 (*MON 88017*), negative control and positive control (Karate – lambda cyhalothrin) revealed no significant differences between Cry3Bb1 and negative control concerning pupation rate (82% against 78%; p = 0.38), hatching rate (74% against 66%; p = 0.084), larval development time 37.94 d against 37.86 d; p = 0.89) and hatching weight (0.064g against 0.064g; p = 0.92). However, all larvae exposed to pyrethroid Karate died within 10 days.

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Potential effects of pollen from stacked *Bt*-maize to non-target Lepidoptera in agrarian systems

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The cultivation of *Bt*-maize expressing lepidopteran-specific Cry proteins poses a potential risk for non-target butterflies. Therefore it is necessary (*i*) to assess the hazard posed by Cry proteins in pollen; (*ii*) to estimate the exposure to pollen deposited on the host plants of larvae in the field; (*iii*) to get data on the occurrence of larvae in relation to maize fields during anthesis.

The small tortoiseshell (*Aglais urticae*) and the peacock butterfly (*Inachis io*) are common in central European agrarian landscapes and were used as test organisms. Their larvae feed on stinging nettle (*Urtica dioica*). The DKC 5143 (event *MON 89034* x *MON 88017*) maize cultivar investigated expresses Cry1A.105 (8.6 µg/g pollen FW), Cry2Ab2 (0.33 µg/g pollen FW) and Cry3Bb1 (3.8 µg/g pollen FW).

Hazard was assessed in single-dose laboratory feeding studies to determine effect thresholds. A dose of 300 pollen/cm^2 did not reveal any significant differences in mortality rates of 3rd instar *A. urticae*.

The average amount of pollen found on nettle leaves was 29±41 grains/cm^2 directly at the maize field edge. In a distance of 5 meters the pollen density was 3±4 grains/cm^2. Pollen load on host plants decreased rapidly with increasing distance.

Patches of nettle and the occurrence of larvae of *A. urticae* and *I. io* were mapped in two agrarian landscapes. Over 72% of nests were found in distances of at least 25 meters to maize fields, corresponding to a pollen density of less than 0.9±1.2 grains/cm^2.

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Important aspects to be considered when assessing the impact of *Bt*-maize on herbivorous insects in the field (Poster)

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During three projects assessing the impacts of *Bt* maize events *MON 810, MON 88017* and *MON 89034* x *MON 88017* (in progress) on non-target organisms (NTOs) in comparison to their near isogenic lines and conventional cultivars in randomised plot designs on different trial locations since 2001, a prevalent community of herbivores was surveyed. This community is usable for environmental risk assessment and for general surveillance. Leaf-feeding (plant bugs, plant- and leafhoppers) and flower-visiting arthropods (thrips) are representatives of this community. They are relevant for an impact assessment of *Bt* maize, as they are exposed to the Cry proteins. To create monitoring schemes for these NTOs, knowledge of their range of density and occurrence in agriculture is important as a baseline.

In our projects, comparing the density of NTOs in *Bt* maize with other cultivars, using differing statistical methods (General Linear Models, tests of equivalence), some ecological and plant physiological aspects were recognised to be of importance for relevant species: (*a*) the management of off-crop areas – the density of the plant bug *Trigonotylus caelestialium* is influenced by the mowing of field margins early in the growing season and (*b*) plant physiological differences – abundances of Thysanoptera in maize panicles are influenced by the relative stage of anthesis of other cultivars nearby.

These aspects need to be taken into account experimentally when developing baselines for these NTOs.

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Comparative aspects of Cry toxin usages in insect control

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Crystalline (Cry) endotoxins from *Bacillus thuringiensis* (*Bt*) and related toxins are currently being used in plant protection as insecticides and in genetically modified plants. While both take advantage of the specificity of Cry lectins against various insect orders, there occur characteristic differences in (*i*) form of application; (*ii*) compatibility with agrotechnologies; (*iii*) the exact active ingredient; and (*iv*) its environmental fate. The clear advantage of insect resistant *Bt*-plants is that they eliminate labor- and energy- demanding field application. In turn, however, *Bt*-plants continuously produce truncated Cry toxin during vegetation. As a result, these *Bt*-plants do not comply with the principle of integrated pest management, as Cry toxin administration cannot be limited to insect pest occurrence. *Bt*-insecticides and *Bt*-plants also differ in their active ingredients: while the former contain protoxins that require metabolic activation in the insect gut, the latter mostly produce preactivated toxin. In case of CrylAb, DIPEL® contains a 131 kDa CrylAb protoxin, along with further Cryl and Cry2 protoxins. In contrast, *Bt*-plants of genetic event *MON 810* express a single truncated CrylAb toxin of 91 kDa. In addition to pesticide registration issues, this difference has pronounced effects on the easy development of insect resistance against CrylAb. Finally, Cry1Ab lectin protected from rapid decomposition in the plant tissue show environmental persistence in stubble.

