AGRICULTURAL BIOLOGY,
2020, V. 56, Iss. 1, pp. 66-77
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.15:581.15:631.543.8

doi: 10.15389/agrobiology.2021.1.66eng
doi: 10.15389/agrobiology.2021.1.66rus

ASSESSMENT OF THE RISKS OF CROSS-POLLINATION DURING CO-CULTIVATION OF MAIZE LINES IN THE SOUTH-EAST EUROPEAN RUSSIA

Yu.S. GUSEV[1], O.V. GUTOROVA[1], E.M. MOISEEVA[1], V.V. FADEEV[1], S.A. ZAYTSEV[2], D.P. VOLKOV[2], E.A. ZUK[2], I.V. VOLOKHINA[1], M.I. CHUMAKOV[1] ✉

[1]*Institute of Biochemistry and Physiology of Plants and Microorganisms RAS*, 13, prosp. Entuziastov, Saratov, 410049 Russia, e-mail gusev_yu@ibppm.ru, olga.gutorova@mail.ru, em-moiseeva@mail.ru, vvf2593@gmail.com, volokhina_i@ibppm.ru, chumakovmi@gmail.com (corresponding author ✉);
[2]*Russian Research, Design and Technology Institute for Sorghum and Maize,* 4, 1-i Institutskii proezd, Saratov, 410050 Russia, e-mail zea_mays@mail.ru, genomix@mail.ru, e.a.zhuk@yandex.ru
ORCID:
Gusev Yu.S. orcid.org/0000-0001-7379-484X
Gutorova O.V. orcid.org/0000-0002-6975-8675
Moiseeva E.M. orcid.org/0000-0001-9234-4000
Fadeev V.V. orcid.org/0000-0002-9725-3439
Zaytsev S.A. orcid.org/0000-0002-6829-1970
Volkov D.P. orcid.org/0000-0001-8055-6516
Zuk E.A. orcid.org/0000-0001-6763-3724
Volokhina I.V. orcid.org/0000-0002-9088-481X
Chumakov M.I. orcid.org/0000-0002-6396-2851
The authors declare no conflict of interests
Acknowledgements:
The authors are grateful to Yu.V. Smolkina for seeds of the hybrid Purpurnii and the line Saratovskaya Purpurnaya, and to I.B. Ivanova and A.V. Ul'yanov for their help in handling the 2019 crop.
Supported financially under the Program of Basic Scientific Research of State Academies of Sciences for 2020, the State registration No. AAAA-A17-117102740101-5 (for GM corn obtaining and risk assessment), and with the grant No. 18-29-14048mk from the Russian Foundation for Basic Research (for modeling GM corn behavior in field conditions)
Received July 3, 2020

Abstract

The new Russian Federal law (No. 358 of 03.07.2016) prohibits the commercial use of GM plants in agriculture, but allows, since 2018, for the first time in Russia their cultivation and testing for research purposes. Consequently, there is a need to assess and develop criteria for safe co-cultivation of non-GM and GM varieties, which are currently absent in Russia. In this paper, it was established for the first time that the 10-15 m distance is sufficient to prevent cross-pollination between maize lines with an acceptable presence of 0.9 % of the donor's genetic material, regardless of the recipient line, donor and recipient time flowering, and the wind direction in Saratov condition (South-West region of European part of Russia). The work aimed to assess the influence of the distance between pollen donor and recipient, wind direction, donor and recipient time flowering, and a buffer zone presence between them on the crossing frequency in mixed maize crops. The maize lines Korichnevyi marker (KM), GPL-1, Zarodyshevyi marker Saratovskii Purpurnyi (ZMS-P), Purpurnaya Saratovskaya (PS), as well as hybrids Purpurnyi (GP), Raduga and Tester 3 were grown (the experimental field of the Rossorgo, Saratov, South-West region of European Russia, 2018-2019). We planted the GP and ZMS-P lines as pollen donors in 2018 on a 3×80 m^2 area with planting density of 7-10 plants per 1 m^2. Between the pollen donor area, maize KM and GPL-1 lines were planted, and around them there were a 1290 m^2 area of yellow-colored grain recipients (Raduga and Tester 3 hybrids). In September, 5-12 ears from each pollen recipient were harvested. The cross-pollination frequency was calculated as the ratio of purple grains (GP pollination result) or yellow grains with a purple spot (ZMS-P pollination result) to the total grain number in recipient lines. In 2018 it was established that the maximum percentage (from 0.1 to 13.2 %) derived from cross-pollination with two pollen donors depends on different factors. At closer distances (1-4 m), the cross-pollination increased 4-fold for the earlier flowering recipient. The percentage of crosses for recipient Raduga decreased 3 times with a 10 m increase in the distance and 11 times at a 40 m distance from the donor plants. Experiments in 2018 indicate that the 10 m distance from the pollen donor guarantees the percentage of crosses not exceeding the 0.9 % GM threshold in food products accepted in the European Union and Russia. In 2019, we used PS inbred line as a pollen donor. The PS was planted on a 3×5 m plot with Sudanese grass (*Sorghum* × *drummondii*) Allegory cultivar as a buffer zone 3 m wide to the East and West and 15 m long to the South-West and North-East. Yellow-grain hybrid Raduga was planted around the buffer zone. The frequency of crosses was calculated as the ratio of the number of purple grains to the total number of Raduga grains per ear. In 2018, the frequency of crosses was also estimated depending on the synchronism of flowering between pollen donors and recipients. The GPL-1 recipient with a 9-day difference from PG (pollen donor) in the beginning of flowering showed a 4 % lower pollination rate compared to a KM line with a flowering period closer to the PG pollen donor (1-day difference). Tall plants of PG donor of pollen prevented spreading pollen from a short ZMS-P donor to the Tester 3 and Raduga recipients in the direction of the wind rose. In 2019, no more than 0.9 % of purple grains were observed for the recipient Raduga when using a buffer zone of 15 m and more from the pollen donor in the wind rose direction. Based on the results of field experiments, the isolation distance from 15 m or more can be recommended to exclude cross-pollination of maize within the threshold of 0.9 % in the conditions of the South-East of the European part of Russia.

