Environmental and Ecological Aspects of First Generation Genetically Modified Crops Regarding Their Impacts in a European Maize Producer Country

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Abstract-The success of the first generation of genetically modified (GM) crops for plant protection purposes in the US has not been followed by similar progress in Europe, where cultivation represents only 3% of the overall worldwide GM cultivation area. As for insect resistant GM plants, protection against numerous important maize pests is yet unresolved e.g., against soil-borne pests. Yield advantages of the MON 810 and SYN-Bt11 maize varieties tested in Hungary, who is the second biggest European maize producer, was lowered by 5%. Resistance against pests to Cry toxins rapidly emerges in the US. As for glyphosate tolerant GM crops certain dangerous weeds resistant to glyphosate are being selected in field applications. First-generation GM crops do not offer a solution to the fundamental ecological conflicts of industrial agriculture, mostly rooted in monoculture-based cultivation.

Keywords-Genetically Modified Crops; Glyphosate Tolerant; Lepidopteran Resistant; Coleopteran Resistant; Bacillus Thuringiensis; Cry Toxins; Pest Resistance

I. INTRODUCTION

The task of crop protection is to control pests feeding on cultivated plants and ecosystems regulating these pests. A long-standing environmental and ecological dilemma of crop protection by agrochemicals is the choice of broad-spectrum or selective agents [1]. Insect control by the use of neurotoxic active ingredients exerts toxicity to all animals. Substances of rapid decomposition are of preference, as only a small segment of the habitat is affected by their use. Another extreme opposing the use of pesticidal substances of global activity is the application super-selective technologies. A typical example is air saturation with sex pheromones (pheromone confusion techniques), mostly targeted directly to a single species and indirectly affecting species related through ecosystem connections. Wide propagation of this technology, however, is limited due to the fact that agrotechnological cases where only a single pest species is necessary to be controlled are rather rare.

A recent development in plant protection is the application of molecular biological approaches in agricultural practices. The introduction of genetically modified (GM) plants are received with high expectations from the aspect of technology developers on the one hand, and high criticism from the aspects of environmental and ecotoxicological safety on the other hand. Thus, on the basis of proposed avoidance of broad spectrum pesticides, GM crops are advocated as a new expansion of integrated pest management (IPM) practices [2, 3]. In contrast, it has also been claimed that, present GM crop varieties cannot fulfill the main ecological principle of IPM that any protection measure should be timed only to the period(s) when pest damage exceeds the critical level, and therefore, regardless how environmentally mild their active ingredient may be, these crops do not comply with IPM [4]. In turn, decision-makers, the general public, and even the scientific community are highly polarized. Besides this societal immoderateness, characteristic regional differences occur. While the overall cultivation area is expanding worldwide, acreage of GM crops in Europe, where high added value crops (e.g., organic produce) are being preferred, is on the decline.

The complexity of the issue from the aspect of environmental protection deserves comparative evaluation. In such analysis, GM crop varieties, the special position of Europe and one of its leading maize manufacturers, Hungary, as well as main and side-effects of first generation GM crops are summarized below.

II. GM CROPS FOR PLANT PROTECTION PURPOSES

The first GM crop authorized by US Environmental Protection Agency (EPA) was bromoxynil tolerant cotton (BXN). The transgene inserted into this variety expressed an enzyme that converts bromoxynil to its metabolite 3, 5-dibromo-4-hydroxybenzoic acid (DBHA). The transgenic enzyme indeed provided tolerance to this herbicide active ingredient, yet DBHA emerged as herbicide residue in the cotton seed pellets used for foraging cattle, and occurred in the milk due to its bioaccumulative properties. As the toxicological evaluation of DBHA has not been completed by deadline by the variety owner, and bromoxynil was also enrolled on the list of substances of suspected carcinogenicity and reprotoxicity, the authorization of the active ingredient was withdrawn. Apart from this, other GM crops for plant protection purposes have been proven successful in the American continents. Members of the first generation of such GM crops contain inserted transgenes that provide intended improvements in agronomic traits, most commonly tolerance to given herbicide active ingredients (e.g., BXN) or resistance to certain insect pests.

