Genetically modified plants – from the laboratory to practical application in European agriculture. Part II*

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Introduction

One of the basic challenges in the 21st century is production of food whose quantity and quality correspond to the needs of the growing human population, with simultaneous preservation of the environment in an unchanged form. The Cartagena Protocol, which stems directly from the Convention of Biological Diversity [1, 2], clearly indicates that it is necessary to develop and implement mechanisms allowing for safe use of modern biotechnology while minimising the potential risks to the environment as well as human and animal health. It was deemed necessary to adopt rules accepted by the international community concerning the transfer, maintenance and use of living genetically modified organisms providing protection to environment and human health. The European Commission also recommended that the Member States prepare the national co-existence measures and best practices for genetically modified, conventional and organic crops (2001/18/EC Directive of the European Parliament and of the Council) together with an obligation to implement them.

The permission to place a given GMO on the market depends on the results of that GMO's mandatory risk assessment regarding human and animal health and the environment. The EU Member States should establish appropriate control and inspection systems in order to guarantee proper implementation of activities promoting safe use of GMOs. The release of genetically modified plants into the environment is not only a subject of legal regulations, but also an important subject matter of the scientific and social debate. Risk assessment regarding the use of GMOs is a complex problem and includes many aspects. The most important ones are: the expression of transgenes in the recipient's genome, the impact of transgenic plants on the non-target organisms and biological diversity, gene flow together with its consequences and the evolution of GMOs. Researching those elements requires scientific methods of risk assessment as well as methods allowing one to monitor GM plants in the environment. Therefore methods which will allow unequivocal identification and quantification of genetically modified organisms approved for use in the European Union must be available.

Environment monitoring is one of the elements of biosafety system mentioned by the Convention.

Impact of GM plants on the environment

The risk assessment related to the use of GMOs

The notion of risk related to the use of GMOs in the environment is to be understood as the relation between the risk posed by unexpected or undesirable changes and the time of exposure to them. Risk assessment should be understood as a procedure aimed at identifying the hazard caused by an action or substance and determining the emergence probability of the hazard [3]. According to the 2001/18/EC Directive and Article 6 of the Polish Act on GMOs, the operation of the contained use of GMOs or deliberate release of GMOs into the environment, including placing GMOs on the market, requires the risks assessment for human and animal health and the environment as well as utilizing such measures necessary to avoid those risks.

The advantages and risks stemming from the use of GMOs can be roughly divided into those related to human and animal health and those related to the environment. Genetically modified plants are subject to individual assessment according to the "case-by-case" principle, i.e. each modification is assessed separately. The assessment of the potential environmental risk which is posed by the use of a given GMO is complicated and requires taking into account numerous factors on which the modification may have an impact. One should pay attention to, i.a., modification of agrotechnical practices, the possibility of gene flow between the related organisms and the possible ecological consequences of it. The first stage of the risk assessment procedure should specify the problem and determine those elements of the environment which should be assessed.

The advantages and risks of cultivation of genetically modified crops have attracted the attention of a wide group of scientists (outside of the avid proponents and emotional opponents) to the need of carrying out the risk assessment ***based exclusively on solid, scientific data.*** The need for such approach has also been pointed out by the experts from the EU Member States, appointed to the European Food Safety Authority, as well as the European representatives of environmental protection organisations (EC, 2011) [4].

The selection of the species for the assessment of unintended effects should provide for various impacts of the transgenic product on the non-target organisms:

- predictable unintended effects, e.g. due to the nature of toxic impact of the Bt protein on the caterpillars of various *Lepidoptera* species
- unanticipated adverse effects, e.g. when a genetic modification triggers changes of other metabolic processes in the plant.

It is postulated for the assessment to include the tritrophic interactions: plant – phytophages – natural enemies (Fig 1).

In many European countries, not only EU Member States, numerous studies were carried out over the past 10 years on the unintended impacts of transgenic varieties on the environment. The studies included the impact of different varieties and lines of genetically modified corn, rapeseed and potatoes, containing the

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genes for resistance to pests, on many phytophages (including snails), parasitoids and predators. The impact of those varieties on soil organisms, including microorganisms, through root exudates was also analysed.