Keywords: GM plants, cross-pollination risks, maize, buffer zones

Maize (*Zea mays* L.) is one of the most important agricultural plants. Since 2010, the world's harvest of maize has grown from 800 to 1,122 million tons [1]. In the Russian Federation in 2019, according to the Ministry of Agriculture, the grain maize harvest amounted to 13.9 million tons, which exceeded the 1995 figure (1.7 million tons) by more than 8-fold (http://mcx.ru/press-service/news/v-2019-godu-urozhaynost-zernovykh-v-rossii-vyrosla-na-4-7/).

The photosynthesis of maize is of the C4 type, which implies a greater efficiency in absorbing carbon dioxide in conditions of good lighting and high temperatures. In addition, maize is drought-resistant, and climate warming, coupled with increasing droughts, will make it possible to expand the area of its cultivation in Russia. In Europe, an increase in the average annual temperature of 1 °C has already pushed the border of maize distribution by 200 km to the north [2].

The use of modern genomic technologies in crop production is one of the priority areas for the use of biotechnology in agriculture in the developed and developing countries [3]. The use of genetically modified (GM) plants for industrial cultivation began in 1996. Currently, almost a third of all cultivated maize is GM maize [4], which gives the largest yield of GM grain.

In the Russian Federation, until June 2018, there was a complete ban on the cultivation of GM crops. According to the Federal Law No. 358 in force since June 2018 (dated July 3, 2016), for the first time in Russia it is allowed to grow and test GM plants in the framework of scientific research. However, the parameters of safe cultivation of GM plants are not regulated and require studying. Each country-participant of the 1993 Convention on Biological Diversity, including Russia, must develop a strategy and program for the conservation and use of biological resources, taking into account their safe reproduction [5, 6]. Therefore, it is necessary to evaluate the criteria for the safe co-cultivation of non-transformed and GM varieties of plants, in particular, maize.

Maize is a monoecious wind-pollinated plant, which is a source of increased risk for the spread of pollen from GM varieties, crossing with which is undesirable in production crops. The world has not developed a unified approach to the legal regulation of the cultivation of GM crops. There are two opposite points of view on the problem of joint cultivation of GM and non-GM plants [7]. In the countries of the European Union and North and South America, regulation is based on fundamentally different risk assessment models for genetically modified organisms (GMOs). These approaches are called principle-based and product-based [8]. In the first case, the risk of genetic modification is recognized, in the second one, it is the risk associated with its result and object (GMO or derivative product) [9, 10]. In a number of countries (USA, Canada, Argentina, Brazil, and Spain), the cultivation of GM plants is permitted. Other states, in particular the countries of the European Union and Russia, have decided to ban the commercial cultivation of GM crops in their territory [11, 12].

The analysis of the world experience, accumulated over the past 20 years on the experimental assessment of the risks of cross-pollination in the joint cultivation of GM and non-GM maize, is given in a number of reviews [13-15]. Buffer distances in different countries can range from 25 to 800 m, depending on legislative acts [16-18]. Moreover, the buffer distances measured in different regions of the world have very insignificant differences [19-21].

The distance between fields was identified as a key factor in spreading GM maize in non-transgenic crops [22]. As a consequence, the existing rules are mainly concerned with maintaining a fixed isolation distance between GM fields and the nearest non-GM fields [23]. However, other factors, such as the synchronicity of flowering of the donor and recipient of pollen, wind, relative sizes and shapes of fields, as well as the spatial distribution of different types of fields, can also affect the buffer distances [24, 25].

In Russia, the co-cultivation of GM and non-GM maize has not been systematically studied. In 2010, in the conditions of Krasnodar Territory, the first experimental assessment of the distribution of GM maize in non-transgenic crops was conducted [26]. The author states that cultivars of maize should be planted at least 200 m from transgenic varieties of maize, and barriers should be created in the direction of pollen flow. However, this work did not consider the question at what distance it is possible to grow GM and non-GM maize in order not to exceed the permissible content (0.9%) in Russia of GM product in the total grain yield. Since in Russia until June 2018 there was a complete ban on the cultivation of GM plants in an open environment, to study the distribution of GM maize pollen in field experiments that began in May 2018, the authors used a technology that simulates the distribution of GM pollen among production varieties under the conditions of free pollination. The essence of the technology is to assess the distribution of donor pollen based on the manifestation of a dominant trait (purple or white grain) in the progeny of yellow-colored grain lines of recipient plants [27-29].

