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RISKS OF POLLEN-MEDIATED GENE FLOW FROM GENETICALLY MODIFIED MAIZE DURING CO-CULTIVATION WITH USUAL MAIZE VARIETIES
(review)

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Abstract

Since 1985, active development of agricultural biotechnology has been associated with genetically modified (GM) plants. After the production of GM maize in the second half of the 1990s, the area of its crops has increased over 100-fold. Therefore, the GM maize spreeding and cross-pollination have become more practically relevant. Almost one third of the total area of all GM plants is occupied by GM maize. The Russian Federal Law No. 358 of 03.07.2016 prohibits the commercial use of GM plants in agriculture but allows their cultivation and testing for research purposes. This necessitates assessing and developing criteria, currently absent in Russia, for the safe co-cultivation of non-GM and GM varieties. This review analyzes the factors influencing pollen dispersion: wind (speed and direction), humidity (rain), physiology (viability), the pollen amount, the character of the landscape, the size, shape and orientation of the recipient fields, and the synchrony of flowering of the pollen donor and recipient. Early studies of gene flow in cross-pollination were reviewed Y. Devos et al., (2005) and O. Sanvido et al. (2008). In particular, the distance between GM and traditional maize recommended in the EU countries, with the same threshold for GM content in food, varies considerably (from 25 to 600 m) (Y. Devos et al., 2009; L. Riesgo et al., 2010). In addition to the distance between crops and the synchronicity of flowering, the frequency of cross-pollination depends on the field size and orientation (M. Langhof et al., 2010). Estimates of the cross-pollination frequency and the pollen counts at different distances from the GM donor allowed the researchers to recommend isolation distances of 10 to 200 m. If the isolation distance cannot be ensured, the recipient and/or donor field should be bordered by a barrier to pollen. In the recipient field, the outer rows of maize plants can be the barrier. After a 10-20 m maize barrier, almost none of the analyzed samples contains more than 0.9 % of GM material. For recipient fields of less than 1 ha in area and/or low-depth fields, an isolation distance of at least 50 m should be recommended, especially in the wind rose direction. Data on spreading GM maize with pollen in Europe, South America, Africa, and Asia provide recommendations for safe co-cultivation of non-GM and GM maize varieties and lines. The cytoplasmic male sterility (CMS) approach for GM- and non-GM maize co-cultivation was developed. The genetic control of CMS (N-, S-, C-types and CRISPR-mediated approach) and the CMS application history are discussed. For CMS hybrids, the isolation distances between GM and traditional maize crops may be significantly reduced (up to 10 m) without violation of the European requirements of a 0.9 % marking threshold. However, GM-maize with CMS is not used for practical cultivation. Russia has yet to develop its own measures and recommendations for the joint cultivation of GM and traditional maize.

Keywords: genetically modified corn, gene flow, pollen, CMS, GM crop co-cultivation, GMO regulations

Derivation of varieties by genetic engineering (GE) methods and their practical application have been actively developing as areas of agricultural biotechnology since 1985. Large-scale industrial production of genetically modified (GM) plants, particularly maize, has begun in 1996. The area of cultivation of agricultural GM crops since the beginning of their use has increased by more than 100 times – from 1.7 million hectares in 1996 to 185 million hectares in 2016, which amounted to about 12% of the world's acreage [1]. In 2017, GM varieties with herbicide resistance (either alone or in combination with insect pest resistance) were grown globally on a total of 166.4 million hectares [2].

Corn (*Zea mays* L.) is one of the most common crops, the production of which in the last 10 years in the world has grown from 600 to 1000 million tons. It adapts well to high temperatures, which in the context of climate warming creates the potential to expand areas of cultivation, including in Russia, where, according to the Federal State Statistics Service, almost 9 times more corn was grown in 2018 than in 1995 (http://www.gks.ru/free_doc/new_site/business/sx/val_1.xls). Among GM crops, maize is the second largest in the world after soybeans in terms of the crop area and the first in terms of harvest. In 2015, GM corn accounted for 53.6 million hectares or almost a third of the total crop area (185 million hectares) in the world [3]. In other words, there is a danger of gene transfer from GM corn to non-GM corn when they are grown together.

According to the Convention on Biological Diversity, 1993 (United Nations 1992; came into force in 1993), each participating country must develop a strategy and program for the preservation and use of its biological resources with their guaranteed and safe reproduction [4]. In particular, it is necessary to establish and approve ways and methods of regulating, managing and controlling the risks associated with the creation, use and distribution of GM varieties, as well as to develop methods for assessing possible risks in the cultivation of GM varieties for biodiversity preservation [5].

Russia is one of the countries that banned the commercial cultivation of GM crops (Federal Law dated July 5, 1996 No. 86-FZ "About State Regulation of Genetic Engineering Activity", Federal Law dated July 3, 2016 No. 358-FZ "On Amendments Being Made to Particular Legislative Acts of the Russian Federation to Improve State Regulation of Genetic Engineering Activity"). Federal Law No. 86-FZ (1996) initially did not provide for the registration of GM varieties and the output of products based on them; but 10 years later, 15 GM crops were studied and allowed in Russia: 8 lines of corn, 3 lines of soybean, 1 line of sugar beet, 1 line of rice and 2 varieties of potatoes (Letter of the Federal Service for Supervision of Consumer Rights Protection and Human Welfare "On Improving the Supervision of Food Products Containing GMO and GMM" dated August 20, 2008 No. 01/9044-8-32). Effective since 2018, Federal Law No. 358 also prohibits the commercial use of GM plants in agriculture but permits their cultivation and testing for scientific and research purposes. Consequently, a real necessity to develop criteria for the joint safe cultivation of untransformed and GM crops, including maize, taking into account the tasks of biodiversity preservation, appeared in Russia. This development includes the analysis of the best practices of experimental evaluation of potential risks in the cultivation of GM plants. This problem has many different aspects, such as considering the effects of herbicides and insecticides on non-target plant and insect species during cultivation of GM crops [6], but in this review, the material will be focused on evaluating the risks of distribution of GM corn with pollen in co-cultivation of untransformed and GM maize varieties, which has not been studied in Russia.

Expansion of GM corn pollen in field conditions. Maize is a monoecious wind-pollinated species; plants reach a height of 3 m (sometimes up to 6-7 m), which increases the risk of spreading pollen of GM varieties carried by the wind for tens and hundreds of meters [7, 8]. Pollen transfer is influenced by many factors: wind (speed and direction), rain (humidity), physiology (viability), pollen quantity, landscape character, size, shape and orientation of the recipient field, the synchronicity of flowering of the pollen donor and recipient [9].

Pollen viability. Pollen viability (ability to germinate) is an important condition for cross-pollination. After leaving the anthers, corn pollen is viable for 1-24 hours [10, 11]. In the moderate climate of Europe (France), pollen viability is maintained up to 24 h [11]. Depending on atmospheric conditions in Iowa (USA), pollen germination decreased by 50% after 1-4 h [12]. In many years of research in the arid conditions of Mexico (area of Nayarit), a decrease in the pollen viability outside by 80% for 1 h and 100% for 2 hours was noted; in the driest conditions, 100% of pollen grains became unviable within 1 h [12].

Wind. Air flows during pollen expansion can lift pollen high into the atmosphere and distribute it over considerable distances. Pollen viability decreases with altitude, but at higher altitudes, lower air temperatures favor the maintenance of pollen viability. The main horizontal flow of pollen in corn was observed at an altitude of 6.5 m, and it was the same at a distance of 3-10 m from the source [13]. A small amount of pollen was recorded at a distance of 800-1000 m from the source, while the settling rate of pollen ranged within 0.0002-0.001 grains/($m^2 \cdot s$) [13, 14]. On average, the frequency of cross-pollination (% of grains) was the same at a distance of 28 m down the wind and at a distance of 10 m against the wind [15].