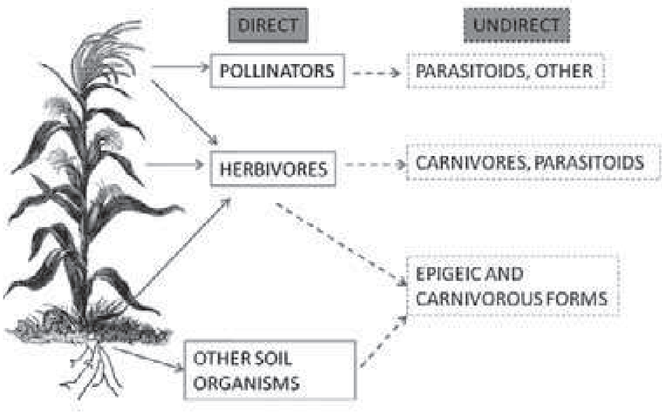

Fig .1. Potential effects of Bt maize on arthropods (based on J. Twardowski)

Between 1995 and 2006 a total of **34** studies of the impact of toxic Cry1Ab and Cry3 proteins on non-target herbivorous arthropods, **32** studies on predators and **6** on parasitoids in laboratory conditions has been carried out. In the case of the Cry1Ab protein with specific toxicity on larvae of *Lepidoptera* butterflies unintended but predictable negative effects were achieved on the development of caterpillars of species such as: Monarch butterfly (*Danaus plexippus*), tobacco hornworm (*Manduca sexta*), Silver Y (*Autographa [Plusia] gamma*), eastern black swallowtail (*Popilio polyxenes* (although in other tests the same authors did not confirm negative effects), Large White (*Pieris brassicae*), Small White (*Pieris rapae*), diamondback moth (*Plutella xylostella*), African cotton leaf worm (*Spodoptera littoralis*) and Greater Wax Moth (*Galleria mellonella*).

For the vast majority of predators preying on herbivores, feeding on GM plants or medium containing Cry1Ab protein **no negative effects were observed**. The opponents of GM plants frequently cite the results of the studies by Hilbeck et al. [5] and Dutton et al. [6] on the toxic impact of the Cry1Ab protein on the development of the green lacewing larvae. Other authors have not confirmed the possibility of the direct toxicity of the Cry1Ab protein on the lacewing larvae, examining also the binding of this protein by their midgut receptors. Despite this discrepancy in the assessment of the impact of the Cry proteins on the examined species, the scientists currently focus on identifying the reason of the discrepancy, rather than giving unequivocal opinions. A good example is the unbiased analysis of differences in the assessment of the impact of the Cry1Ab protein on the development of green lacewing [7]. At the moment the case of presence of pollen from the MON18 corn in honey is being analysed. Although the majority of studies indicate no toxic impact of this pollen on the survival rate and behaviour of honeybees, even trace volumes of pollen from GM plants are enough to raise controversy.

Critical case study of the Monarch butterfly in the United States

Due to numerous reports on the Internet concerning the harmful impact of GM plants on the environment, it was decided to address the most sensational concerns.

A classic example, frequently cited on the Internet by ecological organisations and even some scientists, is the proved toxicity of the pollen of one transgenic corn variety on the caterpillars of the Monarch butterfly in the United States. The results obtained by the researchers from the Department of Entomology of Cornell University indicated that the pollen of the Bt176 corn with the gene from *Bacillus thuringensis* (Bt) placed on the leaves of the Tropical Milkweed (*Asclepias curassavica*) had impact on: reduced intensity of feeding of larvae, reduced growth rate and statistically significant mortality [8]. However, further research and critical analysis of the methodology employed by Losey's team carried out by other research teams in the United States revealed a number of inaccuracies:

- the basic host plant for Monarch butterfly larvae is the Common Milkweed (*Asclepias syriaca*), not the Tropical Milkweed (*A.curassavica*)
- the leaves of *A. curassavica* were sprinkled with large volumes of pollen collected from the transgenic corn
- pollen was collected only from the Bt176 corn which indeed produced high quantities of the toxic Cry1Ab protein in the pollen as compared to other transgenic corn varieties
- the tests on young caterpillars were carried out as non-choice bioassay
- the Bt176 corn varieties were grown on a small area that constituted only 2% of the total area of transgenic corn crops
- in the areas of mass corn cultivation in the Midwestern corn belt in the United States the corn pollination overlaps with the feeding period of Monarch larvae on host plants only for a relatively short period of time
- data cited by Losey et al. [8] on the 60-meter range of spreading of corn pollen have not been confirmed by other researchers (i.a. University of Guleph or Iowa State University) [9]. As it turned out, the majority of the pollen spreads on the weed growing in the middle of the field and the pollen volumes decrease rapidly already within 2-3 meters of the field edge
- also, the spatial distribution of the Common Milkweed was analysed on the areas of mass corn cultivation in the Midwestern Corn Belt in the USA. The analysis revealed that the population of those plants growing around corn fields is very small compared to populations growing around soybean fields and 85% of the population grows at the roadsides. Particularly dense clusters of *A. syriaca* were found on idle lands and protected ecological areas, such as recreational areas or state landscape parks.

