

TWO-YEAR MONITORING OF RUDERAL RAPESEED POPULATIONS IN AREAS POTENTIALLY CONTAMINATED WITH GENETICALLY MODIFIED PLANTS AND COUNTRY-WIDE POLLEN SAMPLE SCREENING FOR GMO CONTAMINATION IN LATVIA

Lelde Grantiņa-Ievina^{1#}, Juris Ķibilds¹, Lilija Kovaļčuka¹, Guntis Boikmanis¹,
Karīna Ortlova¹, Artjoms Mališevs¹, Evija Bebre¹, and Gederts Ievinsh²

¹ Institute of Food Safety, Animal Health and Environment “BIOR”, 3 Lejupe Str., Rīga LV–1076, LATVIA

² Faculty of Medicine and Life Sciences, University of Latvia, 1 Jelgavas Str., Rīga LV–1004, LATVIA

Corresponding author, lelde.grantina-ievina@bior.lv

Communicated by Aivars Bērziņš

Latvia has documented cases of unintentional release of genetically modified plants (GMP), including contamination of rapeseed with GM varieties such as GT73. Due to significant import and export of rapeseed (over 120 thousand tons annually from 2020 to 2022), monitoring is essential to evaluate accidental GMP spread in the environment. Similar studies in Germany, Switzerland, the Netherlands, and Lithuania have been conducted to monitor GM rapeseed near processing plants, ports, and transport routes, generally finding minimal or no GM contamination in ruderal populations. By using bees as natural pollen collectors, pollen sampling serves as an indicator of GMO presence and spread while also providing insight into gene-flow risks within terrestrial ecosystems. Given Latvia's recent detection of accidental GM rapeseed admixtures and limited annual testing, this study prioritised pollen analysis and monitoring of ruderal rapeseed plants. This study presents a comprehensive two-year monitoring effort of ruderal rapeseed populations in Latvia, focusing on potential contamination with GMPs near ports, railway cargo areas, and rapeseed processing factories. It also includes a nationwide screening of pollen samples to detect GMP presence, aiming to assess the environmental impact and spread of GMP in Latvia. None of the samples were considered to contain GMP screening elements.

Keywords: environmental risk assessment, pollen monitoring, gene flow, hybridisation, biosafety, *Brassica napus*.

INTRODUCTION

According to EFSA (2011) guidance on Post-Market Environmental Monitoring, monitoring of ruderal, feral, or volunteer genetically modified (GM) plants is required as part of General Surveillance to detect unanticipated environmental effects beyond cultivated fields. Such plants may arise from seed spillage, transport losses, or persistence of volunteers, leading to unintended establishment along roadsides or in ruderal habitats. Their occurrence can serve as an early indicator of environmental exposure and potential

gene flow to compatible wild relatives, thereby testing key assumptions of the environmental risk assessment. Monitoring the establishment, persistence, and hybridisation potential of these plants is therefore essential for verifying containment effectiveness, identifying unforeseen ecological consequences, and ensuring the protection of biodiversity and ecosystem functions.

In recent years, several cases of unintentional release of genetically modified plants (GMP) in the environment have been documented in Latvia. In 2021, as part of the monitor-

ing of seed and plant propagating material, rape seed contamination with GT73 rape seeds was detected. The total area of sowing was 843.33 ha. All fields were destroyed by farmers (Dieziņa, 2021). Earlier, in 2017, in the framework of the project “Assessment of possible risks of genetically modified seeds and propagating material in the territory of Latvia and development of risk management recommendations in accordance with Latvian agro-economic conditions”, two small lots of seeds (maize and soybean) containing GM seed impurities were found and the sown fields were destroyed. In 2017, genetically modified petunia seedlings and seeds were detected as well. Latvia is exporting and importing rapeseed; in 2020–2022 the import was over 120 thousand tons per year (according to Central Statistical Bureau of Latvia). Therefore, the need to evaluate the spread of unintentional GMOs in the Latvian environment is relevant and justified.

Similar studies have also been conducted in other European countries as well. For example, in a study in Germany, the task was to monitor the presence of GM rapeseed in the environment as a result of the spilling of imported seeds. The most important rapeseed crushing and processing companies were identified and monitored from April to May. An inventory of rapeseed and potential hybridisation partners was carried out at the study sites. The number of samples collected was proportional to the size of the corresponding plant group at a given site. Wild rapeseed was found in all study areas, both in the territory of processing companies and in publicly accessible places in their surroundings. The wild cruciferous species (mainly *Sinapis arvensis*) was found only in some places. Most rapeseed plants were observed and sampled along roads and railroad tracks. Only one plant out of 1918 tested was GM positive (Roundup Ready™) – in the port of Neuss (Franzaring *et al.*, 2016).

A study in Switzerland was aimed to carry out risk-oriented monitoring of GM rapeseed along the railway lines from the Swiss border with Italy and France to the rapeseed processing plants. In the risk assessment, based on the ability to potentially persist under Swiss conditions, rapeseed *Brassica napus* was recognised as the most important (ability to germinate outside agricultural areas and form wild populations, ability to cross with related wild and cultivated species). Two rapeseed processing plants and railway sections used for their supply in the south and north of the country, as well as two transshipment points at the Rhine port in Basel, were selected for monitoring. Railroad crossings and sharp bends were considered as hotspots. GT73 rapeseed was found at three locations. The GM canola plants found contained *gox* and CP4 *epsps* genetic elements and were resistant to herbicides. None of the collected plants of potential hybridisation partner species were GM (Hecht *et al.*, 2014).

A study in Netherlands was carried out to find whether and to what extent GM *B. napus* is imported into the Netherlands and to determine the transport routes; to determine whether *B. napus* populations exist in connection with transshipment sites and transport routes; to analyse the persistence and size of populations formed as a result of seed

spillage; and to check the possible presence of GM *B. napus*. It was concluded that the size and type of processing plants should be taken into account for the monitoring: in large oil presses located in close proximity to ports and where high temperatures and chemicals are used, spillage of seeds could only occur as a result of transshipment operations, but not with processing waste products. Small oil presses use the mechanical pressing method, as a result of which viable seeds can remain in the waste products. Riverbanks, the vicinity of ports, roadsides, railway areas have been identified as potential places for seed spillage outside transshipment points. In total 160 combined samples of five leaves from different plants were analysed. Molecular analyses did not show a conclusive presence of GM *B. napus* in ruderal populations (Luijten *et al.*, 2019).