A. Herbicide Tolerant GM Crops
Total herbicides (e.g., glyphosate, glufosinate, etc.) kill nearly all plants they come into contact with. Two practically applicable GM strategies have been developed to create tolerance to these substances. One is the placement of a modified, less susceptible active site of the herbicide in plant affected; the other is the induced production of a metabolizing enzyme in plants that convert the herbicide active ingredient into its less phytotoxic derivatives. Thus, one group of the GM crops tolerant to the herbicide active ingredient glyphosate contain a mutant epsps gene that expresses a target enzyme in the plant to which glyphosate cannot bind to, and therefore, its inhibitory activity on amino acid biosynthesis does not occur. Such epsps transgenes may be of bacterial (cp4-epsps) or maize (m-epsps, 2m-epsps) origin. Because these GM plants can survive treatments with glyphosate, residues of glyphosate occur in them. The other group of glyphosate tolerant (GT) plants produces a transgene expressing one of two types of metabolizing enzymes. Thus, these GM plants contain either gox or gat transgenes. In these cases, glyphosate metabolises amino-methyl-phosphonic acid (AMPA) and glyoxalate (gox) or N-acetyl-glyoxalate (gat) are expected to occur [5]. Several GM plants resistant to another herbicide compound, glufosinate have been developed (containing transgene pat codes for the enzyme phosphinothricin acetyltransferase and leads to increased tolerance to herbicides with glufosinate-ammonium as the active ingredient), but glufosinate is a rather costly active ingredient, therefore, the market competitor potential of these crops is low. The gat and pat genes are commonly used as marker genes, as the use of selection genes encoding resistance to antibiotics (bla – ampicillin, nptII – kanamycin) meets excessive criticis m. Nonetheless, environmental consequences of tolerance to glyphosate or glufosinate, introduced for marker gene assisted selection of transgenic plants may also be substantial.

B. Insect Resistant GM Crops

The so-called cry genes of Bacillus thuringiensis strain express crystalline (Cry) protoxins showing pathogenicity to insects with specificity at insect order level. Several sprayable pesticides contain such cry toxin as active ingredient, i.e., Cry 1 + Cry2 toxins (e.g., Dipel), Cry 3 toxin (e.g., Novodor) and Cry 4 toxin (e.g., Teknair) specific to Lepidopterans, Coleopterans and mosquitoes (Dipterans), respectively. In the alimentary canal of susceptible insects these Cry protoxins are enzymatically activated and subsequently bind to lectin receptors in the midgut, causing perforating microwounds there, and allowing microorganisms of the alimentary tract to enter the insect body cavity and cause lethal sepsis. Cry toxins produced by GM plants can also trigger this effect: these so-called Bt plants produce an already active, truncated toxin protein, not necessarily requiring enzymatic activation. Of these Bt plants, varieties resistant to the European corn borer (Ostrinia nubilalis Hübner) or the corn rootworm (Diabrotica virgifera virgifera LeConte) have reached the European registration system.

III. EUROPE AND GM CROPS

GM crop are cultivated on 160 million hectares’ of land worldwide. Approximately 90% of this area is located in the American continents (US, Brazil, Argentina, Canada, Paraguay, and Uruguay), 10% in Asia (India, China, Pakistan) and Africa (South Africa). Europe takes an expressly repressive position, and represents only 3% of the overall worldwide GM cultivation area (mostly in Spain), which may be considered the most significant failure so far of the application of GM crops. Austria, Greece, Germany, Hungary and France announced national moratoria to a given genetic event (MON 810), while Poland and Italy joined GMO-free regions with their entire territory. Among European countries not Member States of the European Union, Switzerland, Norway and Serbia announced moratoria. The range of first generation GM crops affects global production of soybean, maize, cotton and canola.