A farm scale verification of models determining the synchronisation of the caterpillar stage of the Monarch butterfly and the dynamics of corn pollination was prepared [10]. The corn pollination period in Midwestern United States usually occurs in 1-2 weeks of July. The Monarch grows two generations on those areas. The egg laying period of the 1st generation is usually May and does not overlap with the corn pollination period. The egg laying period of the 2nd generation takes place in July and August, and partially overlaps with corn pollination. The percentage overlap of those periods is from 5-10% in southern Iowa to 50-100% in southern Minnesota. At the same time it was shown that the content of the toxic Cry protein in other corn varieties with the Bt gene was significantly lower than in the pollen of the Bt176 variety, which also reduced the negative impact on Monarch's caterpillars. In conclusion, the risk of the impact of the pollen of corn with Bt genes currently grown in the United States has been assessed as low [11].

Differences in the interpretation of results of studies on the impact of herbicide tolerant varieties on the biological diversity in Great Britain

European scientists specialising in the risk assessment of GM plants introduction into environment frequently consider the methodology of farm scale experiments and collecting data from 3-year farm scale research carried out on large areas of Great Britain to be the universal standard. The program was initiated in Great Britain in 1999 with the objective of assessing the impact of genetically modified herbicide tolerant (GMHT) varieties on specific elements of agrocenoses - "The farm scale evaluation of spring-sown genetically modified crops". The results of this interdisciplinary evaluation were published by The Royal Society [12]. However, it has been emphasised that the conclusions arising from the assessment of the results should be considered separately from the reaction of NGOs and the decisions of state administration authorities.

The objectives of introducing GMHT varieties into cultivation were: the profit of the farmer, higher flexibility and efficiency in coping with weed and reducing the negative consequential effects for the environment by reducing the use of long-lasting synthetic herbicides. However, those varieties can prove both highly effective in the protection of crops from weeds and cause potential disruptions in the delivery of food to other organism and thus increase the rate of the downward trend of abundance and species diversity of invertebrate and vertebrate populations in agrocenoses, as observed in Great Britain in the 20th Century [13].

The density of weed seeds after the crops of GMHT spring rape and GMHT sugar beet was approx. 20% lower than after conventional crops [14]. The biomass of weeds and the number of falling seeds in GMHT crops was between 17% and 33% respectively in comparison to conventional crops. The differences in the number of falling seeds in both species persisted in the autumn analysis of the seed bank. The impact of GMHT corn crops was different. The weed density was higher throughout the entire vegetation period of GMHT crops. The biomass of weeds at the end of the vegetation period was 82% higher, while the number of falling seeds was 87% higher in comparison to conventional varieties. However, those differences could not have been confirmed by the analysis of the seed bank in the soil, since the total volume of produced seeds was low due to corn crops. The differences in weed density and number of produced seeds in GMHT crops of sugar beet and spring rape in comparison to conventional crops were essential to the volume of food available to organisms on higher trophic levels. The presence of blooming weeds might have had impact on the presence of pollinators or *Heteroptera*.

A significantly lower number of certain groups of insects was observed in the fields sown with herbicide tolerant (HT) varieties: a) HT beets – *Apidae*, butterflies and *Heteroptera*, and (b) HT spring rape – butterflies. An increased number of springtails (*Collembola*) was observed on the fields sown with GM corn. The observed fluctuations in the populations of invertebrates appear to correspond to the changes observed when changing the species of plant grown in the given field. The main factors responsible for the changes in the invertebrate populations included: chemical weed control programme, agrotechnical procedures, crop rotation and long-term interactions between weed and invertebrates. All those potential interactions depend mostly on the given crop system and the environment of the given farm.

Comparing the reactions of the populations of studied invertebrates to GMHT crops sprayed with herbicides, it appears that butterflies are the group with the strongest reaction to changes of the plant species composition and should therefore be used as indicators in future studies on changes in agrocenoses [15].

Decisions of the British Department for Environment, Food and Rural Affairs (DEFRA)

The conducted research indicated certain negative impacts on the specific groups of plants and animals within the field with GM crops and its vicinity. One of the key conclusions from those studies referred to the significant effectiveness of weed control in herbicide tolerant GM crops and thus to the decreased production of weed seeds. This raised concern among organisations fighting for the protection of butterflies feeding on blooming weeds, as well as birds which do not have sufficient weed seeds to feed in autumn and winter. The objective indicator of the weed seed production reduction was the so-called soil seed bank.

DEFRA took the following decision: "Growing conventional beet and spring rape was better for many groups of wildlife than growing GM herbicide-tolerant (GMHT) beet and spring rape. Some insect groups, such as bees (in beet crops) and butterflies (in beet and spring rape), were recorded more frequently in and around the conventional crops because there were more weeds to provide food and cover." According to the British authorities, sustainable agriculture should also ensure the biological diversity of weeds in agrocenoses.