In Lithuania monitoring was carried out in 2019–2020 as part of the project “Assessment of the impact of GMO risks on the environment”. One of the project’s goals was to prepare methodological instructions for the creation of a monitoring programme for spontaneous GM plant populations, summarising the regulatory framework and the experience of European countries for the management of potentially self-sowing GM plants. The study was based on a technical report by the European Network of Environmental Protection Agency Managers (EPA Network), the European Nature Conservation Agency Managers Network (ENCA) and the Joint EPA ENCA Interest Group on Genetically Modified Organisms (Zünd *et al.*, 2019). The target audience of the developed programme was competent state authorities and other persons tasked with monitoring GM plants. Five sites close to port and processing companies were chosen in priority order, as well as one railway section and A1 highway. Not only rapeseed plants, but also the charlock *Sinapis arvensis* and the wild radish *Raphanus raphanistrum* were taken for analysis, mostly in cases where there were no rapeseed plants in the previously selected monitoring sites. In 2019, 64 samples were taken and analysed with molecular biology methods, and in 2020 — 29 samples. In the 1st year, samples were tested for the presence of the following GM rapeseed events: GT(RT)73, MS8, RF3, T45. In the 2nd year, samples were tested for a wider spectrum of GM rapeseed events: GT(RT)73, MS8, RF3, T45, MS11, DP073496, OXY235, and MON88302. No genetically modified plants were found (Paulauskas, 2020).

Pollen has been used in GMO research elsewhere. For example, Germany looked for the admixture of GM corn in pollen samples by comparing those collected with special devices to those collected by bees. Pollen collected by bees was found to have a similar proportion of GM maize pollen to samples collected with pollen collectors (Hofmann *et al.*, 2010). As a result of this research, special regulations were developed in the European Union regarding the presence and detection of GM corn pollen in honey (Binimelis and Wickson, 2018).

Pollen can be used as a verifiable indicator to determine the possible spread of transgenes in the environment in the context of the goal of protecting terrestrial ecosystems — the

protection of biological diversity (genetic variability, the diversity of species and their functions, the diversity of habitats and ecosystems), as well as the prevention of air pollution. Bee colonies can be used as a biological form of pollen extraction similar to technical pollen extraction devices (Züghart *et al.*, 2013).

The bee colony collects nectar and pollen within a radius of 2 km from the location of the apiary (Mizis, 2015), but 3 to 10 km is mentioned in other literature sources as well.

The spectrum of plant species that may have been modified and have accidentally entered and spread into the environment varies significantly in different regions of the world. For example, in New Zealand, the main nectar plants that could theoretically have been genetically modified and accidentally entered the environment are considered to be white clover, rapeseed, eucalyptus, kiwi, apple and maize (Malone, 2002). In China, however, scientific experiments have shown that Chinese honey bees (*Apis cerana cerana* Fabricius) collected pollen from GM rapeseed to a similar extent as pollen from non-modified rapeseed (Liu *et al.*, 2017). In the European Union, of course, the most relevant GM crop is maize MON810, which has been cultivated for a long time in Spain and Portugal (Arpaia *et al.*, 2021).

However, taking into account that in Latvia in 2021, accidental admixtures of GM rapeseed were detected in summer rapeseed material, and that only 40 seed samples are tested for the presence of accidental GM admixtures in Latvia each year, as well as the conclusions of experts in this and the previous project (2016–2018), that GM rapeseed in Latvia is more likely to survive and spread among other GM plants, increased attention was paid to the monitoring of ruderal rapeseed plants and pollen samples specifically in relation to rapeseed cultivation and import and export in Latvia.

The aim of the present study was to monitor ruderal rapeseed populations in areas potentially contaminated with genetically modified plants in Latvia and to include pollen samples in the country wide GMPs monitoring as well. The following tasks were determined: 1) to carry out monitoring and collect samples of ruderal rapeseed plants in surroundings of ports, railway cargo handling areas, rapeseed processing factories, etc.; 2) to determine the presence of GMO screening elements in the collected plant and pollen samples.

MATERIALS AND METHODS

Study sites, monitoring and plant samples. A methodology developed in Germany was chosen for the study (Sukopp and Schmitz, 2013). This floristic mapping method includes a 2 km zone around grain processing plants, ports, transshipment areas. If the company is located by the river, then this zone includes a stretch of the river 2 km upstream and 3 km downstream. Access roads, railway lines and relevant river sections are surveyed within a radius of 2 m around the respective company. Mapping of found rapeseed

plants was carried out with notes, photos and GPS coordinates.

Information in the mass media and on the websites of the companies working in the industry were used to find out where the ports, railway cargo transshipment points, rapeseed processing plants and rapeseed receiving points are located. Ports where grain transshipment takes place were located in Rīga, Liepāja, Ventspils, and Mērsrags. In the Rīga Freeport, grain transshipment takes place in 13 terminals on both banks of the Daugava river. Grain harvests of Lithuanian origin are also loaded onto ships in the ports of Rīga. In larger towns like Rīga, Liepāja, and Ventspils several grain logistic and processing companies were located close to each other with the same main streets for logistic traffic. The most important railway cargo transshipment points in terms of grain transport were Bolderāja 2 and Zemitāni. Intensive freight train traffic takes place on the railway section Zemitāni – Šķirotava. Each urban/port area (Rīga, Ventspils, Liepāja) was treated as a “sampling cluster” rather than individual companies (Table 1). Other monitoring objects located all around the country included grain acceptance points and cooperation partners of the agricultural services’ cooperative societies like LATRAPs and VAKS, and companies Baltic Agro, Linas Agro, Balticovo, and Vidzeme Oil Factory.

Increased attention was paid to objects located near protected natural areas: 1) “Alfa Port” in Rīga, located near the Nature Reserve Vecdaugava; 2) Mērsrags port, located near

Table 1. Number of sampling sites in each town or county, total population of *Brassica napus* plants and total number of sampled leaves

Town / Municipality	Number of sampling sites in town / county	Total population of <i>Brassica napus</i>	Total number of sampled leaves
Rīga	82	5177	764
Ventspils	24	621	234
Liepāja	19	883	201
Bauska Municipality	13	288	125
Jelgava Municipality	13	520	130
Madona Municipality	13	642	125
Aizkraukle Municipality	12	160	120
Jēkabpils Municipality	11	1668	109
Valmiera Municipality	9	100	92
Talsi Municipality	7	118	58
Rēzekne Municipality	6	68	57
Saldus Municipality	4	246	36
Augšdaugava Municipality	4	91	45
Dobele Municipality	4	41	40
Gulbene Municipality	4	2080	41
Tukums Municipality	2	71	20
Ogre Municipality	1	23	1
Salaspils Municipality	1	18	6
Kuldīga Municipality	1	8	8
Cēsis Municipality	1	11	10

Engure Nature Park; 3) Vidzeme Oil Factory, located near the territory of the Gauja National Park; and 4) Otaņķi Dzirnānieks, located approx. 500 m from the NATURA 2000 nature area by Lake Liepāja. Natura 2000 is a network of protected areas across the European Union aimed at preserving biodiversity. Contamination with GMPs in such areas is particularly undesirable (EFSA, 2011).