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Determination of Cry1Ab toxin content of *MON 810* maize pollen by enzyme-immunoassay

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Activated Cry1Ab toxin was measured in the pollen of maize of *MON 810* genetic event using two enzyme-linked immunosorbent assays (ELISAs). Commercial 96-well microplate ELISAs, EnviroLogix Cry1Ab/Cry1Ac QuantiPlate® and Abraxis Bt-Cry1Ab/Ac ELISA were applied and optimized for pollen. Due to its high protein and starch quantity, pollen was found to be a difficult biological matrix, reflected in low but reproducible recoveries in sample preparation: 51-55% and 48-49% in spiked pollen relative to spiked pollen extract and buffer, respectively. To assess the role of extraction conditions on the digestibility of pollen grains as solid and hardly destructible particles, the efficacy of various protocols were compared. Concentration of activated Cry1Ab in pollen was calculated with Cry1Ab activated toxin/protoxin cross-reactivities in ELISA, 41% and 56%, for the EnviroLogix and Abraxis kits, respectively. Purity of the pollen fraction is an essential factor: in one batch of DK-440 BTY pollen, toxin content was 108±7 ng Cry1Ab/g dry pollen, while the corresponding level was over 100-fold higher (13030±1690 ng Cry1Ab/g dry weight) in the pollen sack. Considerable variability was found in Cry1Ab production in two, apparently different DK-440 BTY cultivar phenotypes with 100-150 and 4-18 ng Cry1Ab/g dry pollen. Cry1Ab content in pollen was severely affected by weather conditions: drought before tasseling might lead to increased Cry1Ab level in pollen, but reduced pollen production.

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A Round Robin interlaboratory comparison of Cry1Ab toxin determination in *MON 810* maize and biological samples by enzyme-immunoassay (Poster)

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A laboratory ring trial was performed for determination of Cry1Ab toxin in four laboratories using a standardized enzyme-linked immunoassay protocol, extended with in-house protocols for comparison. Identical standards and samples were distributed among participating laboratories. Botanical samples were lyophilized or frozen homogenized leaf material of *MON 810* maize. For comparison, *Bacillus thuringiensis* preparation DIPEL® was analyzed in two laboratories. Statistical analysis of the results was carried out by the ISO 5725-2 guidelines. Sample standard deviation and standard error (SD, SE), within-laboratory SD and SE (WLSD, WLSE), and interlaboratory SD and SE (ILSD, ILSE) were calculated. Measured interlaboratory average (IA) values were 12.9±5.0, 16.6±7.1 and 67.8±14.0 µg Cry1Ab/g for three lyophilized samples, and 29.1±3.0 µg Cry1Ab/g for a frozen plant sample. Yet the participating laboratories determined Cry1Ab concentrations that ranged between 63.0% and 163.5% of the corresponding IA using the joint protocol. Relative SDs in parallel determinations within given laboratories, WLSDs and ILSDs were 0-10.5%, 0.6-16.6% and 15.5-31.6%, respectively. The data emphasize the importance of a standardized protocol among laboratories for comparable quantitative Cry1Ab toxin determination. However, even when using a standardized protocol, significant differences still occur among toxin concentrations detected in different laboratories although with a smaller range of variation.

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Can transgenic *Bt*-maize and biological control be combined to reduce pest populations of western corn rootworm in maize?

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The Western corn rootworm (WCR) *Diabrotica virgifera virgifera* is one of the most serious pests of maize. *Bt*-maize expressing Cry3 proteins against this pest reduces rootworm populations, but the toxin is not always lethal and rapid development of resistance may be expected. Using entomopathogenic nematodes as biological control agents together with *Bt*-maize may help delay resistance development. The success of this combination depends on the emission of herbivore-induced volatiles by the attacked maize roots, which are known to attract the nematode *Heterorhabditis megidis*. We carried out a field study using five different maize lines, two of them expressing the mCry3A against *D. virgifera*, one the Cry1Ab against the European corn borer, their near isogenic control, and the conventional line Delprim, which is known to be attractive to *H. megidis* due to the high emission of the sesquiterpene (*E*)-β-caryophyllene in roots upon WCR attack. Maize plots received one of three treatments: (*i*) WCR eggs were added, (*ii*) WCR eggs and entomopathogenic nematodes (*H. megidis*) were added, and (*iii*) neither herbivores nor nematodes were added. *Bt*-maize expressing the mCry3A successfully prevented root damage and reduced adult emergence. Adding nematodes was successful in reducing root damage to Delprim, but had no additional effect on root damage or adult emergence in any of the other plots. *Bt*-maize lines that emit more (*E*)-β-caryophyllene may help to further decrease *D. virgifera* survival.