Change of the weed control system vs. bird protection

The priority in the agrocenoses of Great Britain is the protection of biological diversity of plants. Therefore, a group of scientists from Brown's Barn Research Station in Higham, Scotland, decided to take up the challenge and compare various weed control programmes in sugar beet crops. The researchers assumed that glyphosate can be used in various, environmentally beneficial ways in the crops of genetically modified herbicide tolerant (GMHT) beet for weed control [16]. The following changes have been introduced to glyphosate application programme on GMHT beet crops: (a) spraying between the beet rows instead of the entire area; (b) delay of spraying in order to provide optimum conditions for birds nesting on the ground. Delayed spraying on the entire field area or between the rows allowed for retaining weeds between rows, thus increasing the plant coverage of the field and protecting nests of birds. However, the Eurasian Stone-curlew prefers dry, open areas; therefore the field area should be sprayed in the spring. The optimum environment for skylark chicks is the plant cover; therefore it is more advisable to spray only the rows. The increased plant population also improved the conditions for the growth of *Carabidae* and *Staphylinidae* beetles. Those insects are an important source of food for chicks of bird species nesting on the ground on the critical stages of development when high-protein diet is essential for the survival of the offspring. Both systems can be applied without the risk of significant decrease of crops in comparison to the current systems of cropping and protection of conventional varieties from weed. The reported results indicate that technologies related to GMHT beet crops can be flexible and adjusted to various programmes of natural wildlife, at the same time ensuring economical levels of production for the farmers.

It appears that more attention should be given to the integration of the recommended practices of intensive agricultural production with retained protection zones for herbaceous plants (including weeds), insects and other organisms to maintain balance between the priorities of the agriculture and the environment.

The results of the first study of the impact of MON 810 corn on non-target organisms in Poland

The research related to the risk assessment of the impact of genetically modified MON 810 corn on non-target organisms was carried out by the Department of Applied Entomology of the Warsaw University of Life Sciences in 2003 [17]. Planning the scope of the study the researchers took into consideration opinion of ecological organisations and scientists who demanded that the study include all arthropod species that may potentially interact with maize and feed produced from GM grains. The first stage of the study, carried out in greenhouses and the laboratory, analysed the following interaction: (a) impact of MON 810 maize flour on the caterpillars of Mediterranean Flour Moth (*Ephestia kühniella*) and its parasitoid, *Venturia canescens*; (b) impact of pollen on the Small White (*Pieris rapae*) as the representative of Lepidoptera (the Cry1Ab protein in MON 810 maize impacts the caterpillars of European Corn Borer and other stalk-boring butterflies); (c) impact of GM maize on bird cherry-oat aphid (*Rhopalosiphum padi*), its predators, such as: two-spot ladybird (*Adalia bipunctata*) and common green lacewing (*Chrysoperla carnea*), as well as its parasitoid – *Aphidius colemani*; (e) red spider mite (*Teranychus urticae*) and its predator, *Phytoseiulus persimilis*.

The obtained results confirmed that red spider mite, as a species harvesting the Cry1Ab protein from GM maize tissues, may be a potential carrier of this protein to non-target predators and should therefore be included in the studies on the ecological risk of GM plants cultivation. Genetically modified MON 810 maize does not have an indirect, negative impact on the biology of *Phytoseiulus persimilis* which feeds on red spider mites from GM plants [18].

No ecological threat by the crops with MON 810 modification to predatory arthropods feeding on bird cherry-oat aphids has been shown. This phytophagus does not constitute an exposure path of the insecticide Cry1Ab protein on the non-target entomophagous organisms [19, 20].

The studies on the impact of the MON 810 maize pollen on the caterpillars of the Small White found high sensitivity to the presence of maize pollen in the caterpillars' diet, regardless of the maize variety. However, additional laboratory tests that we carried out in the years 2010-2011 with the use of the pollen of the MON 810 variety have confirmed its moderate toxicity to the caterpillars of the Small White.

In their most recent publication a team of Swiss researchers, who have studied the risk of GM plants to non-target organisms for many years, emphasised the difficulties of carrying out the applicable laboratory tests on the impact of GM plants on non-target organisms and the need to be careful when making conclusions [21].

At the same time, the studies carried out by the Authors of this publication have confirmed that it is essential to quantify the Cry1Ab protein content in the analysed maize plants in order to interpret the results of laboratory and field tests. As of the moment of commencing this study, the literature of the subject has not yet emphasised the role of the protein exposure level in various organs of GM plant for the non-target organisms. As of now, the impact of the maize cropping conditions on the expression of the toxic protein in specific plant tissues has been documented. Therefore, it is recommended for the risk assessment to take into consideration the quantitative data on the expression of the transgenic products in various organs of the GM plant.