Two different trends may be spotted in the registration processes in Europe. One is the direction of the variety owners, promoted by the World Trade Organization (WTO) and manifested by the decisions of the GMO Panel of the European Food Safety Authority (EFSA). These decisions have been considered in given cases tendentious by civilian organizations, claiming that the authority relies overwhelmingly on the documentation by the variety owners, and often takes a position in opposition to independent studies. Nearly half of the GM plant variety groups under registration process in Europe belong to Monsanto Corporation, additional significant exploiters are Bayer Crop Science, Syngenta Seeds SAS and Pioneer Hi-Bred (DuPont, Dow AgroScience and Mycogen Seeds). European Union Member States promoting the registration of GM crops include Great Britain and the Netherlands, two most common rapporteur countries for GM variety documentations. It is an absurdity of the present European registration system that the evaluation of the documentation submitted to the authorization process is not dominated by countries determinant in the production of the given crop (e.g., France, Hungary, Italy in case of maize), but by countries selected by the variety owner, often without an insignificant role in cultivation of the crop in Europe. Moreover, it is also an inconsistency in the scientific regulation process that a decision-making board in food safety is responsible for environmental issues as well. If a given problem occurs in at least two member states in Europe, the European Parliament assumes the competency of the European Committee (EFSA as its advisory organ). Member States attempt to maintain their sovereignty in decision-making, reflecting their view on the basis of their crop production. In today’s Europe, struggling with food overproduction, cultivation of crops with greater labor demand, supporting rural communities, healthy and reserving regional characteristics are preferred.

A. Registration Possibilities in Europe

There are more than one hundred single or stacked genetic event GM plant varieties enrolled under the registration process in Europe, and about half of the authorizations concern maize (Fig. 1). The predominant variety owner among main multinational firms registering GM plants is Monsanto Corp. (Fig. 2).
producers are not keen on introducing first generation GM crops. Today’s parallel technologies is the task of agrotechnologists. Today’s

Agricultural, Industrial, and Environmental Gene Technology

B. Hungary and GM Crops

The regulatory authority of GM plants in Hungary is the Agricultural, Industrial, and Environmental Gene Technology Authority managed by the Ministry of Rural Development. Their activity is in conformity with the decisions of the European Union administration. A precautionary national sowing moratorium on the MON 810 maize variety group is effective in Hungary since 2005 [6]. Seed inspection for genetic modification, on the basis of highly sensitive RT-PCR technique, is presently very strict in Hungary, especially after 2011, when several seed batches of Monsanto and Pioneer Hi-Bred were found as not labeled items. This does not affect the range of GM plants with authorization for import: GM soybean originated mostly from South America is added to feed mixtures. GM plant content in feed is not indicated, violating the obligatory labeling requirement. As for human consumption, tofu and Mexican type corn chips of US origin are of GM plant content in all likelihood, moreover, batches unlabeled for GM plant content may occur among canned food (less than 5% of the measured samples was more than 0.9% GMO-content), as well as processed or creamed meat products enriched with soybean.

IV. MAIN EFFECT AND SIDE-EFFECT ANALYSIS OF GM CROPS

A. Main Effects

The assessment of the main effects of first generation GM crops produced by molecular biological methods of plant biotechnology and corresponding comparative analysis with parallel technologies is the task of agrotechnologists. Today’s plant gene technology provided solutions that reached agricultural application for weed control in maize and canola, as well as control of maize pests such as the European corn borer or the Western corn rootworm. Thus, protection against numerous important pests is yet unresolved e.g., against soil-borne pests, and further plant pests, as the maize leaf weevil (Tanytarsus dilacticollis Gyll.), aphids (Aphidoidea) and spider mites (Tetranychidae) in maize, as well as the turnip sawfly (Athalia rosae L.), flea-beetles (Phyllotreta and other species), aphids and rape pollen beetle (Meligethes aeneus F.) in canola. Moreover, these first generation GM crops do not offer a solution against plant pathogens, either. It has been documented that suppression of the damage by the European corn borer reduces Fusarium infection of maize kernels. In the Pannonian Biogeographic Region of the European Union (e.g., Hungary), however, this fungal infection is not dominantly linked to damage by this pest, as the European corn borer causes substantial damage in this region only approximately once in a decade and only sporadically. All susceptible larvae of the European corn borer perish on the leaves of MON 810 maize [7], but kernel damage is also common by the corn earworm (Helicoverpa armigera Hübner) [8]. This species consumes the milky maize seeds along the pistil, where the concentration of Cry1Ab toxin is low in MON 810 maize [9]. National surveys of distinctness, uniformity and stability (DUS) in Hungary, not including main effect studies, revealed that yield advantages of the MON 810 (DKC 4442YG by Monsanto, PR37R71 by Pioneer Hi-Bred) and SYN-Bt11 (Alpha Bt by Syngenta) varieties tested was 3% or below. As for herbicide tolerant GM crops, populations of certain dangerous weeds resistant to glyphosate are being selected in field applications due to the variability of the epsps gene.