In 2015, Russia developed an “Intra-industry standard for growing seed plots of maize, checking and preparing maize seeds for first-generation hybrids” (VOST 01.09.GK. Coordination Council for the development of maize seed production in Russia, 2015), which could serve as a guide for safe growing distances for maize. However, the standard lacks information about the experimental work that underlies these recommendations and does not take into account the possibility of the presence of acceptable 0.9% GMO products, which can significantly affect the safe distances for joint cultivation.

In this work, in the conditions of the Saratov Region (southeast of the European part of Russia), for the first time, it was found that a distance of 10-15 m was sufficient to prevent cross-pollination of maize lines with an acceptable presence of 0.9% of the donor's genetic material, regardless of the recipient line, the flowering time of the donor and the recipient, as well as wind direction.

The aim of the work was to assess the influence of the distance between the donor and recipient of pollen, the direction of the wind, the synchronism of the flowering of the donor and the recipient, and the presence of a buffer zone between them on the frequency of crossing of different maize lines in mixed crops.

Materials and methods. The experimental maize plants of the lines Korichnevyi marker (KM), GPL-1, Zarodyshevyi marker Saratovskii Purpurnyi (ZMS-P), Purpurnaya Saratovskaya (PS), as well as hybrids Purpurnyi (GP), of the selection of the Saratov State University named after N.G. Chernishevsky, as well as hybrids Raduga and Tester 3 of the selection of the Russian Research and Design Institute of Sorghum and Maize (RosNIISK "Rossorgo"), were grown in 2018-2019 on the experimental field of RosNIISK "Rossorgo" (Zonalny settlement, Saratov District, Saratov Region).

In 2018, the pollen donors were the Purpurnyi (GP) hybrid and the

Zarodyshevyi marker Saratovskii Purpurnyi (ZMS-P) line. GP was obtained by crossing the Purpurnaya Saratovskaya (PS) line and the Raduga hybrid and subsequently self-pollinated for several years (Gutorova, personal communication).

The planting density was 7-10 plants per 1 m^2. GP and ZMS-P were planted on an area of 3×80 m. Between the pollen donor areas, maize of the Korichnevyi marker (KM) and GPL-1 lines was planted, forming a buffer zone, and the areas with recipient lines were located around them (hybrids Raduga and Tester 3) with yellow caryopses, with a total area of 1290 m^2. In September 2018, 5-12 ears of each recipient line were taken, photographed in two projections, all caryopses were separated from the ear and analyzed. The frequency of cross-pollination was calculated on the ears of the recipient lines, calculating the ratio of the number of purple (the result of pollination with GP) or yellow grains with a purple spot (the result of pollination with the ZMS-P line) to the total number of grains in the recipient lines.

In the 2019 experiment, the inbred line Purpurnaya Saratovskaya, created on the basis of the American line, served as a pollen donor. The PS line was planted on an area of 3×5 m, around which a buffer zone was created with the sowing of Sudanese grass Allegory *Sorghum* × *drum-mondii* (Steud.) Millsp. & Chase, 3 m wide to the eastern and western directions and 15 m long to the southwest and northeast directions. Yellow grain maize of the Raduga hybrid was planted around the buffer zone. The calculation of the distances from the corners of the rectangular area of the donor to the corners of the rectangular plots of the recipient (near and far points) A5, A6, A10, A11 and F5, F6, F10, F11 was conducted by calculating the hypotenuses. The distances to the near and far points from the corresponding corner of the rectangular pollen donor site to the recipient plots A6, A11, F6, F11 were 6 and 12 m, to the A5, A12, F5, F12 plots 10 and 16 m. The frequency of crossings was calculated as the ratio of the number of purple grains to the total number of grains on the ears of the Raduga hybrid. In September 2019, the authors collected all the ears on each plot of the recipient line and counted the total number of ears on the plot and the total number of grains from the ears. The average number of grains per ear was calculated by dividing the total number of grains per plot by the number of ears.

The total number of harvested and analyzed grains in 2018 was 17,409 pieces, in 2019 201,284 pieces.

The statistical processing was carried out in Microsoft Excel 2010 using a statistics package. The arithmetic means (*AM*) and standard deviations (±SD) were calculated. The significance of differences was determined using Student's *t*-test ($p \leq 0.05$). In the 2018 experiment, each arithmetic mean was obtained on the basis of 5-12 repetitions. In 2019, for each distance, 5 repetitions (plots) were used, in each of which from 3 to 17 ears were analyzed.

Results. The layout of experimental maize crops on the experimental field of RosNIISK Rossorgo in both years of research is shown in Figure 1.

In 2018, the pollen donors were the Purpurnyi (GP) hybrid and the Zarodyshevyi marker Saratovskii Purpurnyi (ZMS-P) line. GP is tall-growing (2.0-2.5 m), has a dominant purple (anthocyanin) color of the stem, leaves, and grains. The genetically marked line of maize ZMS-P [30] has a purple coloration of vegetative parts of the plant, panicles, embryo, and endosperm; it is shorter (1.5 m) in comparison with GP and is mid-late ripening. The *R-g* gene provides the purple color of the aleurone layer of the endosperm and the embryo. When ZMS-P was crossed with yellow-grain recipient lines, hybrid caryopses had endosperm stained with a purple spot. In the 2019 experiment, the inbred line Purpurnaya Saratovskaya (PS), created on the basis of the American line Purple Tester [31] and the early-ripening line of the Saratov selection GPL-1, served as a pollen donor. The mid-ripening tall-growing (1.8 m) line PS has a purple (anthocyanin) coloration of grain, leaves, and stem, determined by the dominant genes *A1*, *A2*, *B*, *Pl*, *Pr*, *R-g*, *C*. In particular, the *R-g* gene controls the purple coloration of grain aleurone [32].