The monitoring was carried out in the period from May until end of September in 2023 and 2024. The surroundings of 69 objects were surveyed (Figs. 1 and 2). Total distance

travelled was 7500 km. The number of combined *B. napus* samples taken was 230 (10 leaves each, if available), of which 223 were rapeseed leaf samples, four were *B. napus* seed samples, but three samples were wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and mustard (*Sinapis alba*) leaves. The pooled samples were from a total of 2200 plants and represented a population from ca. 12,800 plants. Plant samples were collected in sterile Nasco Whirl-Pak bags and kept in the foam box with cold elements during transportation and later in the refrigerator till the extraction of DNA. Seed samples were collected in paper bags.

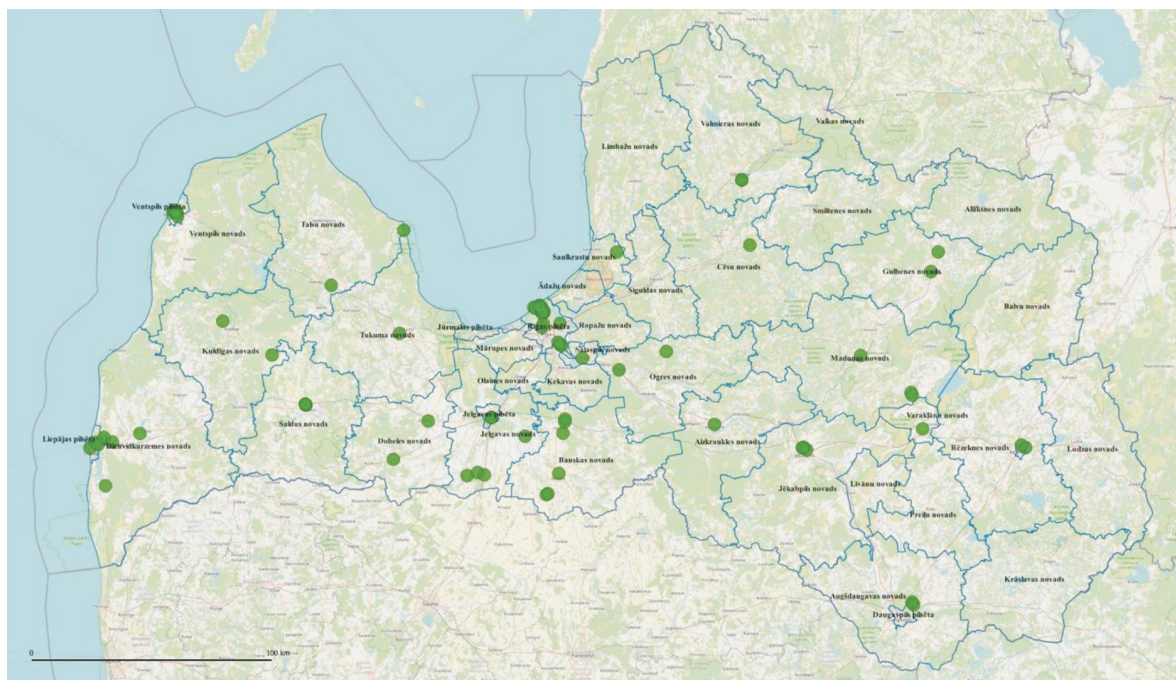


Fig. 1. Location of 69 monitoring objects on the map of Latvia — surroundings of ports, railway cargo handling areas, and rapeseed processing factories.



Fig. 2. Location of monitoring objects within the territory of Rīga.

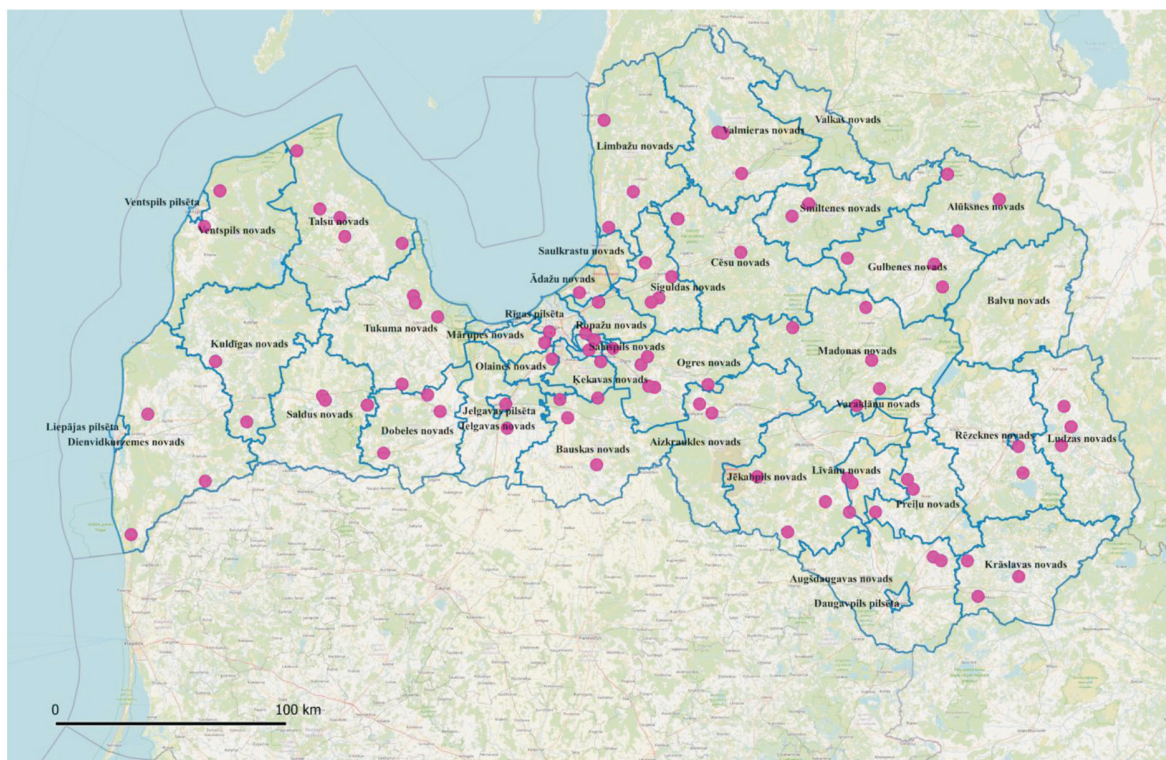


Fig. 3. Places of the origin of 97 pollen and three bee bread samples on the map of Latvia.