Wind speed is the main variable that determines the amount of pollen dispersed after the release of pollen grains from anthers. The time and distance of the pollen grain fall on the snout of the recipient plant depend on gravity, on the one hand, and turbulence and airflow, on the other [16]. Corn pollen grains in comparison with pollen of other wind-pollinated species are relatively large (average diameter 90 microns) and heavy (0.25 rg), so corn pollen has a high settling rate [14, 17]. About 95-99% of the pollen is set at a distance of about 30 m from the source. At a distance of more than 30-50 m, its amount is significantly reduced, but the clear distance beyond which it is not detected is unknown [13, 14, 17]. The relative density of pollen in experiments in Massachusetts (USA) fell to about 2% at a distance of 60 m from the edge of crops and remained within 0.50-0.75% at a distance of 500 m from the donor culture [18]. In another paper, it was reported on the settling rate of pollen downwind at a distance of 10 m from the source – 10-100 grains/($m^2 \cdot s$), 800 and 1000 m – 0.001-0.0002 grain/($m^2 \cdot s$) [13]. In a study carried out in Mexico, no correlation between wind speed and cross-section percentages was observed, meaning that the role of wind speed may not be relevant to cross-section frequency [8].

Humidity, rain. In the air stream, pollen can be captured by water droplets and/or fall on wet flowers, where it bursts and dies. As a rule, corn pollen falls out of anthers in dry conditions, mainly from morning to noon [13, 14]. When it rains, pollen release is delayed because anthers do not crack in wet conditions [13, 14]. However, the published data to quantify the impact of rain on anthers opening and maize pollen flow is not still available.

Field dimensions of the GM pollen source and the recipient field. A cloud of pollen of recipient plants over the field of the recipient competes with donor pollen. The larger the field, the greater the mass of the recipient's pollen. Field tests have shown that for a donor of pollen with a given size, the cross-pollination frequency decreases as the size of the recipient field increases [14, 19, 20]. This indicator, expressed as a percentage of GM plants, decreased from 1.8% to 0.8% when the size of the pollen recipient field increased from 0.25 to 1 ha [19]. The results of research with fodder corn in Germany, when the donor's fields of GM corn were surrounded by an isogenic non-GM variety, confirmed these results [14]. However, later in Germany, it was found that the size of the donor field 0.3-23 ha did not affect the frequency of cross-pollination [21]. In experiments in Spain, the size of the donor field of corn influenced the number of GM grains found in the recipient field, but to a lesser degree than the size of the recipient field [22].

Shape and orientation of pollen donor and recipient fields. The study of the influence of the shape of the fields of recipients and pollen donors showed that the volume of their cross-pollination can be easily reduced by changing the square shape of the recipient field to a rectangular of the same area and placing it accordingly [14, 23]. For example, if a 5-hectare recipient field had a long side facing the pollen source, the cross-pollination frequency was 10.7%, if the short side – only 3.4% [24].

Synchrony of the donor and recipient flowering. Synchronization of pollen dispersion and snouts release is crucial for determining the survival rate of corn pollen [14, 25]. The better the synchronicity between pollen donor flowering and the snouts release of the recipient plant, the higher the probability of cross-pollination [11, 14, 25, 26]. Different sowing times can lead to a difference in flowering time, limiting cross-pollination. In Spain, the difference in sowing time on average of 1 week reduced the frequency of cross-pollination in the first row of recipient fields by 50%, 3 weeks – by 75% [20, 27]. In Mediterranean European countries, this approach can be applied without crop losses [20, 27].

Barriers to Pollen. Plants around the source or recipient of pollen can act as barriers. Thus, the presence of several rows of taller plants on the outside of the donor field reduces the frequency of crosses, i.e., the effect is similar to increasing the distance to the recipient plants. In addition, barriers introduce competing pollen if they are plants of the same species and/or physically impede pollen transfer through the air by affecting flows and filtration [18, 28]. A barrier of corn and trees reduced cross-pollination (immediately behind the barrier) by 50% more efficiently than the sown earth [14]. A barrier of tall sorghum plants reduced the safe distance of the joint cultivation of GM and non-GM corn from 35 to 20 m with a threshold value of the frequency of cross-pollination below 0.9% [29].

Landscape influence. The highly diverse nature of the landscapes of the European Union (EU) suggests that measures to ensure the coexistence of GM and non-GM plants should be adapted to regional conditions under the SIGMEA program for the sustainable introduction of GM crops into European agriculture. In 2010, four landscape-specific maize growing zones in Alsace and Aragon (France) were compared and regional landscapes were classified by risk degree for different threshold values of 0.1; 0.4, and 0.9% [30].

Risk analysis of the spread of GM corn varieties with pollen in different countries. *Europe. EU Countries.* Directive 2001/18/EC dated March 12, 2001 on the deliberate release of genetically modified organisms into the environment is in force in the European Union and was substantially amended in 2015. Directive 2015/412/EC established the right of EU member states to restrict or prohibit the cultivation of GMO approved in the EU in their territories. The prohibition can be imposed not only for scientific but also for political, socio-economic reasons. Permission to grow a GM crop can be obtained only after an individual assessment of the potential danger of each GMO to human and animal health.

A study in 2000-2003 in 15 counties of England showed a quick reduction in the rate of cross-pollination in corn at a distance of the first 20 m from the donor culture; however, marker genes of GM varieties of fodder and sweet corn were detected by PCR at distances of 80 and 200 m [31, 32]. SCIMAC (Supply Chain Initiative on Modified Agricultural Crops, UK), an organization established to regulate the use of GM plants in England in the production of agricultural products, recommended a separating distance of 80 m for the joint cultivation of GM and non-GM corn varieties [31].

Due to the inclusion of 17 GM corn varieties in the EU catalogue of crop varieties on September 8, 2004, an increase in the commercial acreage of transgenic maize was expected in some countries [14] and thresholds (0.9%) were set for allowing the accidental and technically unavoidable presence of GM material in non-GM products. To reduce pollen dispersion, the EU has adopted a regulation that establishes 200 m as the minimum isolation between the pollen source and the recipient field (with the use of physical barriers – 100 m) [14]. The distances originally proposed by EU member states for isolation of traditional and GM corn ranged from 15 to 800 m [33].

An empirical one-dimensional model of gene flow mediated by corn pollen allowed establishing the practice of growing GM and traditional plants while maintaining the threshold value of 0.9% for GM grain in non-GM grain [34]. Based on the model describing the decrease in the rate of germination of pollen grains in the atmosphere, it can be assumed that the decrease in the settling rate is accompanied by a decrease in the viability of pollen [35]. A simple dispersion model is proposed to illustrate the possible effects of changes in the deposition rate and germination rate on pollen propagation and cross-pollination of corn. The results show that modern pollen propagation models that do not take into account these changes overestimate cross-pollination rates [35]. An approach to modelling pollen-mediated gene flow from multiple sources is proposed [8]. It is based on generalized linear mixed models that quantify variability across years and locations to determine which isolation methods will effectively meet the 0.9% threshold. Data for the new model were obtained from a database of experiments conducted in 2000-2010 in Canada, France, Germany, Italy, the Netherlands, Poland, Romania, Slovakia, and the United States. According to this model, for 1 ha of a non-GM field, surrounded from all four sides by GM fields, 12 m of border rows in combination with a 12-meter band out of crop are enough to ensure a threshold of 0.9% (at the 95% significance level) established by the EU [9]. Based on a comparison of four methods for assessing the presence of GM corn in the fields of non-GM recipients with mathematical processing of the results, a more reliable method for recording GM crosses over the entire depth of the field was proposed [36].

Cultivation of GM corn in Europe until 2005 was limited to Spain (about 58 thousand hectares in 2004). Since 2005, GM corn varieties have been cultivated in the Czech Republic, Slovakia, France, Germany, and Portugal [36]. However, since 2008, France has prohibited the cultivation of the GM corn variety MON810 resistant to insect pests [33]. Germany, after further research and public debate, also prohibited the use of the MON810 on its territory [37]. GM corn crops in the Czech Republic and Slovakia have only declined since 2008 until a total prohibition in 2017 [2]. In Portugal and Spain to 2017, the area under GM corn decreased by 10% relative to 2016 (https://www.infogm.org/6391-europe-GMO-drop-of-transgenic-crops?lang=fr).

Italy. In Italy (Lombardy), experiments were conducted to study the effect of different flowering periods of the GM donor and non-GM recipient of corn on the number of GM plants in the recipient's crops. A slight or complete absence of pollen flow reduction was found with the difference in flowering time between the source and the recipient up to 3 days. For the 4-5 days period, the flow of pollen decreased by 25%, for 6 days — by 50%, for more than 7 days — by 100%. The cross-pollination threshold (0.9%) was reached at a buffer zone of 25 m, with corn plants being a more effective barrier rather than fallow (out of crops) lands [38].