Powles et al. described a Lolium rigidum (Gaud.) population resisting 7-11-fold dosage of glyphosate in Australia [10]. Shrestha and Hemree found GT subpopulations of 5-8 leaf stage Conyza canadensis (L.) Conq. surviving only 2-4-fold glyphosate doses [11]. According to Powles [12], it is not coincidental that in countries, where GT crops are on the rise (Argentina and Brazil), the occurrence of GT weeds is more frequent. Moreover, he considers this as one of the main obstacles of the spread of GT crops in the agricultural practice (Fig. 3). Glyphosate tolerance is an inherited property. Therefore, accumulation of weeds in the treated areas is to be expected. Genomics studies of the GT populations revealed that mutation of the gene (epsps) encoding the target enzyme responsible for tolerance is not infrequent in nature. Reduced or modified uptake or translocation of glyphosate has also been observed, and the metabolic fate of the compound may also become altered in the cell [13], possibly resulting in GT populations. The most important representatives of these weeds of emerging glyphosate tolerance include the Indian goosegrass (Eleusine indica L.) horseweed (C. canadensis), Johnsongrass (Sorghum halepense) (L. Pers.), ribwort plantain (Plantago lanceolata L.), Italian ryegrass (Lolium multiflorum Lam.) and the common ragweed (Ambrosia artemisiifolia L.). It is not difficult to predict, that prolonged cultivation of GT crops will necessitate supplemental herbicide administrations with active ingredients other than glyphosate.
Resistance to Cry toxins also rapidly emerges, although its stabilization is slower. During 2005-2006, field-evolved Cry1 toxin resistance has been documented on three noctuid species: Spodoptera frugiperda (J. E. Smith) to Cry1F toxin in Puerto Rico, Busseola fusca (Fuller) to Cry1Ab toxin in Bt maize in South Africa, and Helicoverpa zea (Boddie) to Cry1Ac and Cry2Ab toxins in Bt cotton in the Southeastern United States [14]. In 2001, Cry1Ab-resistant individuals of O. nubilalis were identified from a field collection from Kandiyohi, Minnesota, based on increased survival at a diagnostic Cry1Ab concentration [15]. The resistant strain exhibited later over 800-fold resistance to Cry1Ab. Resistance was primarily autosomal, and was controlled by more than one locus or multiple alleles at one locus.

Bt maize varieties producing Cry3 toxin were developed against Diabrotica species. Cassmann et al. reported field evolved resistance [16]: Western corn rootworm displayed significantly higher survival on Cry3Bb1 maize in Iowa in 2009, MON 863 maize variety having been commercialized since 2003. No significant correlation was found among populations for survival on Cry3Ab1 (DAS-59122) and Cry3Bb1 (MON 853, MON 88017) maize, suggesting a lack of cross-resistance between these Cry3 toxins.

As seen, elimination of possible resistance is not sufficiently solved for first generation GM crops. On the contrary, the occurrence of resistance problems is reported. Therefore, these herbicide tolerant or insect resistant varieties are subject to obsolescence.

B. Side-effects

Evaluation of side-effects is about the competency of environmental analysis, ecologists/ecotoxicologists, dietetics and economists.