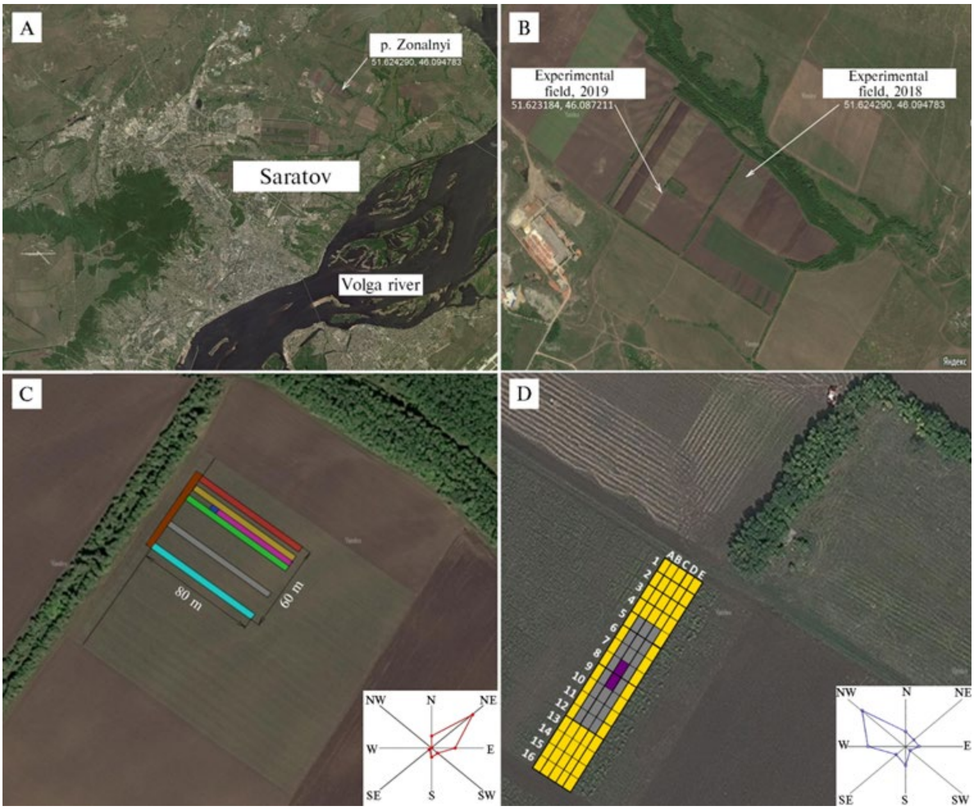

Fig. 1. The location of the experimental maize crops, where the risk of cross-pollination was assessed when growing genetically modified (GM) plants (https://yandex.ru/maps/194/saratov). The wind rose during the flowering period is given according to http://weatherarchive.ru. The orientation of the map (cardinal points) is indicated on the wind rose diagram.

A — the location of the experimental fields in 2018-2019 (Zonalny settlement) in relation to the city of Saratov and the Volga River.

B — general view of the experimental fields in 2018-2019.

C — the scheme of maize planting in the experimental field in 2018: green color corresponds to hybrid Purpurnyi (GP, pollen donor), yellow color to Zarodyshevyi marker Saratovskii Purpurnyi line (pollen donor); red color to hybrid Raduga (pollen recipient, 10 m from the GP to the east), turquoise color to hybrid Raduga (pollen recipient, 40 m from the GP to the west), brown color to hybrid Raduga (pollen recipient, 1-4 m from the GP to the north), gray color to hybrid Tester 3 (pollen recipient, 20 m from GP to the west), blue color t0 GPL-1 line, and pink color to Korichnevyi marker line.

D — planting scheme on the experimental field (2019): purple color corresponds to Purpurnaya Saratovskaya line (pollen donor, 30 m^2), gray color to buffer zone with the sowing of Sudanese grass of the Allegory variety (330 m^2), and yellow color to hybrid Raduga (pollen recipient).

In 2018, there was no rain during the maize flowering period, and the air humidity during the release of pollen from the anthers varied from 75% in the morning hours to 36% in the afternoon. The maximum wind speed during the flowering period was 5.7 m/s (July 31), the average wind speed was 3.1 m/s. The wind direction until August 1 was predominantly northeastern, from August 2, predominantly north and northwest (see Fig. 1, C). The temperature during the flowering period of maize in the morning hours ranged within 16-22 °C, in the daytime within 22-29 °C.

In 2019, it only rained on July 26 during the study period. The relative humidity in the flowering period during the release of pollen from the anthers fluctuated from 38 to 77%. The main wind direction was northwest, but in the period from 25 to 27 July, it was northeast (see Fig. 1, D). In the morning hours, the temperature ranged from 14-21 °C, the daytime temperature was 18-30 °C.

It is known that the distribution of pollen is influenced by temperature, air humidity, precipitation, viability, the total volume of pollen, wind flows, landscape profile, the configuration of the recipient field, and simultaneous flowering of paternal and maternal plants [12, 33]. However, there is no data on how the height of the pollen donor and the angle of arrangement of the leaves of the recipient affect the frequency of cross-pollination.