Romania. In 2008-2009, the distribution of GM corn pollen of the MON810 variety was studied in the South (Călărași district), East (Brăila district) and West (Timiș district) of Romania to assess potential risk [42]. A plot sized 300×250 m planted with transgenic plants was surrounded by crops of non-transgenic seeds. The average distance at which the content of GM grains was less than 0.9% in all four directions (north, south, east, west) was 20 m in 2008, and 25 m in 2009 [39]. On this basis, for the spatial isolation of commercial GM varieties, the authors considered a distance of 35 m sufficient. In 2016, Romania stopped growing GM corn [2].

The Netherlands. In 2006-2007, field tests of the effect of the two insulating distances (25 and 250 m) specified in the Dutch Coexistence Committee (Holland) resolution were conducted in three regions of the Netherlands [40]. A field with the genetically modified donor (DKC3421YG, a modified version of the MON810 variety, 1 ha) was surrounded in four directions by fields (0.25 ha) with traditional corn (variety DKC3420), located at a distance of 25 and 250 m. PCR analysis showed the presence of 0.080-0.084% GM grains for 25 m distance and 0.005-0.007% for 250 m.

It is important to note that the European Union has adopted a stricter regulatory framework for GM plants compared to other countries [41], despite the fact that Europe grows less GM corn than on all other continents except Australia, although, according to Italian scientists who analyzed 1,783 publications for the period from 2002 to 2012, no direct threat from the use of GM plants was revealed [42]. Directive 2015/412/EC of the European Parliament and the Council dated March 11, 2015, as already noted, allows the EU member states to determine independently the amount and rules of cultivation of GM plants [43]. By October 2015, 19 of the 28 EU countries had applied to abandon the cultivation of GM corn MON810 [44]; by 2017, this variety was grown only by Portugal and Spain [45].

Russia. In 2012, the Russian Federation approved the Comprehensive Program for the Development of Biotechnology (Including Agricultural Biotechnology) for the Period up to 2020 [46]. In 2013, Russia ranked 12th in the world in corn production [47]. As per the Federal State Statistics Service, in 2016, the yield of corn grain was almost 9 times higher than in 1995 (respectively 15.0 million tons against 1.7 million tons), but over the next 2 years it decreased, reaching 13 million tons in 2017, and 11 million tons in 2018 (http://www.gks.ru/free_doc/new_site/business/sx/val_1.xls). At the same time, the acreage for corn in the period from 1995 to 2018 in Russia, according to the Federal State Statistics Service, increased by nearly 4 times (from 643 thousand hectares to 2452 thousand ha) (http://www.gks.ru/free_doc/new_site/business/sx/posev_pl1.xls).

Currently, GM corn in Russia is not officially grown, although in 2008, 8 lines of GM corn of American origin were ready for mass production (Letter of the Federal Service for Supervision of Consumer Rights Protection and Human Welfare “On Improving the Supervision of Food Products Containing GMO and GMM” dated August 20, 2008 No. 01/9044-8-32).

For comparison, the area of the world's biotechnological crops increased from 1.7 million hectares in 1996 to 189.8 million hectares in 2017 (by more than 100 times) [48]. In 2016, the total world income of farmers from using GM corn amounted to 2.1 billion USD, the total return for the years 1996-2016 was 13.1 billion USD, among which 4.5 billion USD (34%) was due to increased yield, the rest – due to the reduction of production costs [49].

The review by Zhuchenko [50] presents an analysis of the risks associated with the creation, cultivation, and use of GM plants in agriculture. The limitations of genetic engineering, as well as possible evolutionary, biological and environmental consequences of the widespread use of genetically modified organisms, are considered. It is important to note that some of the limitations and dangers of genetic engineering mentioned in the review (in particular, the lack of site-directed gene integration methods, or insertion of introduced genes into a specific host DNA site) have already been overcome by modern CRISPR/Cas technologies. For modeling the microevolution of a population, in which the GM species are presented, Zhivotovsky [51] considered two scenarios of the behavior of mixed populations depending on the adaptation potential of GM plants. According to one scenario, GM plants that carry useful adaptations (for example, heat and salt resistance) may gain an advantage over other populations for a short period of time and displace them, but then die due to their own narrow adaptation with a significant and varied change in conditions ("Trojan horse hypothesis"). Similar conclusions were given earlier by other authors [52]. However, experimental risk assessment of GM corn distribution in Russia has not been carried out; criteria and recommendations for its safe cultivation together with untransformed varieties in Russia are absent.

North America. Mexico. Mexico is considered the center of origin and biodiversity of corn, the domestication of which began in its territory more than 8 thousand years ago [53]. In Mexico, more than 9 thousand species have been created and stored [54], including the populations of subspecies of corn (*Zea Mexicana, Z. parviglumis*) and teosinte plant species that participated in the breeding of modern corn in Mexico [55].

As early as 2001, GM corn imported from the United States was found in local corn populations in Northern Mexico (Oaxaca state) [56], although no GM corn was detected in a later 2-year study of 125 Oaxaca fields using PCR [57]. However, no more than 10 plants (corn ears) from each field were analyzed and combined samples (from 300 to 810 and even 5,630) were used [57]. The authors also do not indicate at what distance the studied fields were from the fields where GM corn varieties were tested in Mexico in 1998. Experimental testing of the ability of donor corn pollen to spread and pollinate recipient plants in Mexico showed the highest frequency of crossing near the pollen source – 12.9% at a distance of 1 m. This value fell sharply to 4.6; 2.7; 1.4; 1.0; 0.9; 0.5 and 0.5% as the distance increased, respectively, to 2, 4, 8, 12, 16, 20, and 25 m. At a distance of more than 20 m, the crossing frequency at all points was below 1% [8]. It should be noted that in Mexico, unlike many other countries, the additional factor of increased risk of uncontrolled spread of GM corn is active: during cultivation, small-scale farmers traditionally exchange grain for planting and sow mixed populations of corn (the so-called creolization) [58]. The observed distribution of GM corn in Mexico under the described conditions showed that in sowing 5 hectares of GM corn in each of the 13 regions, in 10 years, GM plants will be present in 87.85% of all crops [55].

To protect the genetic resources of corn in Mexico, the GM Biosafety act was drafted in 2008, which provides for the establishment of isolation zones for areas that are considered centers of corn origin; the protection of species for which Mexico is considered a center of origin and genetic diversity; the use of isolation distances (250 m) for experimental field studies with transgenic corn, with additional restrictions in regions identified as centers of origin [8]. Since 1996, Mexico has approved 170 biotech crops for food, feed, and cultivation, including 75 GM corn lines [2]. In 2017, Mexico approved five GM corn lines for production.

The USA. The marked gap between American and European approaches to GM crops is observed. The USA has 1.4 times more arable land than the EU, with almost 600 times more land allocated to GM crops [45]. This huge difference reflects the different attitudes towards genetically modified organisms (GMOs) in Europe and the United States. In Europe and America, regulation is based on fundamentally different models of risk assessment inherent in GMOs. These approaches are called process-based (principle-based) and product-based [59]. In the first case, the risk character is recognized for the process of genetic modification, in the second – for its result, the object (GMO or derivative products) [60]. Legislation of all countries can be divided according to this criterion into two types. The first includes regulation in the EU, the second – in the United States and Canada. Most of the countries that follow the process-oriented approach participate in the Cartagena Protocol on Biosafety (2000), while the countries implementing the product-oriented approach largely do not participate. This causes significant differences in the requirements for GM crops but does not directly affect the possibility of their cultivation. The process-oriented approach is adopted in Brazil and India, which are among the leaders in the use of biotechnology in agriculture [61].

In the United States since 1986, the Coordinated Framework for Regulation of Biotechnology, updated in 1991, is active. It serves as a guideline for federal oversight and regulation of GM foods, including GM crops, food products, and GMO use. In implementing the regulation, U.S. regulatory authorities should rely solely on the risks inherent in the final product, without regard to biotechnological processes of its manufacture [62]. The USA has no federal legislation on GMOs in the United States and the United States is not a party to the Cartagena Protocol on Biosafety and the Convention on Biological Diversity [2].