Other *Brassicaceae* species detected in the monitored territories were, for example, tall rocket *Sisymbrium altissimum*, warty-cabbage *Bunias orientalis*, mustard *Sinapis* sp., shepherd's-purse *Capsella bursa-pastoris*, and field pennycress *Thlaspi arvense*. Other plants detected along road sides in few cases were field beans *Vicia faba* and peas *Pisum sativum*. Maize *Zea mays* and soybean *Glycine max* plants were not detected.

Pollen samples. In the first year of the project, 49 pollen samples were collected from retail outlets in person and on-line. In the second year of the project, 48 pollen samples and three bee bread samples were collected in collaboration with the Latvian Beekeeping Society. Places of the origin of 97 pollen and 3 bee bread samples on the map of Latvia are given in Figure 3. Distribution of the samples by counties is given in Table 2.

Total sample coverage. Figure 4 illustrates the location of the pollen samples and monitoring sites around the country and in relation to Natura 2000 areas. Several apiaries were located within the protected nature territories like Slitere National Park territory, Gauja National Park Nature Reserve and Landscape Protection Zone, and Talsi Hills Nature Park. One sample was declared to be from an organic farm.

GMO screening and further analysis. Rapeseed leaves and pollen samples were crushed using a mortar and a pestle. DNA was isolated from 200 mg of the homogenised sample in duplicate using the NucleoSpin Food, Mini kit (Macherey-Nagel).

DNA concentration and absorbance were determined with a NanoDrop ND-1000 at 230, 260, and 280 nm. The next

DNA quality control step involved amplification of a plant chloroplast gene, which indicates whether the sample contains amplifiable plant DNA, and/or amplification of a plant taxon-specific gene:

- 1) Plant chloroplast intron gene *trnL* – according to ISO 21569:2005 Annex A.2. “Determining the sequence of a multicopy gene (*trnL* intron) in plant chloroplasts”;

Table 2. Distribution of 97 pollen samples and 3 bee bread samples across the municipalities of Latvia

Municipality	Number of samples	Municipality	Number of samples
Aizkraukle	3	Madona	6
Alūksne	3	Mārupe	1
Augšdaugava	2	Ogre	8
Ādaži	1	Olaine	2
Bauska	3	Preiļi	3
Cēsis	3	Rēzekne	2
Dienvidkurzeme	3	Rīga	2
Dobele	3	Ropaži	3
Gulbene	3	Salaspils	2
Jelgava	2	Saldus	4
Jēkabpils	3	Sigulda	5
Krāslava	3	Smiltene	2
Kuldīga	4	Talsi	5
Ķekava	2	Tukums	4
Limbaži	2	Valmiera	3
Līvāni	3	Ventspils	2
Ludza	3	-	-

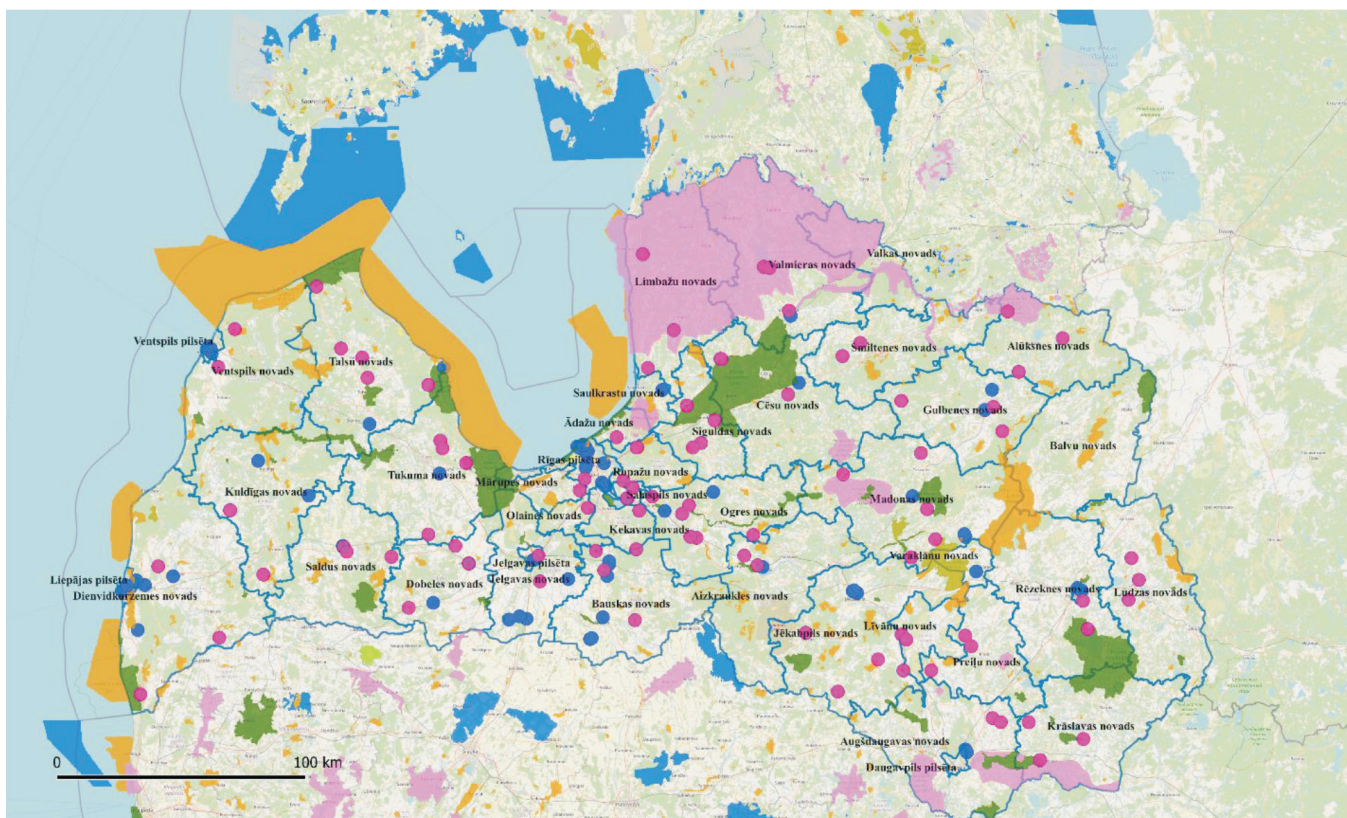


Fig. 4. Location of the pollen samples and monitoring sites around the country and in relation to Natura 2000 areas. The Natura 2000 areas map layer was used from: https://bio.discomap.eea.europa.eu/arcgis/rest/services/ProtectedSites/CDDA_Dyna_WM/MapServer.