1) Environmental Analysis:

Due to its water solubility, the herbicide active ingredient glyphosate and its decomposition products rapidly permeate among environmental matrices. Its main metabolite AMPA persists for longer periods in the environment [5]. Glyphosate is a hormonal modulating substance. Its formulated herbicide product, Roundup has been documented to kill special cells in the human placenta under in vitro conditions, which effect is further acerbated by formulation additives.

With the occurrence and expanding application of GT crops, and with the new agrotechnological element, post-emergence application of glyphosate these crops facilitate, the overall use of glyphosate may double. This jeopardizes the quality of surface and ground waters, the basis of drinking water assets.

It has not been proven in agricultural practice that chemical pressure on environment would be reduced by herbicide tolerant GM crops. Due to the lengthy germination cycle of weeds, at least two glyphosate treatments are being used in maize cultivation. Clarification of the herbicide residue issue is unavoidable, and if special metabolites emerge, their toxicology also needs to be explored and documented.

Open pollinated plants may get cross-pollinated, and thus, the transgene may spread within the species. Traditional or organic maize pollinated with the pollen of Bt maize produces Cry1Ab toxin already in the year of pollination in the seeds. Cry toxins from Bt plants decompose in the environment only when the plant cells containing these truncated toxins have broken down. Bt crops release orders of magnitude more bioavailable toxin into the environment than the amount released with a treatment with a Bt-based bioinsecticide (e.g.,
Dipel), and approximately 1-4% of Cry IAb toxin content is still detected from the stubble even one year upon harvest [17].

A single treatment of Dipel bioinsecticide at the registered dosage (1 kg/ha) contains 5-60 mg/ha (average 21 mg/ha) of bioavailable Cry IAb toxin, while the amount of bioaccessible amount of Cry IAb toxin is 0.09-8.16 g/ha. In contrast, the production of plant-expressed Cry IAb toxin was found to be 147-456 g Cry IAb toxin/ha (Fig. 4), representing 18-56 treatments with Dipel. The level of plant-expressed Cry IAb toxin can be further elevated by soil fertilization (2-7-fold) and the use of long maturation maize varieties (3-6-fold), representing, in worst case scenarios, in 600-1900 treatments with Dipel [17].

Environmental risk assessment of agrochemical or agrobiotechnological substances is based on the identification and estimation of negative effects and a subsequent evaluation of real exposures in agricultural practice. Secondary effects on non-target species are often tested experimentally on model organisms to describe the potential effects. In the assessment of possible non-target effects, regulatory frameworks should advocate a tiered approach. Early tier (laboratory) tests are assumedly conducted under worst case conditions [2], yet laboratory conditions with ample food supply and favorable climatic circumstances usually do not represent worst cases, where additional stressors (low temperature, rain, food shortage, parasitoids and diseases) are likely to exacerbate the effect [22]. For example, N. io larval populations are regularly strongly reduced by an endemic pathogen (cypovirus 2) and certain parasitoids e.g., Sturmia bella (Meigen), Tachinidae and Microgaster subcompleta Nees, Ichneumonidea and Pteromalus puparum L., Pteromalidae) in the Pannonian Biogeographic Region. These natural controlling agents may divide a single N. io population into different susceptible and tolerant subpopulations, modulating the effect of an additional pathogenic factor such as food containing Cry IAb toxin. Moreover, active substances may also exert indirect tritrophic effects. Although several of the proposed conclusions and recommendations were claimed restrictive and premature [23], it is essential for a suitable environmental risk assessment to include direct and indirect effects on natural enemies, which may not be resolved in a mechanistic and simplified decision procedure.

If any element of the ecosystem is eliminated (see glyphosate), the entire food-chain it is contained in is affected in a network of indirect effects. Thus, destruction of the host consequently reduces the population(s) of its natural enemies, particularly specialized parasitoids. Total herbicides exert even more drastic effects by liquidating food-chains based on all eliminated weed species.

3) Dietetics:

The attention of the world was called upon possible dietetics affects of GM crops by Árpád Pusztai [24]. Toxicological assessment of the variety documentations is not unambiguous either, as seen for example in the case of maize variety MON 863. In 2002, Monsanto Company submitted an application to the German authorities to import MON 863 maize into the European Union. The submission contained a 13-week rat feeding study, performed by a third company (Covance Labs Inc.), with statistical analysis by Monsanto [25]. Based on the results EFSA GMO Panel stated that there are no concerns over the safety of this variety.