The first commercial herbicide-resistant GM corn appeared in the U.S. market in 1998. By 2009, genetically modified corn in the United States accounted for 85% [63]. According to the evaluation of American researchers, the yield of GM corn from 1996 to 2010 in the central corn belt of the United States only slightly increased, unlike GM soybeans. However, whether this was due to the use of GM corn or other factors is not analyzed in detail [64]. The US legislation on GM plants is the most liberal, although in the US, there is a theoretical danger of crossing corn with eastern gamagrass *Tripsacum dactyloides* L., which is widespread in the eastern and northern part of the United States [65]. Eastern gamagrass belongs to the same genus of the family *Poaceae* as corn *Zea mays* L., and grows naturally in the same region of the United States where corn is commercially produced. To study the gene flow from corn to gamagrass, experimental crosses were made between transgenic corn used as a male parent and gamagrass as a female parent to assess the possibility of interspecific hybridization [66]. No evidence of gene flow from transgenic corn to gamagrass was observed in nature, although the two species grew in close proximity for many years and had ample opportunities for interbreeding [66].

South America. Colombia. The Colombian Agricultural Institute (Instituto Colombiano Agropecuario, ICA) in 2010 established a distance requirement of 500 m between local and transgenic corn fields [67]. In 2015, Colombia reviewed 60 randomly selected plots of corn in the valley of San Juan planted in groups at distances of 2.2-4 km in the area of 15×8 km. The areas of ordinary corn varied from 8 to 114 hectares, GM corn – from 95 to 125 hectares. The effects of the distribution of GM corn with pollen were analyzed by three independent methods (two enzyme immunoassay and PCR). The results showed the presence of transgenic sequences in leaves (more than 88% of plots) and seeds (12% of plots) of non-GM corn [68].

Uruguay. Uruguay's government regulations stipulate that when GM corn is grown, at least 10 percent of the area should be occupied by non-GM corn as a biodiversity safety zone. Government resolutions (2003-2004) set a minimum distance of 250 m between GM and non-GM corn [16]. In 2010, Uruguay studied cases and determined the frequency of cross-pollination between commercial GM and non-GM varieties. The technique included detection of GM varieties by sandwich-ELISA and PCR methods. The proportion of transgenic seedlings in the offspring of non-GM crops was 0.56; 0.83 and 0.13% at three sampling points at a distance of 40, 100 and 330 m from GM crops, respectively [16].

Argentina. Argentina is the third country in the world in terms of the cultivation volume of GM crops [69]. In 2010, their GM crop acreage here accounted for 21% of the world's biotech crops (99% for soybeans, 83% for corn, 94% for cotton) [70]. In 2016, this value was already 97% for corn, almost 100% for soybeans and 95% for cotton [3].

Data on environmental and agrotechnical risks obtained in EU countries based on assessments and the need to have certain isolation zones are perceived in Argentina as an obstacle to the realization of national interests [52], as the use of GM crops in Brazil and Argentina has resulted in a higher average income for farmers. The combined economic impact of 24.8 billion USD for Brazil and 21.1 billion USD for Argentina was achieved mainly by reducing the cost of selective herbicide and achieving higher yields [3].

Brazil. Since the first GM crop was grown in Brazil in 1998, their share in crops has increased to 88% for corn, 96% for soybeans and 78% for cotton crops [3]. Brazil requires a buffer distance of 100 m from the edge of the GM corn field to the beginning of the non-GM corn field. Alternatively, a 20-meter buffer consisting of at least 10 rows of non-GM corn along the edge of the GM corn field can be used. Buffer zones were established by the Brazilian National Technical Biosafety Commission on the basis of gene flow studies, as well as taking into account national legislation, which set a threshold of 1% for GM crops [71].

Africa. Compared to other African countries, the Republic of South Africa has adopted GM crop biotechnology from the very beginning. The first field tests of GM crops were conducted in South Africa in 1989; the first commercial production of GM corn was approved in 1997 [46]. The main legislation in South Africa concerning GMOs, including their limited use, trial release, commercial release, and import and export, is the GMOs Act 1997 (Genetically Modified Organisms Act No. 15 of 1997, Statutes of the Republic of South Africa – Agriculture) [46]. South Africa is currently the eighth producer of GM crops in the world. The study of the level of cross-pollination between GM and non-GM corn in South Africa showed that <0.1-1.0% of pollen reached 45 m distance, < 0.01-0.1% of pollen moved to a 145 m distance, and <0.01-0.001% of pollen to a 473 m distance [72].

Asia. *China and Japan*. At a threshold of 0.9%, the insulation distance in China was estimated to be 50 and 25 m on a two-stage model [73]. Japanese food labeling rules for GM products are not particularly strict. New varieties of GM crops are grown on isolated farmland. Once the safety of such varieties is proven, they are allowed to grow on traditional farms. In Japan, however, the introduction of GM crops for food production has met strong public opposition. More than 70% of Japanese consumers surveyed are against growing GM varieties and eating them [74].

The use of corn varieties with male sterility to limit the spread of corn with pollen. One of the approaches for the joint safe cultivation of GM and non-GM corn is the use of plants with cytoplasmic male sterility (CMS), in which pollen is defective (functionally defective) or is formed in small quantities. CMS is inherited through the maternal line. At first, three types of CMS were known: T-type (Texas), S-type (USDA) and C-type (Charrua type) [75, 76]. In the 1990s, the corn mitochondrial gene *T-urf13* was found to encode a small protein URF-13 (13 kDa) expressed in mitochondrial crystals, which is responsible for the T-type of corn CMS and also increases susceptibility to fungal pathogens [76, 77]. The mitochondrial genome of the male-sterile corn S-cytoplasm contains a repeating R-region of DNA having two open reading frames [76]. The restoration of the fertility of S-type CMS is mainly controlled by the nuclear restorer *Rf3*, which is located in the 2nd chromosome [78]. For CMS of C-type, *Rf4*, located in the 8th chromosome, is a dominant gene restoring fertility [79].

T-type CMS was widely presented in corn hybrids in North America in the 1950s and 1960s, mainly to limit seed self-reproduction by farmers [76]. In addition, for the introduction of CMS corn to agricultural practices, it was necessary to ensure comparable yields. However, weather conditions in the early 1970s contributed to the development of leaf blight in the varieties of corn with T-type CMS, which led to a loss of up to 15% of the crop in the states of the "corn belt" of the United States (more than 6 billion USD at the current rate). The varieties without T-type CMS were immune to infection, which limited the use of CMS in practice [80]. Due to the susceptibility of T-type CMS to the race of the fungus *Bipolaris maydis* (formerly known as *Helminthosporium maydis*, race T), other cytoplasm groups providing corn CMS were looked for. In the United States, S-type CMS (often referred to as USDA-type) was found in the offspring of the variety Teopod (Iowa) [76]. C-type CMS, identified in the Brazilian corn of the variety Charrua in 1970, was used in the creation of hybrids, but C-type CMS was sensitive to the race of *B. maydis*, found only in China so far [76].

To assess whether the CMS of corn hybrids reliably reduces pollen-mediated gene flow from GM plants [81], two-year field experiments with three corn hybrids with CMS were conducted in three regions of Germany. The characteristics of panicles, pollen viability, and cross-pollination frequency were investigated. CMS stability depended on the genotype, weather conditions of the year and location. One CMS corn hybrid showed high CMS-C stability and very low cross-pollination rate (<1%). The other two hybrids with the CMS types S and T were characterized by high variability and formed fertile panicles with small or large amounts of pollen, respectively. In all corn hybrids with CMS, cross-pollination was by 84-99% lower than in a conventional fully fertile variety. It was found that when using corn hybrids with CMS, the isolation distances between adjacent GM and non-GM fields can be greatly reduced (up to 10 m) while maintaining the set threshold of 0.9% [81].

If the corn hybrid with CMS and the pollinator plant provide another genetic background, yield can be increased significantly. The so-called Plus-Hybrid-Effect combines the potential benefits of CMS and the Xenia effect (increased female fertility associated with resource redistribution or greater seed viability) [81-83]. In the review by Wan et al. [84], the main achievements in the identification and characterization of CMS genes in corn, Arabidopsis, and rice are summarized. In particular, corn to date is characterized by 17 CMS genes, 13 of which are orthologs of CSM genes of rice and/or Arabidopsis.