- 2) The plant taxon-specific gene – *cruA* (cruciferin A gene for rapeseed), according to GMOMETHODS: EU Database of Reference methods <https://gmo-crl.jrc.ec.europa.eu/gmomethods/>, *cruA* – CRL-GMFF: Protocol RT73.

The DNA extracts of the samples were screened for GMO on six genetic elements:

- 1) cauliflower mosaic virus CaMV 35S promoter and nopaline synthase terminator T-nos – ISO 21569:2005 Annex B.9. “Determination of certain DNA sequences often used in GM organisms, originating from CaMV 35S promoter (P35S), as well as *Agrobacterium tumefaciens* terminator (T-nos), in food products – screening method”;
- 2) phosphinothricin N-acetyltransferase gene *pat* – “Quantitative PCR method for detection of phosphinothricin N-acetyltransferase gene”, JRC Compendium of Reference Methods for GMO Analysis;
- 3) figwort mosaic virus 34S promoter pFMV, ISO/TS 21569-5:2016 “Real-time PCR-based screening method for determination of FMV promoter (P-FMV) DNA sequence”;
- 4) E9 terminator of the pea ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) gene *tE9*, GMO Methods: EU database of reference methods. Qualitative PCR method for detection of *tE9* terminator (2016);

- 5) phosphinothricin acetyltransferase gene *bar*, ISO 21569:2005 Annex B.8. “Real-time PC R-based screening method to detect *Streptomyces hygroscopicus bar* gene”;
- 6) for pollen samples two closely related genes within the *cryI* family, both targeting *Lepidopteran* insects – *cryIAb* and *cryIAc* genes – GMO Methods: EU database of reference methods, Qualitative PCR method for detection of *cryIAb/Ac* gene (2017);
- 7) in addition, all samples were screened for the presence of genetic material specific to the GM rapeseed events DP-073496-4 and MON94100, as there are no screening genes (Jacchia *et al.*, 2014).

DNA obtained from certified reference material traceable to SI units was used as a positive control in PCR reactions. Reference materials were purchased from European Reference Materials (ERM) and the American Oil Chemists’ Society (AOCS). Extraction blank controls and negative real time PCR controls were used in every reaction.

The estimated limits of detection (LOD) for all screening elements were as follows: CaMV 35S promoter < 0.07 %, FMV 35S gene (pFMV) 1.00 %, NOS terminator < 0.07 %, *pat* gene 0.09 %, *bar* gene 0.10 %, *tE9* 0.01 %, and *cryIAb/Ac* 0.001 %. One 100 %-GMO plant among 10 equal-mass leaves contributes about 10 % GMO DNA to the pool. All of the listed LODs (0.07% – 1%) are much lower than 10%, so each of those targets should be detectable if a single GMO plant is present in a 10-leaf pool.

Those DNA extracts from canola leaves that showed any amplification for the screening elements were tested for particular canola events using event-specific methods according to the GMO Methods database (<https://gmo-crl.jrc.ec.europa.eu/gmomethods/>): RT73, MON88302, Ms11, RF1, RF3 in 2023 and RT73, MON88302, Topas19/2, T45, Ms1, Ms8, Ms11, RF1, RF2, RF3 in 2024 (depending on the screening element combinations). These samples were tested for the natural CaMV 35S promoter as well (Chaouachi *et al.*, 2008).

The upper 95% confidence intervals for combined plant samples and pollen samples were calculated using the “3/N” method (Rule of Three: when 0 positives in N independent tests, a simple 95% upper bound is $\approx 3/n$).

Data analysis. Maps were created using QGIS 3.10.14-A Coruna. The Natura 2000 areas map layer was used from: https://bio.discomap.eea.europa.eu/arcgis/rest/services/ProtectedSites/CDDA_Dyna_WM/MapServer.

RESULTS

Results of the rapeseed monitoring. Out of 69 facilities (Fig. 1), 58 facilities (84%) were found to have rapeseed plants at their surroundings in at least one of the monitoring years. Rapeseed plants were not detected in the vicinity of 11 surveyed objects. Flowering of rapeseed plants were observed from the second half of June, but recently sprouted plants were found in large numbers at the beginning of harvesting. The territories close to objects located near protected natural areas had few (4 to 10 plants) or no rapeseed plants at all.

During the monitoring in 2023, four places in Rīga were found, where the existing rapeseed populations could be described as permanent populations, and the situation was monitored also in the next year. In these territories it was observed that rapeseed plants grow at a distance of several meters (up to 8 m) from the edge of the roadway, and rapeseed plants in various stages of growth were found, including flowering plants and plants with ripe seeds. The plant density was variable (Table 3). Individual plants did not have many seeds and mostly they were poorly developed and infested by pests (example from Vecāķi Prospectus in Table 4). Seed counts per individual plant at Vecāķi Prospectus ranged from 52 to 236 seeds, with pod counts between 9 and 29 pods per plant.

In total, 216 of 230 monitored samples showed no amplification for any of the six screening elements (CaMV 35S promoter, T-nos, *pat*, pFMV, tE9, *bar*), as well as the rapeseed event DP-073496-4 and MON94100. During both monitoring years in total 14 pooled rapeseed leaf samples from port surroundings in Rīga, Liepāja, and Ventspils showed late amplification (Ct 39) for one or more screening genes in one or both duplicate DNA extractions in one or several repetitions of particular real-time PCR reactions (Table 5). According to the methodology developed by Luijten and colleagues (2019), such Ct values should be

evaluated as negative. To check the reliability of the method developed in the Netherlands, for sample 8.5. DNA was extracted from each rapeseed leaf individually in two replicates, and all screening reactions were performed except for the *bar* gene (Table 6). The Ct values obtained for the individual leaf samples were also late (39) and were evaluated as negative.

Results of the pollen monitoring. Amplification on the cruciferin A gene *CruA* was obtained for all samples, except for one sample from Rēzekne district. Real-time PCR amplification Ct values of the cruciferin A gene *CruA* ranged from 22.45 to 38.27 (mean 26.27), indicating that nearly all samples contained pollen from canola and other cruciferous plants.

With the method used, positive amplification can be obtained not only for canola, but also for plants of other cruciferous plant species: *Arabidopsis thaliana*, various subspecies of mustard *B. juncea*, black mustard *B. nigra*, various subspecies and cultivars of garden cabbage *B. oleracea*, various cabbage *B. rapa* subspecies, radish *R. sativus* and white mustard *S. alba* (Wu *et al.*, 2010).