A Court of Appeal action in Germany in 2005 allowed public access to the raw toxicity data, and in an independent analysis, Séràlina et al. [26], concluded that the MON 863 consumption affected the main organs of detoxification. The EFSA GMO Panel re-evaluated the statistical methods, and stated that the observed differences in test parameters were not indicative of adverse effects, and the new statistical analysis had not raised toxicologically relevant issues. Additional studies with maize of MON 863 and other genetic events also came to the same conclusion [27, 28]. Further statistical analysis done by de Vendômois et al., however, clearly revealed new, sex- and often dose-dependent side-effects (mostly associated with the kidney and liver, but to some extent also with the heart, adrenal glands, spleen and the hematopoietic system) for three genetic events (MON 810 –
cry1Ab gene, MON 863 – cry3Bb gene, MON 603 – cp4-epsps gene) [29], assumedly due to unpredictable insertional mutagenesis or metabolic effects, or to new pesticide residues [30]. Therefore, as chronic health effects including cancerous, hormonal, reproductive, nervous or immune diseases are increasing worldwide, gender differences and the non-linear dose- or time-related effects should be particularly considered in toxicology, mainly in attempts to reveal hormone-dependent diseases and first signs of toxicities [31]. Upon commercialization of GM crops, especially stacked events, the standard toxicological evaluation is even more seriously inadequate as the so-called “cocktail effects” are not taken into consideration.

4) Economy:

The European corn borer is not significantly pest-related to its effective natural parasitization in the Pannonian Biogeographic Region of the European Union; therefore, Hungarian farmers do not even take protective steps against this insect. Weed control with the use of glyphosate does not solve any so far unresolved issue. Glufosinate represents an expensive technology, while glyphosate is of medium price. Bt crops resistant to the corn earworm are the closest to be in principle considered. Here targeted comparison with crop rotation and the use of conventionally bred varieties (e.g., variety SUM 1352) has to be performed.

Separated cultivation of GM crops lays a heavy burden on the countries authorizing public cultivation. Authorization and regulatory authorities need to be operated, GM produce need to be labeled, moreover, transported and stored separately from conventional varieties, as well as certified on the basis of special instrumental analyses, which requires a laboratory network of sufficient analytical capacity, while the cost can reach USD 140-270 per sample, and certification of a trade consignment may require the analysis of 4-8 samples. This brings producers of organic (ecological) goods and seed producers into an unmaintainable situation, and conventional plant breeders become outworkers of GM variety owners due to the patent status of the genetic events. In parallel, farmers also fall into a dependant position to the variety owners through their seed contracts and forbiddance of saving seeds of GM crops, while consumers pay significantly higher price, due to the increased costs of quality control for a practically unamended product quality.

V. CONCLUSIONS

Both environmental and human toxicological side-effects of GM crops vary by each genetic event and each GM variety. That is why GM crops are evaluated on a case-by-case basis. Nonetheless, certain conclusions can be generalized.

On the one hand, first-generation GM crops are directly related to agrochemical technologies: herbicide tolerant GM crop rely on the use of given herbicides, and insect resistant GM crops produce entomopathogenic toxins that are closely related to similar toxins registered as active ingredients of bioinsecticides. In consequence, the use of these GM crops have to comply with all chemical safety regulations regarding the active ingredient(s) their use is related to, and also with all genetic safety requirements that apply to agricultural biotechnology.

On the other hand, first-generation GM crops do not offer a solution to the fundamental ecological conflicts of industrial agriculture, which mostly rooted in monoculture-based cultivation. Instead, they provide a novel technological means for intensive agriculture. Therefore, ecological concerns regarding agrochemical protection against agricultural pests also apply (although in cases to a lesser extent) to GM crops, particularly where an immediate comparator (such as Bt bioinsecticides in the case of Bt maize) is available.

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