In 1998, DuPont (USA) developed the GM lines DP-32138-1 (commercial name 32138 SPT maintainer), PH-ØØØ676-7, PH-ØØØ678-9, and PH-ØØØ68Ø-2, and Bayer Crop Science (USA) introduced the GM line ACS-ZMØØ1-9 (commercial name InVigor™ Maize) and ACS-ZMØØ5-4 (commercial name InVigor™ Maize), approved for commercialization by ISAAA (GM Approval Database, http://www.isaaa.org/gmap-provaldatabase/default.asp). The line DP-32138-1 (commercial cultivation in the USA in 2011 and in Brazil in 2015) has the transferred genes *ms45* (restores fertility due to the normalization of development of the cell wall of microspores), *zm-aa1* (its expression in the immature pollen hydrolyzes starch and makes the pollen sterile) and the marker gene *dsRed2*, encoding red fluorescent protein for easy selection. The lines PH-ØØØ678-9, PH-ØØØ676-7, and PH-ØØØ68Ø-2 (commercial cultivation in the USA in 1998) have the transferred *dam* gene, which provides CMS, preventing the formation of functional anthers and pollen, and the *pat* gene for resistance to phosphinothricin. The lines ACS-ZMØØ1-9 and ACS-ZMØØ5-4 have the transferred *barnase* gene (causes male sterility; fused with a tapetum-specific promoter TA29 and activated in tissue during the formation of pollen, the product has ribonuclease activity, inhibiting the synthesis of RNA in the tapetum cells of the anther), the gene *bla* (neutralizes β-lactam antibiotics, such as ampicillin) and the *bar* gene (in its presence, the enzyme phosphinothricin-N-acetyltransferase is produced, which acetylates herbicide phosphinothricin on the amino group, making it non-toxic to plants).

In 2016, DuPont Pioneer (USA) developed a system for the production of hybrid seeds using male sterility to produce hybrids of corn and other cross-pollinating crops [85]. The main element of this system is the so-called Seed Production Technology (SPT), which includes several stages. First, using the vector bearing the SPT design (contains the gene for male fertility of wild type *Ms45* to restore fertility, the mortality gen of pollen *ZmAA* that violates its normal development, and the gene of the fluorescent marker *DsRed2*, which facilitates the sorting of seeds), the method of agrobacterium transformation of immature embryos of corn is used to receive an SPT transgenic corn maintainer line. When the male sterile line (with a mutant gene *ms45*/*ms45*) is pollinated with pollen of the line that contains the SPT design, almost 100% of the resulting seeds have the genotype *ms45*/*ms45* and can be used as female lines with CMS for crossing and produce hybrid seeds. A Multi-Control Sterility (MCS) hybrid seed production system has been developed to limit the rate of transgenic transfer through pollen lines containing the SPT design. The transgenic line is obtained with the agrobacterium transformation of mutants at the genes *ms7* or *ms30* using a vector containing the gene of male fertility (*ZmMs*) to restore fertility, two genes for fertility disturbance of pollen, which are able to inhibit the formation or function of transgenic pollen, the fluorescent marker (*DsRed2*) and the gene of resistance to herbicides (*bar*) to prevent contamination of the seeds [86]. In lines containing these structures, the rate of transmission of the transgene is reduced significantly.

In 2014, Monsanto (USA) developed the Roundup Hybridization System (RHS) for the production of hybrid seeds [87]. The RHS mechanism is based on obtaining plants resistant to this herbicide through the introduction of the *CP4-EPSP* gene from *Agrobacterium* sp. CP4 that encodes 5-enolpyruvyl-shikimate-3-phosphate-synthase insensitive to glyphosate, with the subsequent selective decrease in the expression of this gene in male reproductive tissues. As a result, when exposed to glyphosate, defective pollen (glyphosate-mediated male sterility) is formed with virtually no negative effects on other organs [87].

Despite the fact that the three-gene hybrid system on the basis of CSM was used in the commercial production of hybrid seeds of GM corn, its widespread use was prevented by serious problems [84]. Most of the developed CMS strategies have not been successfully applied due to the lack of a cost-effective, environmentally friendly and genetically stable strategy for large-scale reproduction of parent lines with CMS [84]. The necessary data was not found in the available literature on the large-scale sale and cultivation of GM corn seeds with CMS, although in 2017 the report on the commercial cultivation of GM rape with CMS was published [2].

Since 2016, CRISPR/Cas9 technology has been used to create corn lines with CMS. Xie et al. [88] studied the *ms33* gene of corn, encoding glycerol-3-phosphate acyltransferase (GPAT), a key enzyme in the synthesis of glycerolipid. The form obtained using CRISPR/Cas9 technology turned out to be a full-fledged mutant with CMS without anthers and mature pollen grains. Loss of *ZmMs33* gene expression in anthers violates the metabolism and development of the tapetum, which causes damage of anther cuticle and blocks the formation of pollen. In 2018, the vector CRISPR/Cas9 for editing the *MS8* gene, encoding β-1,3-galactosyltransferase that affects the meiotic stage of anther development, was developed [89]. The authors obtained eight transgenic lines (T_0). The results of sequencing showed that genes *MS8* in these lines of the T_0 generation are not mutated. However, mutations have been identified in the gene *MS8* among the descendants of the transgenic lines H17 to F_1 (obtained by crossing with the inbred line Zong31) and F_2 (reproduced by self-pollination of F_1). Mutations in the *MS8* gene and CMS can be consistently inherited in generations and passed on to other elite inbred lines to produce hybrid products [89]. With the help of the CRISPR/Cas9 technology, corn mutants at the *MS45* gene required for pollen development were also produced [90]. Plants of the generation T_0 containing two-allelic mutations in the *MS45* gene were with CMS. CMS lines created using CRISPR/Cas9 technology have not yet been tested in practical production. Thus, CMS has been used for more than half a century to limit the crossing of the donor and recipient of corn pollen, but this approach has not yet been widely used to create GM corn for various reasons. Perhaps, attempts to create corn varieties with CMS using CRISPR/Cas9 technology will be more successful.

Early studies of gene flow in cross-pollination are discussed in summarizing papers [14, 91]. However, differences in approaches, analytical methods, and experimental schemes impede comparison of results and complicate the identification of measures to limit cross-pollination in the field conditions. In particular, the distance between GM and non-GM corn recommended in EU countries at the same threshold of GM content in food varies significantly (from 25 to 600 m) [92, 93]. In addition to the distance between crops and the synchronicity of flowering, the frequency of cross-pollination depends on the size and orientation of the fields [94]. Most studies of the distribution of GM varieties were based on a single source of donor pollen, often with a smaller or equal field size relative to the recipient [34, 91]. Later, models based on multiple pollen sources were created, based on numerous data of field experiments [95]. The established requirements to the minimum distance and flowering time when grown transgenic and non-transgenic corn discriminate farmers, but the additional costs of these requirements (up to 300 Euro/ha) severely limit the economic benefits of the growers of GM corn in Europe [92, 96].

Thus, since the second half of the 1990s, the area under GM corn has increased by more than 100 times; therefore, the issues of its distribution and cross-pollination have become more relevant in practical terms. Based on cross-pollination and pollen counting data at different distances from the source, a range of isolation distances from 10 to 50 m is recommended. If the proper isolation distance cannot be ensured, the area of the recipient and/or donor may be bordered by a pollen barrier. In the recipient field, the outer rows of corn can be considered the pollen barrier. After the 10-20 m wide corn pollen barrier, almost no analyzed sample contains more than 0.9% of GM material. For recipient areas of less than 1 ha and/or shallow depth, an isolation distance of at least 50 m may be recommended, especially in the direction of the wind pattern. When using corn hybrids with CMS, isolation distances between adjacent GM and non-GM corn fields can be significantly reduced (to 10 m) while maintaining compliance with European requirements (marking threshold 0.9%). However, for practical mass cultivation of GM corn and corn modified with the CRISPR/Cas9 technology with CMS is not yet used. Russia has yet to develop its measures and recommendations for the joint cultivation of GM and non-GM corn.