All 97 pollen and three bee bread samples were negative for the screened genes tested, as well as for the individual canola events DP-073496-4 and MON94100.

The study power. Each “sampling cluster” around the largest urban/port areas (Rīga, Ventspils, Liepāja) had the highest numbers of sampling sites and highest numbers of combined rapeseed leave samples. However, since there are relatively few beekeepers in urban areas, the number of pollen samples in these areas was small.

The 95% confidence levels for plant samples, pollen samples and across all samples together were as follows:

- Pollen samples (n = 100): the 100 pollen samples were treated as independent, so the 95% upper bound on the proportion of pollen samples that would contain GMO screening elements was 2.95%;

Table 3. Average rapeseed plant population density in four locations in Rīga

Population	Number of plants/m ²
Daugavgrīvas Street	2.0
Lejasvoleri port	5.0
Bolderāja, Gaigalas Street	6.6
Vecāķi Prospectus	13.0

Table 4. Number of pods and seed of individual plants in Vecāķi Prospectus

Sample	Number of pods	Total number of seeds
26.2	26	64
26.3	29	221
26.4	9	52
26.5	25	236

Table 5. Examples of late amplifying samples in 2023 and 2024

Sample	<i>CruA</i> , Ct average	Cut-off, Ct	Screening elements						DP-073496-4	Natural CaMV p35S
			p35S	tNOS	<i>pat</i>	tE9	pFMV	<i>bar</i>		
Samples in 2023										
4.11.	23.84	31.16	neg ¹	neg	neg	40	neg	neg	neg	NA ³
5.3.	24.47	30.79	neg/ > 40	neg	neg	neg	neg	neg	neg	
7.1.	26.16	33.48	neg/ > 40	neg	neg/ > 39	neg	neg	neg	neg	neg
8.4.	24.15	32.47	neg/ > 39	neg	neg	neg	neg	neg	neg	neg
8.5. ¹	23.57	30.89	neg/ > 40	39	neg	> 40	neg	neg	neg	neg
8.6. ¹	23.73	31.05	neg	neg	neg	> 40	> 40	neg	neg	NA
8.9.	23.20	30.52	neg/ > 40	neg	neg	neg	neg	neg	neg	neg
10.6.	24.91	32.23	neg	neg	> 39	neg	neg	neg	neg	NA
12.7. ¹	24.94	32.26	neg	neg	neg	> 40	neg	neg	neg	NA
Samples in 2024										
8.3.	23.69	31.01	neg/ > 39	neg/ > 40	> 37	neg/ > 39	neg	neg	neg	neg
8.4.	22.25	29.57	neg/ > 38	neg/ > 39	> 39	neg	neg	neg	neg	neg
8.5.	22.01	29.33	neg	neg/ > 39	neg/ > 38	neg/ > 39	neg	neg	neg	NA
8.6.	25.41	32.73	neg/ > 40	neg	neg /39	neg	neg	neg	neg	neg
8.7.	26.43	33.75	neg/ > 40	neg	neg	neg	neg	neg	neg	neg
Other (n=216)	24.33 ²	31.55	neg	neg	neg	neg	neg	neg	neg	NA

¹ neg – negative; ² – for the wheat and barley sample positive amplification of the chloroplast intron gene trnL; ³ NA – not assessed

Table 6. Results of individual leaf examinations of Sample 8.5 in 2023

Sample	CruA, Ct average	Cut-off, Ct	Screening elements				
			p35S	tNOS	pat	tE9	pFMV
8.5.-1	28.21	32.21	neg	neg	neg	> 40	neg
8.5.-2	27.03	31.03	neg	neg	neg	neg/ > 40	neg
8.5.-3	25.22	29.22	> 40/neg	neg	neg	neg	neg
8.5.-4	23.3	29.30	neg	neg	neg	neg/ > 40	neg
8.5.-5	24.23	28.23	neg	neg	neg	neg	neg
8.5.-6	24.7	28.7	neg	neg	neg	neg/ > 40	neg
8.5.-7	23.83	27.83	neg	neg	neg	neg/ > 40	neg
8.5.-8	24.18	28.18	neg	neg	neg	neg/ > 40	neg
8.5.-9	23.58	27.58	neg	neg	neg/ > 39	> 40	neg
8.5.-10	24.74	28.74	neg	neg	neg/ > 39	neg	neg

- Combined plant samples (n = 230): for the 230 combined plant samples, the 95% upper bound on the proportion of combined samples that would test positive was 1.29%;
- Across all samples together (n = 330): pooling evidence across pollen plus plant samples (assuming independence), the 95% upper bound on the proportion of all tested samples that would be positive was 0.90%.

DISCUSSION

The findings of this monitoring align with other international studies indicating that while GM oilseed rape can unintentionally disperse and establish ruderal populations, actual GMP contamination events in the environment were

rare or minimal. However, monitoring ruderal rapeseed populations and screening pollen samples for GMO contamination are critical for understanding and managing the environmental impact of GMP plants.

Studies have shown that oilseed rape (*B. napus*) can unintentionally disperse during transportation, leading to the establishment of feral populations. For instance, a pilot project in Italy monitored possible GM oilseed rape along transport routes and found evidence of seed dispersal of non-GM oilseed rape and potential interspecific gene flow with species like *Raphanus raphanistrum* (Rastelli *et al.*, 2025).

In Canada, surveys along railways and roadsides revealed that transgenic herbicide-resistant oilseed rape is prevalent in ruderal areas, with a significant proportion of plants be-

ing transgenic (Yoshimura *et al.*, 2006). This persistence is influenced by factors such as traffic intensity and proximity to grain elevators (Knispel and McLachlan, 2010). The establishment of GM oilseed rape in non-crop areas poses risks of gene flow to wild relatives and other *Brassica* species, potentially leading to hybridisation and persistence of transgenes in the environment (Elliott *et al.*, 2004; Wurbs *et al.*, 2010).

Pollen from GM crops can travel significant distances, contributing to gene flow. For example, a study on maize pollen deposition found that pollen can be detected several kilometres from the source, indicating the need for buffer zones to prevent contamination (Hofmann *et al.*, 2014). Effective monitoring involves both technical and biological sampling methods. For instance, pollen mass filters and honey samples collected by bees have been used to detect the presence of GM pollen, although improvements in purification methods are needed for routine use (Kuhlmann and Beismann, 2004; Köllner *et al.*, 2006). In Serbia, a case-specific monitoring protocol was developed to detect herbicide-tolerant GM oilseed rape in seed and feed products. This study found GM material in feed samples, highlighting the need for continuous monitoring to ensure compliance with labelling thresholds (Nikolić *et al.*, 2010).