The viability of pollen, or the ability of pollen to germinate through the pistil filaments into the embryo sac, is an important prerequisite for cross-pollination. The duration of the viability of maize pollen (from 1 to 24 h) is influenced by humidity and temperature [34, 35]. As a rule, pollen spills out from the anthers in dry hot conditions, mainly from morning to noon [16, 36]. The flowering of maize pollen donor lines in the Saratov Region in 2018 continued from July 25 to August 5. Air temperature and humidity during this period were favorable for pollination.

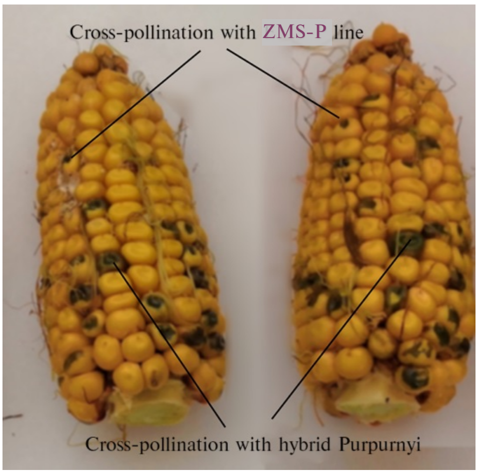

Fig. 2. The appearance of maize ears (*Zea mays* L.) obtained as a result of free crossing between pollen donors (hybrid Purpurnyi GP, Zarodyshevyi marker Saratovskii Purpurnyi ZMS-P) **and the recipient line GPL-1** (experimental field of RosNIISK Rossorgo, Zonalny settlement, Saratov District, Saratov Province, 2018). The purple spots on yellow grains are the result of pollination with the ZMS-P line, the completely colored purple grain results from pollination by the hybrid Purpurnyi GP.

The distance to which the pollen will spread after being ejected from the anthers largely depends on the wind speed. During the period of experiments in 2018, the wind speed in the morning and afternoon was 2.4-5.7 m/s, and if the viability of the pollen was maintained for 1 hour, it could spread over 14 km. However, since maize pollen is rather large (average diameter 90 μm) and relatively heavy (0.25 μg), its sedimentation rate is high and, in real field conditions, pollen is spread relatively close [17, 39]. In 90-93% of all pollen from the donor line ZMS-P, its size was 120-140 μm and differed little from the size of pollen from other lines of diploid maize of the Saratov selection [37], that is, this trait probably did not affect the distribution of pollen.

In hot and dry conditions of the Saratov Region in 2018, the frequency of cross-pollination with pollen from donor lines ranged from 0.1 to 13.2% for different recipient lines (Table 1). The maximum frequency of cross-pollination was observed for the lines KM and GPL-1 located in close proximity to pollen donors: 13.2% for KM when pollinated with Purpurnyi hybrid pollen and 10.5% for GPL-1 when pollinated with ZMS-P pollen. The appearance of maize ears obtained as a result of cross-pollination is shown in Fig. 2.

The frequency of crosses in the Raduga hybrid at a distance of 10 m was 3 times less (0.3%) ($p \leq 0.05$) than at a distance of 1-4 m when pollinated with GP pollen (0.9%) ($p \leq 0.05$), whereas when pollinated with ZMS-P pollen at the same distance, the indicator was 0.05% ($p \leq 0.05$) and was 4 times less than at a distance of 1-4 m (0.2%) ($p \leq 0.05$). At a distance of 40 m from the GP, the crossing frequency decreased 11 times and amounted to 0.08% ($p \leq 0.05$), and we did not find plants pollinated with pollen from the ZMS-P line. Interestingly, in the Raduga hybrid, even at a close distance, the content of purple grains in the mass of yellow ones in the progeny practically did not exceed the threshold of 0.9% of the presence of GM products in food (harvest) accepted in the countries of the European Union and Russia.

In the work conducted in the conditions of the Krasnodar Territory [22], the question remained unclear, at what distance it is possible to grow GM and non-GM maize in order not to exceed the permissible content of GM product in Russia in the total grain yield. If it is taken into account that the total grain yield may contain 0.9% of the GM product, then the distance of 200 m, indicated by the author to prevent cross-pollination of maize, would be significantly reduced.

1. Frequency of purple grain occurrence in the progeny of yellow grain recipient lines of maize (*Zea mays* L.) with free pollination (*AM*±SD, experimental field of RosNIISK Rossorgo, Zonalny settlement, Saratov District, Saratov Province, 2018)

Recipient	A	B	C	D	E	F
KM	1-4 m, east	07/24-08/06	332±93.0	44.3±20.90	3.3±2.90	13.2/1.0
GPL-1	1-4 m, east	07/16-07/26	175±92.0	6.0±5.60	18.4±15.00	3.4/10.5
Raduga	1-4 m, north	07/23-08/01	539±206.0	5.0±3.20	1.3±1.50	0.9/0.2
Raduga	10 m, east	07/23-08/01	378±185.0	0.7±0.60	0.2±0.20	0.3/0.1
Raduga	40 m, west	07/23-08/01	506±213.0	0.4±0.40	0.0	0.1/0.0
Tester 3	20 m, west	07/24-08/01	590±70.0	1.0±0.80	0.0	0.2/0.0

Note. A — distance and direction from pollen donors; B — flowering period of recipient lines; C — the average number of all grains per 1 ear, pcs; D — the number of purple grains from the GP per 1 ear, pcs; E — the number of purple grains from ZMS-P per 1 ear, pcs; F — the proportion of purple grains by GP/ZMS-P donors, %. GP — hybrid Purpurnyi (donor), ZMS-P – Zarodyshevyi marker Saratovskii Purpurnyi line (donor). The flowering period of the GP is from July 25 to August 5, ZMS-P from July 21 to August 7. The location of the experimental plots is shown in Figure 1.