REFERENCES

1. Pellegrino E., Bedini S., Nuti M., Ercoli L. Impact of genetically engineered maize on agronomic, environmental and toxicological traits: a meta-analysis of 21 years of field data. *Scientific Reports*, 2018, 8(3113): 1-12 (doi: 10.1038/s41598-018-21284-2).
2. *ISAAA. Global Status of Commercialized Biotech/GM Crops: 2017. Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 years. ISAAA Brief No. 53.* ISAAA, Ithaca, NY, 2017.
3. *ISAAA. Global Status of Commercialized Biotech/GM Crops: 2016. ISAAA Brief No. 52.* ISAAA, Ithaca, NY, 2016.
4. Golikov A.G., Stepanova N.G., Krasovskii O.A., Skryabin K.G. *Biotekhnologiya*, 1997, 1: 53-58 (in Russ.).
5. Chesnokov Yu.V. *Vavilovskii zhurnal genetiki i selektsii*, 2011, 15(4): 818-827 (in Russ.).
6. Romeis J., Naranjo S.E., Meissle M., Shelton A.M. Genetically engineered crops help support conservation biological control. *Biological Control*, 2019, 130: 136-154 (doi: 10.1016/j.biocontrol.2018.10.001).
7. Bannert M., Stamp P. Cross-pollination of maize at long distance. *European Journal of Agronomy*, 2007, 27: 44-51 (doi: 10.1016/j.eja.2007.01.002).
8. Baltazar B., Castro Espinoza L., Espinoza Banda A., de la Fuente MartHnez J.M., Garzyn Tiznado J.A., González García J., Gutiérrez M.A., Guzmán Rodríguez J.L., Heredia Díaz O., Horak M.J., Madueco Martínez J.I., Schapaugh A.W., Stojšin D., Uribe Montes H.R., Zavala García F. Pollen-mediated gene flow in maize: implications for isolation requirements and coexistence in Mexico, the center of origin of maize. *PloS ONE*, 2015, 10: e0131549 (doi: 10.1371/journal.pone.0131549).
9. Marceau A., Gustafson D.I., Brants I.O., Leprince F., Foueillassar X., Riesgo L., Areale F.-J., Sowaf S., Kraicg J., Badeah E.M. Updated empirical model of genetically modified maize grain production practices to achieve European Union labeling thresholds. *Crop Science*, 2013, 53: 1712-1721 (doi: 10.2135/cropsci2012.04.0224).
10. Luna S., Figueroa J., Baltazar B., Gomez R., Townsend R., Schoper J.B. Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Science*, 2001, 41: 1551-1557 (doi:m10.2135/cropsci2001.4151551x).
11. Angevin F., Klein E., Choimet C., Meynard J., de Rouw A., Sohbi Y. Modélisation des effets des systиmes de culture et du climat sur les pollinisations croisйes chez le maпs. Isolement des collectes et maotrise des dissйminations au champ. In: *Rapport du groupe 3 du programme de recherche: pertinence йconomique et faisabilitй d'une filiure sans utilisation d'OGM, INRAFNSEA.* J.-M. Meynard, M. Le Bail (eds.). Thiverval-Grignon, France, 2001: 21-36.
12. Aylor D. Survival of maize (*Zea mays*) pollen exposed in the atmosphere. *Agricultural and Forest Meteorology*, 2004, 123: 125-133 (doi: 10.1016/j.agrformet.2003.12.007).
13. Jarosz N., Loubet B., Durand B., Foueillassar X., Huber L. Variations in maize pollen emission and deposition in relation to microclimate. *Environmental Science & Technology*, 2005, 39: 4377-4384 (doi: 10.1021/es0494252).
14. Devos Y., Reheul D., De Schrijver A. The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. *Environmental Biosafety Research*, 2005, 4(2): 71-87 (doi: 10.1051/ebr:2005013).
15. Ma B.L., Subedi K.D., Reid L.M. Extent of cross-fertilization in maize by pollen from neighboring transgenic hybrids. *Crop Science*, 2004, 44: 1273-1282 (doi: 10.2135/cropsci2004.1273).
16. Galeano P., Debat C.M., Ruibal F., Fraguas L.F., Galván G.A. Cross-fertilization between

genetically modified and non-genetically modified maize crops in Uruguay. *Environmental Biosafety Research*, 2010, 9(3): 147-154 (doi: 10.1051/ebr/2011100).
17. Aylor D., Schultes N., Shields E. An aerobiological framework for assessing cross-pollination in maize. *Agricultural and Forest Meteorology*, 2003, 119: 111-129 (doi: 10.1016/S0168-1923(03)00159-X).
18. Emberlin J., Adams-Groom B., Tidmarsh J. A report on the dispersal of maize pollen. In: *Report commissioned by and available from the Soil Association National Pollen Research Unit*. Bristol House, Bristol, UK, 1999: 40-56.
19. Mele E. Spanish study shows that coexistence is possible. *Agricultural Biotechnology International Conference*, 2004, 3: 2.
20. Ortega Molina J. *Results of the studies into the coexistence of genetically modified and conventional maize. COPA-COGECA colloquy on the co-existence and thresholds of adventitious presence on GMOs in conventional seeds, 2004.* Available http://www.copa-cogeca.be/pdf/9.pdf. No date.
21. Weber W.E., Bringezu T., Broer I., Eder J., Holz F. Coexistence between GM and non-GM maize crops — tested in 2004 at the field scale level (Erprobungsanbau 2004). *Journal of Agronomy and Crop Science*, 2007, 193: 79-92 (doi:10.1111/j.1439-037X.2006.00245.x).
22. Palaudelmas M., Mele E., Monfort A., Serra J., Salvia J., Messeguer J. Assessment of the influence of field size on maize gene flow using SSR analysis. *Transgenic Research*, 2012, 21(3): 471-483 (doi: 10.1007/s11248-011-9549-z).
23. Ingram J. The separation distances required to ensure cross-pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape. *Plant Varieties and Seeds*, 2000, 13(3): 181-199.
24. Novotny E., Perdang J. Report on a model for pollen transport by wind. In: *Report for the Chardon LL hearing*. London, 2002.
25. Westgate M., Lizaso J., Batchelor W. Quantitative relationship between pollen-shed density and grain yield in maize. *Crop Science*, 2003, 43: 934-942 (doi: 10.2135/cropsci2003.9340).
26. Bock A.-K., Lheureux K., Libeau-Dulos M., Nilsagard H., Rodriguez-Cerezo E. *Scenarios for co-existence of genetically modified, conventional and organic crops in European agriculture*. IPTS-JRC, Seville, Spain, 2002.
27. Brookes G., Barfoot P., Melé E., Messeguer J., Bénétrix F., Bloc D., Foueillassar X., Fabié A., Poeydomenge C. *Genetically modified maize: pollen movement and crop coexistence*. PG Economics Ltd., Dorchester, UK, 2004.
28. Du M., Kawashima S., Matsuo K., Yonemura S., Inoue S. Simulation of the effect of a cornfield on wind and pollen deposition. In: *International Congress on Modelling and Simulation*. F. Ghassemi, P. Whetton, R. Little, M. Littleboy (eds.). Australian National University, 2001: 899-903.
29. Liu Y., Chen F., Guan X., Li J. High crop barrier reduces gene flow from transgenic to conventional maize in large fields. *European Journal of Agronomy*, 2015, 71: 135-140 (doi: 10.1016/j.eja.2015.09.005).
30. Le Bail M., Lecroart B., Gauffreteau A., Angevin F., Messean A. Effect of the structural variables of landscapes on the risks of spatial dissemination between GM and non-GM maize. *European Journal of Agronomy*, 2010, 33: 12-23 (doi: 10.1016/j.eja.2010.02.002).
31. Henry C., Morgan D., Weekes R., Daniels R., Boffey C. *Farm scale evaluations of GM crops: monitoring gene flow from GM crops to non-GM equivalent crops in the vicinity: Part I: Forage maize*. DEFRA report EPG, 2003.
32. Weekes R., Allnutt T., Boffey C., Morgan S., Bilton M., Daniels R., Henry C. A study of crop-to-crop gene flow using farm scale sites of fodder maize (*Zea mays* L.) in the UK. *Transgenic Research*, 2007, 16(2): 203-211 (doi: 10.1007/s11248-006-9036-0).
33. Devos Y., Demont M., Sanvido O. Coexistence in the EU — return of the moratorium on GM crops? *Nature Biotechnology*, 2008, 26(11): 1223-1225 (doi: 10.1038/nbt1108-1223).
34. Gustafson D.I., Brants I.O., Horak M.J., Remund K.M., Rosenbaum E.W., Soteres J.K. Empirical modeling of genetically-modified maize grain production practices to achieve European Union labeling thresholds. *Crop Science*, 2006, 46: 2133-2140 (doi: 10.2135/cropsci2006.01.0060).
35. Chamecki M., Gleicher S.C., Dufault N.S., Isard S.A. Diurnal variation in settling velocity of pollen released from maize and consequences for atmospheric dispersion and cross-pollination. *Agricultural and Forest Meteorology*, 2011, 151: 1055-1065 (doi: 10.1016/j.agrformet.2011.03.009).
36. Makowski D., Bancal R., Bensadoun A., Monod H., Messéan A. Sampling strategies for evaluating the rate of adventitious transgene presence in non-genetically modified crop fields. *Risk Analysis*, 2017, 37(9): 1693-1705 (doi: 10.1111/risa.12745).
37. Bohn T., Primicerio R., Traavik T. The German ban on GM maize MON810: scientifically justified or unjustified? *Environmental Sciences Europe*, 2012, 24(22): 1-7 (doi: 10.1186/2190-4715-24-22).
38. Della Porta G., Ederle D., Bucchini L., Prandi M., Verderio A., Pozzi C. Maize pollen mediated gene flow in the Po valley (Italy): Source—recipient distance and effect of flowering time. *European Journal of Agronomy*, 2008, 28: 255-265 (doi: 10.1016/j.eja.2007.07.009).
39. Popescu S., Leprince F., Ioja-Boldura O., Marutescu A., Sabau I., Marcela Badea E. Quantification of pollen mediated gene flow in maize. *Romanian Biotechnological Letters*, 2010, 15: 5351-5360.
40. Van de Wiel C.C.M., Groeneveld R.M.W., Dolstra O., Kok E.J., Scholtens I.M.J., Thissen J.,