Remote sensing and GIS techniques can help locate rapeseed fields and potential sites of gene flow, improving the efficiency of ground surveys and spatially explicit modelling of pollen dispersal patterns (Elliott *et al.*, 2004; Schmidt and Schröder, 2008). Programmes like BINATS in Austria provide baseline data on biodiversity and habitat configuration, which are crucial for assessing the long-term impacts of GM crop cultivation on biodiversity (Pascher *et al.*, 2011).

In the present study, the widespread presence of rapeseed plants in the vicinity of 84% of the monitored facilities indicates that rapeseed is a common species in these environments, from which many were located in or in vicinity of protected nature sites, like Natura 2000 territories. This high prevalence suggests that rapeseed plants are well-adapted to the local conditions and may have established stable populations in these areas. The identification of permanent rapeseed populations in four locations in Rīga further supports this observation, highlighting the ability of rapeseed to persist and thrive in urban settings.

In summary, monitoring ruderal rapeseed populations and screening pollen samples for GMO contamination are vital for managing the environmental impact of GM crops. These efforts require a combination of field surveys, molecular analysis, and advanced monitoring techniques to ensure effective detection and mitigation of gene flow.

CONCLUSIONS

In general, evaluating the data obtained by analysing ruderal rapeseed samples and pollen samples, it can be concluded

that there are no indications that an accidental and undetected spread of GM plants has taken place in Latvia so far.

The spread of rapeseed in the environment is due to accidental seed dispersal, which can lead to the formation of a persistent soil seed bank. In areas without regular maintenance (mowing or herbicide treatment), rapeseed plants can flower and produce seeds that can spread further. According to the literature, if other plants of the Brassicaceae family grow naturally in these places, their spontaneous hybridisation with rapeseed may occur. The establishment of persistent wild rapeseed populations increases the possibility that genetic material from GM rapeseed can spread in the environment through hybridisation. Continuous monitoring remains essential to manage these risks.

This comprehensive monitoring effort provides valuable baseline data on the status of GMO presence in Latvia's environment, supporting informed decision-making for biosafety and environmental protection.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Institute of Food Safety, Animal Health and Environment "BIOR" for the support in carrying out this study. This study was done within the project Nr.23-00-S0INZ03-000038 "Monitoring of unintentional release of genetically modified plants in the environment and evaluation of environmental monitoring programmes available in Latvia in connection with general monitoring of GMOs" (2023–2024).

REFERENCES

- Arpaia, S., Smaghe, G., Sweet, J. B. (2021). Biosafety of bee pollinators in genetically modified agro-ecosystems: Current approach and further development in the EU. *Pest Manag. Sci.*, **77** (6), 2659–2666.
- Binimelis, R., Wickson, F. (2019). The troubled relationship between GMOs and beekeeping: An exploration of socioeconomic impacts in Spain and Uruguay. *Agroecol. Sustain. Food Syst.*, **43** (5), 546–578.
- Chaouachi, M., Fortabat, M. N., Geldreich, A., Yot, P., Kerlan, C., Kebdani, N., Audeon, C., Romaniuk, M., Bertheau, Y. (2008). An accurate real-time PCR test for the detection and quantification of cauliflower mosaic virus (CaMV): Applicable in GMO screening. *Eur. Food Res. Technol.*, **227** (3), 789–798.
- Dieziņa, S. (2021). Pirmo reizi Latvijā iznīcināti vasaras rapša sējumi. Kāds varēja būt risks videi? [For the first time in Latvia, summer rapeseed fields have been destroyed. What could have been the risk to the environment?] *Latvijas Avīze*, 06.07.2021. <https://www.la.lv/gmo-del-iznicina-sejumus> (accessed 10.05.2026).
- EFSA Panel on Genetically Modified Organisms (GMO). (2011). Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. *EFSA Journal*, **9** (8), 2316.
- Elliott, L. J., Mason, D. C., Wilkinson, M. J., Allainguillaume, J., Norris, C., Alexander, M., Welters, R. (2004). The role of satellite image-processing for national-scale estimates of gene flow from genetically modified crops: rapeseed in the UK as a model. *J. Appl. Ecol.*, **41** (6), 1174–1184.
- Franzaring, J., Wedlich, K., Fangmeier, A., Eckert, S., Zipperle, J., Krah-Jentgens, I., Hünig, C., Züghart, W. (2016). Exploratory study on the presence of GM oilseed rape near German oil mills. *Environ. Sci. Pollut. Res.*, **23** (22), 23300–23307.