In our experiments under the conditions of the Lower Volga region, the permissible proportion of crossings (0.9%) was observed for the tall-growing recipient Raduga even at a distance of 1-4 m from the donor (Table 1). The study conducted in 2000-2003 in 15 counties of England also showed a rapid decrease in the rate of cross-pollination in the first 20 m from the donor crop [38, 39]. In the experiments with maize in Mexico, the highest values of crosses were observed near the pollen source (12.9% at a distance of 1 m). The degree of crossing dropped sharply to 4.6, 2.7, 1.4, 1.0, 0.9, and 0.5% as the distance from the pollen source increased to 2, 4, 8, 12, 16, 20, and 25 m, respectively. At a distance of more than 20 m, the crossing frequency at all points was 0.9% ($p \leq 0.05$) and below [19].

In the dry summer of 2018, for the Tester 3 hybrid at a distance of 20 m to the west from the GP donor, the percentage of crosses was 0.2% ($p \leq 0.05$), which was significantly lower than the threshold value (see Table 1). It is interesting to note that approximately the same frequency of crossings (0.3%) ($p \leq 0.05$) occurred at a 10 m distance from the GP in the Raduga hybrid, but in the eastward direction, from where the winds mainly blew during flowering (see Table 1).

The effectiveness of the crossing depends on the simultaneous release of pollen from the anthers of the donor and the appearance of pistil filaments in the recipient [16, 40]. According to the literature, asynchronous flowering led to a decrease in the frequency of pollination in the recipient with a difference of 4-5 days by 25%, 6 days by 50% [34, 41]. The authors also observed a 4-fold decrease in the proportion of cross-pollination in the GPL-1 line (the difference at the beginning of flowering is 9 days with GP) compared to the KM line with a closer (1 day difference) flowering period. At the same time, the frequency of cross-pollination in the recipient line GPL-1 (early flowering) and donor ZMS-P (mid-flowering) increased by 10 times compared with KM (recipient) and ZMS-P (donor) (see Table 1).

The height of pollen donor plants averaged 2.0-2.5 m for the Purpurnyi hybrid, 1.8 m for the PS line, and 1.5 m for ZMS-P. In 2018, the number of purple grains after pollination with GP in recipient lines was the maximum for the forms in close proximity and decreased with distancing. For the pollen donor ZMS-P, the percentage of crossings with recipient lines fell strongly in the north and north-east directions, and for the Raduga and Tester 3 recipient lines located in the west and southwest (see Fig. 1, C), cross-pollination from the ZMS-P line was not observed at all. Possibly, this is due to the fact that the tall-growing GP donor prevented the spread of pollen of the low-growing donor of the ZMS-P line in these directions. It can be noted that in ZMS-P, already at a distance of 1-4 m, the frequency of crossings with the tall-growing recipient Raduga was less than 0.9% ($p \leq 0.05$), and with the short-growing KM line slightly exceeded this value (see Table 1).

One of the possible factors limiting the flow of maize pollen is a buffer zone between the donor and the recipient in the form of an area not sown or sown with another plant. In 2019, the authors conducted an experiment to create a buffer zone with the sowing of the Sudanese grass Allegory, which is less tall than the donor. The maximum proportion (1.7-2.1%) ($p \leq 0.05$) of purple grains in the recipient (Raduga hybrid) pollinated by the PS donor was observed in the directions to the west (blocks 7A-12A) and east (blocks 7F-12F) with a buffer zone 3 m wide from the pollen donor (see Fig. 1, D, Table 2). For the south-western direction, with a buffer zone width of 15 m, the percentage of crosses (0.9%) ($p \leq 0.05$) did not exceed the permissible threshold (blocks 13A-13F, see Fig. 1, D).

2. The frequency of occurrence of purple grains in the progeny of the yellow grain maize (*Zea mays* L.) hybrid Raduga with free pollination by the donor of the line Purpurnaya Saratovskaya (*AM*±SD, experimental field of RosNIISK Rossorgo, Zonalny settlement, Saratov District, Saratov Province, 2019)

No. of row, plot	Direction and distance from the pollen donor, m	Number of ears, pcs.	Average number of yellow and purple grains per ear, pcs.	Proportion of purple grains, %
1A-1E	30-35 m, northeast	14	288.0±82.31/0.00	0.0
2A-2E	25-30 m, northeast	17	159.0±37.04/0.5±1.36	0.3
3A-3E	20-25 m, northeast	42	226.0±85.60/0.2±0.42	0.1
4A-4E	15-20 m, northeast	49	234.0±84.35/0.1±0.11	0.1
5A, 12A	10-16 m, west	26	419.0±29.63/1.2±0.62	0.3
6A, 11A	6-12 m, west	16	368.0±70.00/1.5±0.12	0.4
7A-10A	3-6 m, west	41	520.0±84.21/10.5±9.32	2.1
7E-10E	3-6 m, east	37	303.0±40.53/4.8±4.85	1.7
6E, 11E	6-12 m, east	28	303.0±7.02/0.9±1.13	0.3
5E, 12E	10-16 m, east	18	302.0±68.54/2.0±1.91	0.7
13A-13E	15-20 m, southwest	59	406.0±53.73/3.4±0.81	0.9
14A-14E	20-25 m, southwest	63	360.0±79.54/1.1±0.52	0.3
15A-15E	25-30 m, southwest	89	443.0±6.31/0.7±0.50	0.2

N o t e. The flowering period of the pollen donor and recipient is July 20-31. The location of the experimental plots is shown in Figure 1.