The first farm scale assessment of the impact of MON 810 maize on non-target organisms was initiated in Poland in 2008 under the programme "Environmental and economic aspects of GM crop cultivation in Poland", coordinated by the Plant Breeding and Acclimatization Institute – National Research Institute. The main focus of the assessment was the impact of genetically modified plants on selected species of *Carabidae* and *Staphylinidae* beetles that play an important role in maize agrocenosis. The analysis of the results of three-year farm scale study carried out in the vicinity of Łańcut and Wrocław (where the European Corn Borer causes substantial economic damage) has confirmed the conclusions from the first year of study on the absence of negative impact of the modified maize crops on the analysed groups of epigenetic beetles [22]. Those results will also be used in the analysis of the selection of indicator species on the basis of their nutritional preferences for the purposes of the future assessment of the impact of maize varieties with other transgenes giving resistance to pests and herbicide-tolerance.

Benefits and risks for the agrocenoses, arising from GM crop cultivation

The analysis of benefits and risks of GM crop cultivation must take into consideration not only the nutritional needs of the expanding human population, but also the environmental aspects and more needs for the implementations of sustainable agriculture policy. The GM plants play an important role in increasing crop output, at the same time reducing the production input. James [23] reports that the global economic profits at the farm level amounted to $65 billion in the years 1996÷2009, of which 45% originated from the reduction of production inputs (reduced number of agrotechnical procedures, less ploughing, reduced number of plant protection activities and smaller workforce input) and 56% from additional crops, amounting to 229 million tons. The additional crops amounted to 83.5 million t of soybean, 130.5 million t of maize, 10.5 million t of cotton and 4.8 million t of rape.

In 2010 in China 6.5 million farmers working on farms with the average area of 0.6 ha grew cotton resistant to caterpillars feeding on flower buds and cotton fruits. It was calculated that growing resistant varieties had reduced the total population of those caterpillars in the cotton cultivation regions as compared to conventional varieties, which brought additional profit of $4.2 billion [23].

Similar calculations were made for Bt maize crops resistant to European Corn Borer in the United States, indicating that in 2010 those varieties were grown on the area of 22.2 million ha., which amounted to 63% of the total maize cultivation area. It was concluded that the observed general reduction of the population of European Corn Borer, both in Bt and conventional crops was related to growing varieties resistant to this pest. The reduction of losses caused by this pest in 5 states (Illinois, Iowa, Nebraska, Minnesota and Wisconsin) in the years 2006÷2009 brought additional profit of $6.9 billion, with $4.3 billion (approx. 62%) originating from reduced losses of conventional crops in those states.

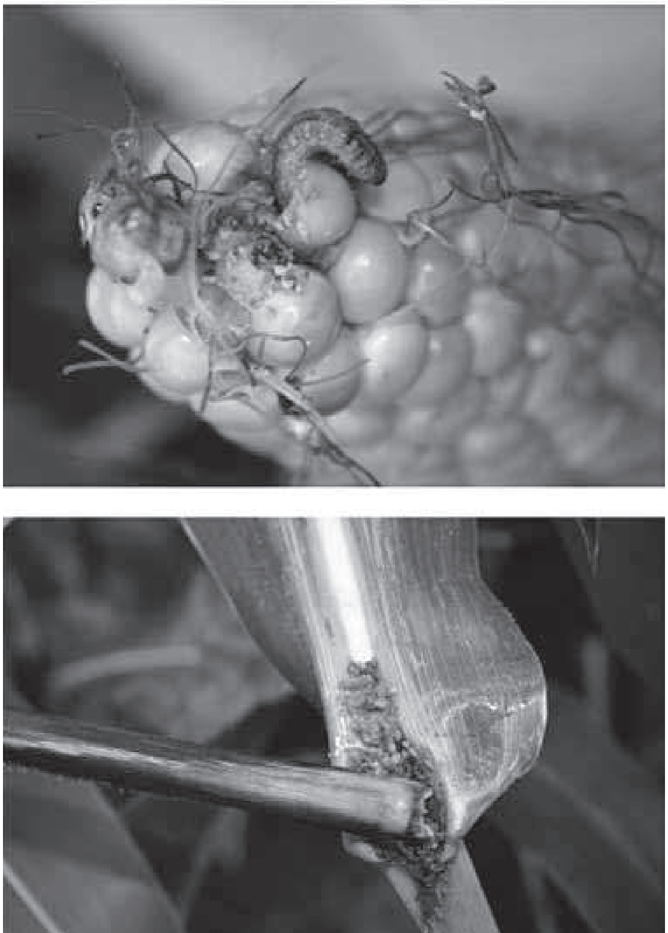

Fig. 2. Damages to maize conventional variety caused by European corn borer (*Ostrinia nubilalis* (Hübner)). (fot. S. Sowa)

It was proven that growing pest-resistant varieties is less expensive, thereby reducing the farmers' expenditures, does not kill natural enemies of those pests and reduces the content of other harmful products, such as aflatoxins produced by pathogenic fungi and bacteria growing on plants affected by the pests (Fig. 2). Due to those beneficial properties, the Bt maize varieties have been quickly accepted by farmers first in the United States and then in other parts of the world.