- Hecht, M., Oehen, B., Schulze, J., Brodmann, P., Bagutti, C. (2014). Detection of feral GT73 transgenic oilseed rape (*Brassica napus*) along railway lines on entry routes to oilseed factories in Switzerland. *Environ. Sci. Pollut. Res.*, **21**, 1455–1465.
- Hofmann, F., Epp, R., Kruse, L., Kalchschmied, A., Maisch, B., Müller, E., Kuhn, U., Kratz, W., Ober, S., Radtke, J., Schlechtriemen, U., Schmidt, G., Schroder, W., Ohe, W. v. d., Vogel, R., Wedl, N., Wosniok, W. (2010). Monitoring of Bt-Maize pollen exposure in the vicinity of the nature reserve Ruhlsdorfer Bruch in northeast Germany 2007 to 2008. *Environ. Sci. Eur.*, **22**, 229–251.
- Hofmann, F., Otto, M., Wosniok, W. (2014). Maize pollen deposition in relation to distance from the nearest pollen source under common cultivation—results of 10 years of monitoring (2001 to 2010). *Environ. Sci. Eur.*, **26** (1), 24.
- Jacchia, S., Bogni, A., Mazzara, M., Kreysa, J. (2014). Event-specific method for the quantification of oilseed rape DP-073496-4 using real-time PCR. European Union Reference Laboratory for GM Food and Feed, Ispra. 29 pp.
- Knispel, A. L., McLachlan, S. M. (2010). Landscape-scale distribution and persistence of genetically modified oilseed rape (*Brassica napus*) in Manitoba, Canada. *Environ. Sci. Pollut. Res.*, **17** (1), 13–25.
- Köllner, B., Brünen-Nieweler, C., Kilwinski, J., Altenbeck, P. (2006). Monitoring of genetically engineered plants—a pilot study in North Rhine-Westphalia (NRW). *Gefahrst. Reinhalt. Luft.*, **66** (7/8), 311–314.
- Liu, F. X., Bu, C. P., Tang, T., Chen, G. M., Gu, S. K., Wang, Y. N., & Zhao, X. X. (2017). Effects of Chinese honeybee foraging on oilseed rape gene flow and honey ingredients. *J. Agric. Sci.*, **155** (10), 1623–1632.
- Luijten, S. H., Seip, L. A., van Beekvelt, C., Fronen, B., Prins, T. W. (2019). What is known about the import, distribution and presence of GM oilseed rape (*Brassica napus*) in the Netherlands? Stichting Science4Nature, Amsterdam, report S4N2019.06. 48 pp.
- Malone, L. A. (2002). Literature review on genetically modified plants and bee products. HortResearch, Palmerston North. MAF technical paper 5, 47 pp.
- Mizis, A. (2015). *Darbi bišu dravā* [Work in the Apiary]. Avots, Rīga. 168 lpp. (in Latvian).
- Nikolic, Z., Vujakovic, M., Jeromela, A. M., Jovicic, D. (2010). Implementation of monitoring for genetically modified rapeseed in Serbia. *Electron. J. Biotechnol.*, **13** (5), 17–18.
- Pascher, K., Moser, D., Dullinger, S., Sachslehner, L., Gros, P., Sauberer, N., Traxler, A., Grabherr, G., Frank, T. (2011). Setup, efforts and practical experiences of a monitoring program for genetically modified plants: An Austrian case study for oilseed rape and maize. *Environ. Sci. Eur.*, **23** (1), 12.
- Paulauskas, A. (2020). 2014–2020 metų Europos Sąjungos fondų investicijų veiksmų programos 5 prioriteto „Aplinkosauga, gamtos išteklių darnus naudojimas ir prisitaikymas prie klimato kaitos“ 05.5.1 APVA V 018 priemonė „Biologinės įvairovės apsauga“, GMO rizikos poveikio aplinkai vertinimas [Evaluation of the environmental impact of GMO risk under Measure 05.5.1 APVA V 018 “Biodiversity Protection” of Priority 5 “Environmental Protection, Sustainable Use of Natural Resources, and Adaptation to Climate Change” of the 2014–2020 European Union Funds Investment Operational Programme]. Kaunas. 84 pp. (in Lithuanian).
- Rastelli, V., Giovannelli, V., Staiano, G., Bianco, P. M., Sergio, A., Lener, M. (2025). Development of a monitoring plan for the accidental dispersal of genetically modified oilseed rape in Italy. *Seeds*, **4** (2), 20.
- Schmidt, G., Schröder, W. (2008). Selection of representative sites for modelling the dispersal of genetically modified plants in Northern Germany. *Umweltwiss. Schadst.-Forsch.*, **20** (1) 9–22.
- Sukopp, U., Schmitz, U. (2013). How to track genetically modified (GM) plants in the field? The VDI standard method of floristic mapping of GM plants as an efficient tool. *BioRisk*, **8**, 89–110.
- Wu, G., Zhang, L., Wu, Y., Cao, Y., Lu, C. (2010). Comparison of five endogenous reference genes for specific PCR detection and quantification of *Brassica napus*. *J. Agric. Food Chem.*, **58** (5), 2812–2817.
- Wurbs, A., Glemnitz, M., Graef, F., Funke, B., Ehler, S. (2010). Regionalisation of flora elements in field boundaries sensitive to hybridisation with genetically modified oilseed rape. *Environ. Sci. Eur.*, **22** (3), 252–263.
- Yoshimura, Y., Beckie, H. J., Matsuo, K. (2006). Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environ. Biosaf. Res.*, **5** (2), 67–75.
- Zueghart, W., Beismann, H., Schroeder, W. (2013). Tools for a scientifically rigorous and efficient monitoring of genetically modified organisms (GMOs) – VDI Guidelines to ensure high quality of GMO-monitoring data. *BioRisk*, **8**, 3–13.
- Zünd, J., Wüst-Saucy, A. G., Züghart, W., Bühler, C. (2019). Monitoring of spontaneous populations of genetically modified plant species in the environment: Experiences and recommendations for the design of a monitoring program. Joint EPA ENCA Interest Group on Genetically Modified Organisms. 81 pp.

Received 7 January 2026

Accepted in the final form 17 March 2026

DIVUS GADUS ILGS SAVVAĻĀ AUGOŠU RAPŠU POPULĀCIJU MONITORINGS TERITORIJĀS, KAS POTENCIĀLI PIESĀRŅOTAS AR ĢENĒTISKI MODIFICĒTIEM AUGIEM, UN VALSTS MĒROGA PUTEKŠŅU PARAUGU SKRĪNINGS ĢMO PIESĀRŅOJUMA NOTEIKŠANAI LATVIJĀ

Latvijā ir dokumentēti gadījumi, kad ģenētiski modificēti augi (ĢMA) nejauši nonāk vidē, tostarp rapša piesārņojums ar ģenētiski modificētām šķirnēm, piemēram, GT73. Ņemot vērā ievērojamo rapša importu un eksportu (vairāk nekā 120 tūkstoši tonnu gadā no 2020. līdz 2022. gadam), monitorings ir būtisks, lai izvērtētu nejaušu ĢMA izplatīšanos vidē. Līdzīgi pētījumi veikti Vācijā, Šveicē, Nīderlandē un Lietuvā, lai uzraudzītu ģenētiski modificēta rapša sastopamību pārstrādes uzņēmumu, ostu un transporta maršrutu tuvumā, un tajos parasti konstatēts minimāls vai neesošs ĢMA piesārņojums savvaļas vai ruderalajās populācijās. Izmantojot bites kā dabiskas putekšņu savācējas, putekšņu paraugu analīze kalpo kā rādītājs ĢMO klātbūtnei un izplatībai, vienlaikus sniedzot ieskatu gēnu plūsmas riskos sauszemes ekosistēmās. Ņemot vērā nesenā nejaušo ģenētiski modificēta rapša piejaukumu konstatēšanu Latvijā un ierobežoto ikgadējo testēšanu, šajā pētījumā prioritāte tika piešķirta putekšņu analīzei un ruderalo rapša augu monitoringam. Pētījumā apkopoti visaptveroši divu gadu monitoringa rezultāti par ruderalajām rapša populācijām Latvijā, īpašu uzmanību pievēršot iespējamam piesārņojumam ar ģenētiski modificētiem augiem ostu, dzelzceļa kravu pārstrādes teritoriju un rapša pārstrādes rūpnīcu tuvumā. Pētījumā iekļauta arī valsts mēroga putekšņu paraugu skrīninga analīze, lai noteiktu ĢMA klātbūtni un izvērtētu to ietekmi uz vidi un izplatību Latvijā. Nevienā no paraugiem netika konstatēti ĢMA skrīninga elementi.