The maximum crossing frequency corresponded to the prevailing wind direction (see Fig. 1, D). With a buffer zone width of 3 m, the authors observed a significant excess of the permissible threshold of 0.9% ($p \leq 0.05$) for purple-colored caryopses in the harvest collected on plots 7A-10A and 7F-10F, which stood 0-3 m from the buffer zone (see Table 2). At distances farther from the donor (6-16 m), the proportion of crosses was within 0.3-0.7% ($p \leq 0.05$) in both directions. With a buffer zone width of 15 m at a distance of 15-20 m in the southwestern direction from the donor, the Raduga hybrid had 0.9% ($p \leq 0.05$) purple grains in the ears, which even taking into account the coinciding wind direction did not exceed the permissible threshold (see Table 2).

It should be noted that the Raduga hybrid in the experiments of 2018-2019 showed a relatively low frequency of crosses (see Tables 1, 2). Recipient maize, especially tall-growing maize, itself serves as a barrier to the spread of donor pollen. Perhaps due to this, with a solid planting in 2018, the distribution of GP pollen was within the normal range already at a distance of 10 m from the donor, and in the presence of a buffer zone sown with a low-growing plant, in 2019 a threshold of 0.9% was recorded at a distance of 15 m. In the works of other researchers, it is also indicated that buffer zones with rupture plants, in contrast to barriers with tall-growing plants, do not lead to a decrease in safe distances [23]. Taking into account our findings, in the conditions of southeastern Russia, in order to eliminate the risks of cross-pollination of maize, it is possible to recommend a minimum isolation distance of 15 m in the presence of buffer (non-sown) zones.

Thus, under the conditions of the southeast of Russia, in order to exclude cross-pollination of maize above 0.9%, an isolating distance of 15 m or more can be recommended. In two independent experiments (2018 and 2019), it was found that at a distance of 10 and 15 m, respectively, from the pollen donor, the crossing frequency remained within the permissible norm, regardless of the recipient line, flowering time, and wind direction. At the same time, the synchronicity of flowering of donors and recipients of pollen had a significant effect on the frequency of crossings. In particular, under the conditions of 2018, in the recipient line GPL-1 (the difference at the beginning of flowering is 9 days with the donor hybrid Purpurnyi), the frequency of cross-pollination was 4 times less than in the Korichnevyi marker line with a period of flowering, which is closer to the donor. It was established that a tall-growing pollen donor, the Purpurnyi hybrid had an advantage in the spread of pollen as compared to the short-growing Zarodyshevyi marker Saratovskii Purpurnyi donor.

REFERENCES

1. United States Department of Agriculture. *World Agricultural Production. Current Report.* Circular Series WAP 9-20. October 2020. Available: https://apps.fas.usda.gov/psdonline/circulars/production.pdf. Accessed: 01.07.2020.
2. Zhao S., Liu B., Piao S., Wang X., Lobell D.B., Huang Y., Huang M., Yao Y., Bassu S., Ciais P., Durand J.L., Elliott J., Ewert F., Janssens I.A., Li T., Lin E., Liu Q., Martre P., Müller C., Peng S., Pecuelas J., Ruane A.C., Wallach D., Wang T., Wu D., Liu Z., Zhu Y., Zhu Z., Asseng S. Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences*, 2017, 114(35): 9326-9331 (doi: 10.1073/pnas.1701762114).
3. 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(1): 3113 (doi: 10.1038/s41598-018-21284-2).
4. ISAAA. *Global Status of Commercialized Biotech/GM Crops: 2016.* ISAAA Brief No. 52. ISAAA, Ithaca, NY, 2016.
5. Golikov A.G., Stepanova N.G., Krasovskii O.A., Skryabin K.G. *Biotekhnologiya*, 1997, 1: 53-58 (in Russ.).
6. Chesnokov Yu.V. *Vavilovskii zhurnal genetiki i selektsii*, 2011, 15(4): 818-827 (in Russ.).
7. Ujj O. European and American views on genetically modified foods. *The New Atlantis*, 2016, 49: 77-92.
8. McHughen A. A critical assessment of regulatory triggers for products of biotechnology: Product vs. process. *GM Crops & Food*, 2016, 7(3-4): 125-158 (doi: 10.1080/21645698.2016.1228516).
9. Ramessar K., Capell T., Twyman R.M., Quemada H., Christou P. Trace and traceability — a call for regulatory harmony. *Natural Biotechnology*, 2008, 26(9): 975-978 (doi: 10.1038/nbt0908-975).
10. 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: 15-56 (doi: 10.1017/CBO9780511976582.003).
11. Chumakov M.I., Gusev Yu.S., Bogatyreva N.V., Sokolov A.Yu. Risks of pollen-mediated gene flow from genetically modified maize during co-cultivation with usual maize varieties (review). *Sel'skokhozyaistvennaya biologiya* [*Aricultural Biology*], 2019, 54(3): 426-445 (doi: 10.15389/agrobiology.2019.3.426eng).