In the years 1965 ÷ 1970 in Poland the European Corn Borer was considered a pest causing occasional economic damages to maize crops in south-western Poland. Currently the territorial range of this pest has increased considerably northwards. The European Corn Borer now causes substantial damage not only in southern regions of the country, but also in Greater Poland and Lubelskie Voivodeship.

According to the experiments carried out in southern Poland, where the European Corn Borer can affect as much as 70% of crops and stimulates the growth of pathogenic fungi which produce very dangerous mycotoxins, the MON 810 maize varieties provide complete protection against this pest [24]. The results of farm scale analyses carried out by entomologists [25] have confirmed that preventing the European Corn Borer caterpillars from damaging the plant has reduced the infestation rate of grains by pathogenic fungi. The currently recommended methods of European Corn Borer manage are based on chemical control during the butterflies' egg-laying period. The efficiency of the biological method, comprising the use of biopreparations based on the *Bacillus thuringensis* bacteria or the release of *Trichogramma* wasps bred on a mass scale, depends on a number of climate and biological factors. In practice this method is unreliable. In the countries of Western Europe farmers growing maize on large areas perform the pest control using special high-wheeled sprayers (each worth several dozen thousand EUR). In Poland pest control is performed manually, if at all, when maize rows reach the height of 1.5-2 m, or using home-made sprayers (without applicable certificates, potentially hazardous for the operator).

Also, the benefits of growing herbicide-tolerant varieties should be considered. In a study carried out in Great Britain it was shown that over 95% of crops were treated with plant protection chemicals (PPC – herbicides, fungicides and insecticides, in Great Britain still collectively referred to as pesticides) and growth regulators. A common practice was to use 6-8 different PPCs on crops (e.g. 2-3 herbicides, 3 fungicides and 1 insecticide). In the study on GMHT varieties of beet and rape, carried out in Great Britain, the number of herbicide applications was reduced by 50% as compared to conventional crops. In the crops of sugar and fodder beets herbicides were applied later and only once to 66% of crops. Weed on GMHT fields appeared later, were smaller and their volume was 23% lower than on conventional fields. Thus, their biomass was lower [26].

The number of herbicide applications in studies on growing GMHT maize and conventional varieties did not differ significantly, but the volumes of the active substance used were approx. 50% lower in GMHT crops. In the crops of the analysed varieties of the spring rape the volume of the active substance used did not differ significantly in both types of crops [27]. The volume of active substance used on rape crops was approx. 50% of the volume used on beet and 80% of the volume used on maize.

The report "The farm level impact of using GM agronomic traits in Polish arable crops" by G. Brookes, Ph.D. and Professor A. Anioł [28] lists the following benefits of growing herbicide tolerant varieties in Poland:

- studies on glyphosate-resistant rape conducted in Poland have shown that a 15-20% increase in yield of this crop is possible
- given the results of farm scale analyses carried out in Poland, the expected crop yield increase should be 15-30% and the costs of direct pest control should be reduced.

At the same time, the authors emphasise that the possibility of glyphosate-resistant self-sown plants and weeds cannot be excluded. This problem will be much less important with sugar beet than with rape [28].

The necessity of following Good Agricultural Practices

Growing GM crops will not be the solution to the problems of agriculture without following the Good Agricultural Practices. Studies over the past 16 years have proven that unfavourable consequences of mass cultivation of GM crops can be avoided using the following methods:

- applying crop rotation, since otherwise there is a risk of emergence of glyphosate-resistant weeds
- applying glyphosate in recommended doses. Doses higher than recommended increase the selection pressure, also on varieties tolerant of this herbicide
- maintaining refugia in pest-resistant crops, i.e. sow conventional varieties on up to 20% of the field (where the potentially resistant pests will cross with the sensitive ones)
- check regularly for the emergence of specimens resistant to the toxic protein in the population of the pest. Long-term studies carried out in Europe indicate a very low level of alleles responsible for overcoming resistance. Even assuming mass cultivation of maize crops resistant to European Corn Borer caterpillars, the genetic simulations indicate that within the next 10 years no resistant population of this pest is expected to emerge in Europe
- eliminating intensive chemical protection with pesticides with broad spectrum of impact on caterpillars feeding on maize or cotton may lead to the emergence of the so-called secondary pests that thus far have been eliminated together with the main pests. Therefore it is recommended to periodically change the applied insecticides and monitor the role of other pest groups.