Smulders M.J.M., Lotz L.A.P. Pollen-mediated gene flow in maize tested for coexistence of GM and non-GM crops in the Netherlands: Effect of isolation distances between fields. *Njas-Wageningen Journal of Life Sciences*, 2009, 56: 405-423 (doi: 10.1016/S1573-5214(09)80007-9).
41. Jaffe G. Regulating transgenic crops: a comparative analysis of different regulatory processes. *Transgenic Research*, 2004, 13: 5-19 (doi: 10.1023/B:TRAG.0000017198.80801.fb).
42. Nicolia A., Manzo A., Veronesi F., Rosellini D. An overview of the last 10 years of genetically engineered crop safety research. *Critical Review Biotechnology*, 2014, 34(1): 77-88 (doi:10.3109/07388551.2013.823595).
43. Sirsi E. Coexistence: a new perspective, a new field. *Agriculture and Agricultural Science Procedia*, 2016, 8: 449-454 (doi: 10.1016/j.aaspro.2016.02.042).
44. Ujj O. European and American views on genetically modified foods. *The New Atlantis*, 2016, 49: 77-92.
45. *EC (European Commission). 2015b. Fact sheet: Questions and answers on EU's policies on GMOs.* Available http://europa.eu/rapid/press-release_MEMO-15-4778_en.htm. Accessed 30.11.2015.
46. *Restrictions on Genetically Modified Organisms.* Washington: Law Library of Congress, Global Legal Research Center, 2014. Available https://www.loc.gov/law/help/restrictions-on-gmos/index.php. No date.
47. Deppermann A., Balkovic J., Bundle S.-C., Di Fulvio F., Havlík P., Leclиre D., Lesiv M., Prishchepov A., Schepaschenko D. Increasing crop production in Russia and Ukraine— regional and global impacts from intensification and recultivation. *Environmental Research Letters*, 2018, 13: 025008 (doi: 10.1088/1748-9326/aaa4a4).
48. Clive J. *Global status of commercialized biotech/gm crops: 2014. ISAAA Brief No. 49.* ISAAA, Ithaca, NY, 2014.
49. Brookes G., Barfoot P. Farm income and production impacts of using GM crop technology 1996-2016. *GM Crops & Food*, 2018, 9: 59-89 (doi: 10.1080/21645698.2018.1464866).
50. Zhuchenko A.A. Role of genetic engineering in adaptive system of plant breeding (myths and reality). *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2003, 1: 3-33.
51. Zhivotovskii L.A. V sbornike: *GMO — skrytaya ugroza Rossii* /Pod redaktsiei I.V. Starikova [In: GMO — the hidden threat to Russia. I.V. Starikov (ed.)]. Moscow, 2004: 94-104 (in Russ.).
52. Muir W.M., Howard R.D. Possible ecological risks of transgenic organism release when transgenes affect mating success: Sexual selection and the Trojan gene hypothesis. *Proc. Natl. Acad. Sci. USA*, 1999, 96 (24): 13853−13856 (doi: 10.1073/pnas.96.24.13853).
53. Piperno D.R., Flannery K.V. The earliest archaeological maize (*Zea mays* L.) from highland Mexico: new accelerator mass spectrometry dates and their implications. *Proc. Natl. Acad. Sci. USA*, 2001, 98: 2101-2103 (doi: 10.1073/pnas.98.4.2101).
54. Cárdenas F. Latin American maize germplasm regeneration and conservation. In: *Proc. Workshop CIMMYT, Mexico, June 4-6, 1996.* S. Taba (ed.). CIMMYT, Mexico, 1997: 74.
55. Serratos-Hernandez J.-A., Islas-Gutierrez F., Buendia-Rjdrigueez E., Berthaud J. Gene flow scenarios with transgenic maize in Mexico. *Environmental Biosafety Research*, 2004. 3: 149-157 (doi: 10.1051/ebr:2004013).
56. Quist D., Chapela I.H. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature*, 2001, 41: 541-543 (doi: 10.1038/35107068).
57. Ortiz-Garcıa S., Ezcurra E., Schoel B., Acevedo F., Soberon J., Snow A.A. Absence of detectable transgenes in local landraces of maize in Oaxaca, Mexico (2003-2004). *Proc. Natl. Acad. Sci. USA*, 2005, 102(35): 12338-12343 (doi: 10.1073/ pnas.0503356102).
58. Bellon M.R., Berthaud J. Transgenic maize and the evolution of landrace diversity in Mexico. The importance of farmers' behavior. *Plant Physiology*, 2004, 134: 883-888 (doi: 10.1104/pp.103.038331).
59. McHughen A. A critical assessment of regulatory triggers for products of biotechnology: Product vs. process. *GM Crops & Food*, 2016, 7: 125-158 (doi: 10.1080/21645698.2016.1228516).
60. Ramessar K., Capell T., Twyman R.M., Quemada H., Christou P. Trace and traceability — a call for regulatory harmony. *Natural Biotechnology*, 2008, 26: 975-978 (doi: 10.1038/nbt0908-975).
61. Tetsuya I., Motoko A. A future scenario of the global regulatory landscape regarding genome-edited crops. *GM Crops & Food*, 2017, 8(1): 44-56 (doi: 10.1080/21645698.2016.1261787).
62. Baram M. Governance of GM crop and food safety in the United States. In: *Governing risk in GM agriculture.* M. Baram, M. Bourrier (eds.). Cambridge University Press. 2011: 15-55.
63. Nabradi A., Popp J. Economics of GM crop cultivation. *Applied Studies in Agribusiness and Commerce*, 2011, 05: 7-19 (doi: 10.19041/Apstract/2011/3-4/1)
64. Xu Z., Hennessy D.A., Sardana K., Moschini G.C. The realized yield eject of genetically engineered crops: U.S. maize and soybean. *Crop Science*, 2013, 53: 735-745 (doi: 10.2135/cropsci2012.06.0399).
65. Eastham K., Sweet J. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. In: *European Environment Agency. Environmental issue report.* D. Gee (ed.). Copenhagen, 2002, 28: 38-42.
66. Lee M.S., Anderson E.K., Stojšin D., McPherson M.A., Baltazar B., Horak M.J., de la Fuente J.M., Wu K., Crowley J.H., Rayburn A.L., Lee D.K. Assessment of the potential for