12. 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(4): 1712-1721 (doi: 10.2135/cropsci2012.04.0224).
13. 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).
14. 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).
15. Meillet A., Angevin F., Bensadoun A., Huby G., Monod H., Messéan A. Design of a decision support tool for managing coexistence between genetically modified and conventional maize at farm and regional levels. *Ecological Informatics*, 2015, 30: 379-388 (doi: 10.1016/j.ecoinf.2015.09.014).
16. 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).
17. 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(8): 780-782 (doi: 10.1038/nbt0810-780).
18. 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).
19. Baltazar B., Castro Espinoza L., Espinoza Banda A., de la Fuente Martínez J.M., Garzón Tiznado J.A., González García J., Gutiérrez M.A., Guzmán Rodríguez J.L., Heredia Díaz O., Horak M.J., Madueño 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(7): e0131549 (doi: 10.1371/journal.pone.0131549).
20. 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).
21. 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(2): 407-426 (doi: 10.1111/1477-9552.12178).
22. Ricci B., Messéana A., Lelièvrec A., Colénod F.C., Angevin F. Improving the management of coexistence between GM and non-GM maize with a spatially explicit model of cross-pollination. *European Journal of Agronomy*, 2016, 77: 90-100 (doi: 10.1016/j.eja.2016.04.008).
23. 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).
24. Duncan B., Leyva-Guerrero E., Werk T, Stojšin D., Baltazar B.M., García-Lara S., Zavala-López M., de la Fuente-Martínez J.M., Meng C. Assessment of potential impacts associated with gene flow from transgenic hybrids to Mexican maize landraces. *Transgenic Research*, 2019, 28(5-6): 509-523 (doi: 10.1007/s11248-019-00160-3).
25. Bøhn T., Aheto D.W., Mwangala F.S., Fischer K., Bones I.L., Simoloka C., Mbeule I., Schmidt G., Breckling B. Pollen-mediated gene flow and seed exchange in small-scale Zambian maize farming, implications for biosafety assessment. *Scientific Reports*, 2016, 6: 34483 (doi: 10.1038/srep34483).
26. Kil' V.I. *Teoreticheskoe obosnovanie i prakticheskoe ispol'zovanie molekulyarno-geneticheskikh metodov v zashchite sel'skokhozyaistvennykh rastenii ot vreditelei i otsenke transgennykh rastenii na biobezopasnost'. Avtoreferat doktorskoi dissertatsii* [Theoretical substantiation and practical use of molecular genetic methods in protecting agricultural plants from pests and assessing transgenic plants for biosafety. DSc Thesis]. Krasnodar, 2010 (in Russ.).
27. 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(4): 1273-1282 (doi: 10.2135/cropsci2004.1273).
28. Bannert M., Stamp P. Cross-pollination of maize at long distance. *European Journal of Agronomy*, 2007, 27(1): 44-51 (doi: 10.1016/j.eja.2007.01.002).
29. Langhof M., Hommel B., Hüsken A., Schiemann J., Wehling P., Wilhelm R., Rühl G. Coexistence in maize: do nonmaize buffer zones reduce gene flow between maize fields? *Crop Science*, 2008, 48(1): 305-316 (doi: 10.2135/cropsci2007.04.0189).
30. Gutorova O.V., Apanasova N.V., Yudakova O.I. *Izvestiya Samarskogo nauchnogo tsentra Rossiiskoi akademii nauk*, 2016, 18(2): 341-344 (in Russ.).
31. Coe E.H. Jr. A line of maize with high haploid frequency. *The American Naturalist*, 1959, 93(873): 381-382 (doi: 10.1086/282098).
32. Smol'kina Yu.V., Serikov L.V., Kalashnikova E.V. *Byulleten' botanicheskogo sada Saratovskogo gosudarstvennogo universiteta*, 2004, 3(1): 144-148 (in Russ.).
33. 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(8): 1055-1065 (doi: 10.1016/j.agrformet.2011.03.009).

34. 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(5): 1551-1557 (doi: 10.2135/cropsci2001.4151551x).
35. Angevin F., Klein E., Choimet C., Meynard J., de Rouw A., Sohbi Y. Modélisation des effets des systèmes de culture et du climat sur les pollinisations croisées chez le maïs. Isolement des collectes et maîtrise des disséminations au champ. In: *Rapport du groupe 3 du programme de recherche: pertinence économique et faisabilité d'une filiure sans utilisation d'OGM, INRAFNSEA* /J.-M. Meynard, M. Le Bail (eds.). Thiverval-Grignon, France, 2001: 21-36.
36. 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(12): 4377-4384 (doi: 10.1021/es0494252).
37. Gutorova O.V. *Byulleten' Botanicheskogo sada Saratovskogo gosudarstvennogo universiteta*, 2016, 14(2): 62-70 (in Russ.).
38. 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.
39. 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).
40. Westgate M., Lizaso J., Batchelor W. Quantitative relationship between pollen-shed density and grain yield in maize. *Crop Science*, 2003, 43(3): 934-942 (doi: 10.2135/cropsci2003.9340).
41. 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(3): 255-265 (doi: 10.1016/j.eja.2007.07.009).