The role of the European Food Safety Authority in GMO risk assessment

The requirements concerning the conditions of GMOs release into the environment in the EU Member States are provided in Directive 2001/18/EC [29], which separates the environmental risk assessment from the monitoring plan (EC 2001). The objective of the environmental risk assessment is to identify and assess the potential consequences of releasing GMOs into the environment that may be harmful to human health and the environment. Such assessment is carried out for each GMO individually and includes direct, indirect, immediate or delayed consequences of marketing the GMO. The assessment may also require consideration of long-term effects on other organisms and the environment.

The European Food Safety Authority (EFSA), through its GMO panel and committees of independent experts appointed by Member States, has drawn up detailed requirements for the scientific data to be provided in the dossier of institutions or persons applying for marketing GMOs. The most recent recommendations are provided in the guidelines for the risk assessment of releasing new GMOs into the environment (EFSA 2010) [30]. The guidelines have taken into consideration several hundreds of comments provided over a year and a half by government institutions, scientists and independent social organisations (EFSA 2009) [31]. According to the recommendations, the potential risk assessment of releasing GMOs into the environment should be carried out by comparing the given modified plant to its original, unmodified parental form. The observed differences in the properties of a GM plant may be the effect of the introduced transgene or its product, or arise from changes caused by transgenesis. The impact on non-target organisms should be assessed with reference to: (a) **species** and (b) **processes in the ecosystem** that should be protected and sustained, but might be subject to negative impact of the GM plant. When selecting species for the risk assessment, the applicant (e.g. a biotechnological company) should first determine the key processes related to biological diversity linked to the given GM plant. The analysis of those processes should first of all include:

- the role of various groups of agrophages in the food chain of the given crop
- the impact of natural enemies on agrophages
- activity of pollinators
- processes of decomposition of dead biological matter and the circulation of nitrogen and phosphorus in the given crop.

Next, all known species and ecological processes important for the functioning of biological diversity should be subjected to transparent procedure based on the synthetic analysis of multiple properties [30, 32]. For instance, for the organisms performing functions important to the sustainability of biological diversity the following steps should be taken:

- gather available information on the species composition of fauna in the given crop
- analyse the role of those species for the functioning of the ecosystem
- establish the order of priority for particular species, according to their role in the environment.

Important properties to be considered in the selection of indicator organisms are the economic role, aesthetic or cultural value, such as the Monarch butterfly (*Danaus plexippus* [L.]) in the United States, as well as whether the given species is under protection or threatened by extinction.

When establishing the order of priority for the environmental risk assessment, the following associations between the non-target organisms and GM plants should be considered:

- exposure of the given species in field conditions to the genetically modified property taking into account all stages of development exposed to the impact of this property
- sensitivity of the given species and its stages of development to the products of the modified property
- association of the given species with the target species in the trophic systems of the given crop
- abundance of the given species in the agrocenosis
- susceptibility of the species to the current stress factors in the environment (e.g. certain shrinking populations that might be further affected by the additional stress factor)
- associations of the given species with the environment, including natural and semi-natural habitats.

In spite of numerous studies on the impact of GM plants on the non-target species in the European Union countries, many NGOs and some scientists believe that further research is required by institutions financed from public funds.

The basics of GMO detection – objectives, control mechanisms and reference laboratories in Europe

One of the conditions of approving a given GMO for growing in the environment or for other use in the European Union is the possession of a method allowing detection and monitoring of such organism in the environment.

The law of the European Union requires labeling and control of GMO products (Regulations (EC) 1829/2003 and 1830/2003) [33, 34]. The EU legal regulations on GMOs assume the development of reliable and sensitive methods for detection and quantification of GMOs. The GMO detection methods can find DNA sequences of modified organisms, the products of those modified genes, i.e. transgenic proteins or other substances present in the plant due to modification (e.g. genetically modified fatty acids). With the approved GMOs already on the market, the implementation of new genetic modifications and the need to detect unauthorised transgenic organisms, modern methods of detection and quantification of GMOs are essential. Those methods must be characterised by high sensitivity, repeatability and reproducibility. Qualitative and quantitative analyses of GMOs are carried out on raw materials such as feed and seeds, as well as on highly processed materials, such as food [35].

The nucleic acids, in particular DNA, are the main objective of qualitative and quantitative analyses of genetically modified organisms. In order to enable the analysis of the GMO, the searched molecules must not be completely degraded. Therefore, the final effect of the analysis is highly dependent on the degree of processing of the material, proper isolation of DNA or proteins and the availability of certified reference materials that are used as positive control [36].