gene flow from transgenic maize (*Zea mays* L.) to eastern gamagrass (*Tripsacum dactyloides* L.). *Transgenic Research*, 2017, 26(4): 501-514 (doi: 10.1007/s11248-017-0020-7).
67. Instituto Colombiano Agropecuario. *Por medio de la cual se implementa el plan de manejo, bioseguridad y seguimiento para siembras controladas de maíz genéticamente modificado. Resoluciyn No. 2894.* Bogota, 2010.
68. Chaparro-Giraldo A., Blanco M.J.T., López-Pazos S.A. Evidence of gene flow between transgenic and non-transgenic maize in Colombia. *Agronomнa Colombiana*, 2015, 33(3): 297-304 (doi: 10.15446/agron.colomb.v33n3.51501).
69. Vicién C., Trigo E. The Argentinian GMO biosafety system: an evolving perspective. In: *Genetically modified organisms in developing countries risk analysis and governance*. A. Adendle, E. Jane Morris, J. Denis (eds.). Cambridge University Press, Cambridge, UK, 2017: 247-257 (doi: 10.1017/9781316585269.022).
70. Burachik M. Experience from use of GMOs in Argentinian agriculture, economy and environment. *New Biotechnology*, 2010, 27(5): 588-592 (doi: 10.1016/j.nbt.2010.05.011).
71. Coelho M.V.S. Coexistence in Brazil. In: *The coexistence of genetically modified, organic and conventional foods.* N. Kalaitzandonakes, P. Phillips, J. Wesseler, S. Smyth (eds.). Natural Resource Management and Policy, Springer, NY, USA, 2016, 49: 87-94 (doi: 10.1007/978-1-4939-3727-1_8).
72. Viljoen C., Chetty L. A case study of GM maize gene flow in South Africa. *Environmental Sciences Europe*, 2011, 23: 1-8 (doi: 10.1186/2190-4715-23-8).
73. Wang C.Y., Kuo B.J., Hsu Y.H., Yiu T.J., Lin W.S. Using the two-step model based on the field border consideration to evaluate pollen-mediated gene flow (PMGF) model and the isolation distance of GM maize in Potzu city of Chiayi county. *Crop, Environment & Bioinformatics*, 2013, 10: 172-189 (doi: 10.1371/journal.pone.0131549).
74. Smyth S.J. Genetically modified crops, regulatory delays, and international trade. *Food Energy Security*, 2017, 6: 78-86 (doi: 10.1002/fes3.100).
75. Bückmann H., Capellades G., Hamouzová K., Holec J., Soukup J., Messeguer J., Melé E., Nadal A., Guillen X.P., Pla M., Serra J., Thiele K., Schiemann J. Cytoplasmic male sterility as a biological confinement tool for maize coexistence: Optimization of pollinator spatial arrangement. *Plant, Soil and Environment*, 2017, 63: 145-151 (doi: 10.17221/761/2016-PSE).
76. Bruns H.A. Southern corn leaf blight: a story worth retelling. *Agronomy Journal.* 2017, 109: 1218-1224 (doi: 10.2134/agronj2017.01.0006).
77. Schnable P.S., Wise R.P. The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends in Plant Science*, 1998, 3: 175-180 (doi: 10.1016/S1360-1385(98)01235-7).
78. Su A., Song W., Xing J., Zhao Y., Zhang R., Li C., Duan M., Luo M., Shi Z., Zhao J. identification of genes potentially associated with the fertility instability of S-type cytoplasmic male sterility in maize via bulked segregant RNA-seq. *PLoS ONE*, 2016, 11(9): e0163489 (doi: 10.1371/journal.pone.0163489).
79. Liu Y., Wei G., Xia Y., Liu X., Tang J., Lu Y., Lan H., Zhang S., Li C., Cao M. Comparative transcriptome analysis reveals that tricarboxylic acid cycle-related genes are associated with maize CMS-C fertility restoration. *BMC Plant Biology*, 2018, 18(1): 190 (doi: 10.1186/s12870-018-1409-z).
80. Herman R.A., Zhuang M., Storer N.P., Cnudde F., Delaney B. Risk-only assessment of genetically engineered crops is risky. *Trends in Plant Science*, 2019, 24(1): 58-68 (doi: 10.1016/j.tplants.2018.10.001).
81. Bückmann H., Hüsken A., Schiemann J. Applicability of cytoplasmic male sterility (CMS) as a reliable biological confinement method for the cultivation of genetically modified maize in Germany. *Journal of Agricultural Science and Technology,* 2013, A3: 385-403.
82. Bückmann H., Thiele K., Schiemann J., Husken A. Influence of air temperature on the stability of cytoplasmic male sterility (CMS) in maize (*Zea mays* L.). *AgBioForum*, 2014, 2: 205-212.
83. Bückmann H., Thiele K., Schiemann J. CMS maize: a tool to reduce the distance between GM and non-GM maize. *EuroChoices*, 2016, 15(1): 31-35 (doi: 10.1111/1746-692X.12116).
84. Wan X., Wu S., Li Z., Dong Z., An X., Ma B., Tian Y., Li J. Maize genic male-sterility genes and their applications in hybrid breeding: progress and perspectives. *Molecular Plant*, 2019, 12(3): 321-342 (doi: 10.1016/j.molp.2019.01.014).
85. Wu Y., Fox T.W., Trimnell M.R., Wang L., Xu R.J., Cigan A.M., Huffman G.A., Garnaat C.W., Hershey H., Albertsen M.C. Development of a novel recessive genetic male sterility system for hybrid seed production in maize and other cross pollinating crops. *Plant Biotechnology Journal*, 2016, 14: 1046-1054 (doi: 10.1111/pbi.12477).
86. Zhang D., Wu S., An X., Xie K., Dong Z., Zhou Y., Xu L., Fang W., Liu S., Liu S., Zhu T., Li J., Rao L., Zhao J., Wan X. Construction of a multicontrol sterility system for a maize male-sterile line and hybrid seed production based on the ZmMs7 gene encoding a PHD-finger transcription factor. *Plant Biotechnology Journal*, 2018, 16: 459-471 (doi: 10.1111/pbi.12786).
87. Feng P.C., Qi Y., Chiu T., Stoecker M.A., Schuster C.L., Johnson S.C., Fonseca A.E., Huang J. Improving hybrid seed production in corn with glyphosate-mediated male sterility.

Pest Management Science, 2014, 70: 212-218 (doi: 10.1002/ps.3526).
88. Xie K., Wu S., Li Z., Zhou Y., Zhang D., Dong Z., An X., Zhu T., Zhang S., Liu S., Li J., Wan X. Map-based cloning and characterization of *Zea mays* male sterility33 (*ZmMs33*) gene, encoding a glycerol-3-phosphate acyltransferase. *Theoretical and Applied Genetics*, 2018, 131: 1363 (doi: 10.1007/s00122-018-3083-9).
89. Chen R., Xu Q., Liu Y., Zhang J., Ren D., Wang G., Liu Y. Generation of transgene-free maize male sterile lines using the CRISPR/Cas9 system. *Frontiers in Plant Science*, 2018, 9: 1180 (doi: 10.3389/fpls.2018.01180).
90. Svitashev S., Schwartz C., Lenderts B., Young J.K., Mark C.A. Genome editing in maize directed by CRISPR–Cas9 ribonucleoprotein complexes. *Nature Communications,* 2016, 7: 13274 (doi: 10.1038/ncomms13274).
91. Sanvido O., Widmer F., Winzeler M., Streit B., Szerencsits E., Bigler F. Definition and feasibility of isolation distances for transgenic maize cultivation. *Transgenic Research*, 2008, 17: 317-335 (doi: 10.1007/s11248-007-9103-1).
92. Devos Y., Demont M., Dillen K., Reheul D., Kaiser M., Sanvido O. Coexistence of genetically modified (GM) and non-GM crops in the European Union (review). *Agronomy for Sustainable Development*, 2009, 29: 11-30 (doi: 10.1051/agro:2008051).
93. Riesgo L., Areal F.J., Sanvido O., Rodriguez-Cerezo E. Distances needed to limit cross-fertilization between GM and conventional maize in Europe. *Nature Biotechnology*, 2010, 28: 780-782 (doi: 10.1038/nbt0810-780).
94. Langhof M., Hommel B., Hüsken A., Njontie C., Schiemann J., Wehling P., Wilhelm R., Rühl G. Coexistence in maize: isolation distance in dependence on conventional maize field depth and separate edge harvest. *Crop Science*, 2010, 50: 1496-1508 (doi: 10.2135/cropsci2009.11.0641).
95. Marceau A., Saint-Jean S., Loubet B., Foueillassar X., Huber L. Biophysical characteristics of maize pollen: Variability during emission and consequences on cross-pollination risks. *Field Crops Research*, 2012, 127: 51-63 (doi: 10.1016/j.fcr.2011.11.006).
96. Venus T.J., Dillen K., Punt M.J., Wesseler J.H. The costs of coexistence measures for genetically modified maize in Germany. *Journal of Agricultural Economics*, 2017, 68: 407-426 (doi:10.1111/1477-9552.12178).