The DNA of genetically modified plants is usually detected with the use of PCR (Polymerase Chain Reaction) which allows for reproducing specific sequences of the transgene in a single enzymatic reaction. The PCR test may be designed to detect every element of the construct – the promoter, the target gene, the terminator sequence, the marker, or event-specific sequences specifically related to the transformation. In order to determine the presence of modifications in the given sample, the Real-time PCR technique is usually employed. In this method the increase of reaction product during the reaction is analysed. During the reaction specific transgenic sequences are reproduced and their quantity is assessed with respect to the endogenous reference gene which contains information on the presence of the DNA of the given species in the sample. In the quantification of GMOs the Real-time PCR system requires primers and probes specific for the transgene, as well as species-specific primers complementary to the endogenous reference gene.

In 2002 the European Network of GMO Laboratories (ENGL) was created in the UE to address GMO-related issues in Europe in cooperation with the European Commission. One of the objectives of ENGL is to develop new methods of detection and quantification of GMOs, as well as to validate methods submitted by companies applying for GMO authorisation in the EU.

Control of the application of European provisions and the Polish Act on GMO is the responsibility of laboratories of national inspectorates, and laboratories set forth in Regulation (EC) No. 882/2004 [37]. In Poland those laboratories are: National Laboratory for Feedingstuffs Research Institute for Animal Production – National Research Institute in Lublin, Laboratory of the Department of Hygiene of Animal Feedingstuffs of the National Veterinary Research Institute in Puławy and Regional Laboratory for Genetically Modified Food Analysis in the Sanitary Inspectorate in Tarnobrzeg.

Additionally, the Commission Regulation (EC) No. 1981/2006 [38] lists the reference laboratories in the Member States that, together with the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF), are responsible for analysing and validating the methods of GMO detection and identification. Those laboratories are obligated to participate in the validation of GMO methods submitted for authorisation by biotechnological companies. In Poland, aside from the units listed above, those laboratories are: Laboratory of Genetic Modification Analyses at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw, and Genetically Modified Organisms Controlling Laboratory at the Plant Breeding and Acclimatization Institute – National Research Institute in Radzików.

The Genetically Modified Organisms Controlling Laboratory at the Plant Breeding and Acclimatization Institute – National Research

Institute is a member of ENGL. The laboratory assists EURL-GMFF and provides scientific data for the European Commission. The Genetically Modified Organisms Controlling Laboratory carries out plant material analyses, including detection and quantification of GMOs, and conducts trainings and consultations on genetically modified organisms. The laboratory implements research projects concerning: the development of qualitative and quantitative assays of GMOs and their validation, and the assessment of environmental and economic impacts of introducing GMOs into the environment.

Summary

Similarly to other European countries, the society in Poland differs on the subject of benefits and risks related to introducing genetically modified plants. In the scientific community the majority of researchers postulate objective risk assessment, based on independent research. Only four members of the Division of Biological and Agricultural Sciences abstained or voted against the draft of the official position of the Polish Academy of Sciences on genetically modified organisms (Resolution no. 2/2012 of the Plenary Session of Division 2 of Polish Academy of Sciences, 18 May 2012). The document justifies the use of biotechnology in agriculture in the following manner (quot.):

- "Experts from the European Commission, OECD, FAO, World Health Organisation, a number of Academies of Sciences, including the Pontifical Academy of Sciences, consider the use of GMO benefits in the agriculture justified. (...) Genetically modified domesticated plants contribute i.a. to: 1. increase of production of feed and food
- 2. improving the nutritional and health values of food products, 3. reducing the power consumption and the volume of chemicals used in agriculture, 4. producing bioenergy, biomaterials and biomedications"
- "Genetic engineering, like any other new, breakthrough technology, is also seen as a potential threat to the natural environment and human health. The European Union has created a number of institutions, including the European Food Safety Authority, to control food and feed produced from GMOs and prevent any adverse consequences of using GMOs. The risk assessment procedures for new genetically modified organisms are constantly improved and provide for the demands of various social groups. Those procedures cover a broad scope of studies on the impact of GMOs on human life and health. Poland is no exception here"
- "After using GMOs for 30 years in the economy and for 15 years in the agriculture we have yet to see reliable and confirmed proof that GMOs have negative side effects. This also applies to fodder containing components of genetically modified plants, such as maize, linen and soybean"
- "Creating rational foundations for economic development in Poland requires adopting relevant legal regulations that would facilitate research and the development of bioeconomy. This should be accompanied by proper social education based on solid, reliable knowledge, since there are no scientific reasons to consider genetic modifications harmful in their own right".

The authors of this paper, having had ten years of professional experience with GMOs, would like to add that when discussing genetically modified organisms we have to look at them individually. The risk to the environment and human health is assessed for each genetic transformation separately. Therefore, both in Europe and in Poland according to the precautionary principles, in case of GM beet or rapeseed cultivation, the risk assessment must include the possibility of gene flow to the related species. This does not apply to maize, as it has no wild relatives in Europe.

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