Genetically modified oilseed rape (*Brassica napus*)

Aspects in relation to the environmental risk assessment and
post-market environmental monitoring of import applications

COGEM advisory report CGM/130402-01

Contents

1 Summary

Brassica napus *(oilseed rape) has the biological characteristics to cross-pollinate with wild relatives and to form feral populations under northern-western European climatic conditions. Spillage of* B. napus *seeds can therefore lead to the establishment of feral* B. napus *populations. These populations occur along transport routes such as roads and railways, and around previous cultivation sites. If a population arises from spilled herbicide tolerant genetically modified (GM)* B. napus *seeds, use of the corresponding herbicides provides a selective advantage for the GM plant.*

B. napus *can cross-hybridise with wild relatives, which may involve the permanent incorporation of transgenes in feral populations. Further cross-fertilisation can eventually lead to several transgenes coming together in one plant (stacking). In the literature, stacking of transgenes in* B. napus *has been reported along roadsides in North America which had been sprayed with herbicides. Interspecific hybridisation between* B. napus *and* B. rapa *(turnip) occurs under natural conditions and has been reported in the Netherlands. It is known that backcrosses between these hybrids and* B. rapa *may occur in the field.*

The environmental risk assessments (ERAs) of the currently authorised GM B. napus *events in the European Union indicate that these events in themselves do not pose a risk to the environment in the Netherlands. However, stacking of transgenes may occur in feral* B. napus. *It cannot be excluded that a possible combination of GM traits and/or a possible interaction between gene products, expressed by the genetic modification, may result in a potentially adverse effect. Therefore, general surveillance of feral GM* B. napus *populations will be needed to identify any unanticipated, delayed or (in)direct adverse effects. As these (stacked) GM* B. napus *plants are most likely to occur in handling areas and along transport routes, in particular when herbicides are used for weed control, general surveillance should focus on these areas. Where GM* B. napus *plants occur, transgene flow to* B. rapa *is possible. Any* B. rapa *populations in the vicinity of GM* B. napus *should therefore be included in the general surveillance plan and monitored for the presence of transgenes.*

2 Introduction

Oilseed rape (*Brassica napus*, 'Koolzaad' in Dutch, 'Colza' in French, 'Raps' in German) is an important oil plant cultivated in the temperate climate regions of the world. Among the genetically modified (GM) crops commercially available in Europe, i.e. maize, soybean, cotton, and oilseed rape,[1] only oilseed rape has biological characteristics that enable it to establish feral populations under northern-western European climatic conditions and to cross-pollinate with wild relatives.

The capability of *B. napus* to form feral populations is still unclear, although the species is included in several European floras, suggesting that *B. napus* has established itself in many countries. In several floras it is pointed out that distribution maps may be unreliable as *B. napus* could have been confused with its closest relative *Brassica rapa* (turnip, 'Raapzaad' in Dutch, 'Navette' in French, 'Rübsen', 'Rübsamen' or 'Rübsaat' in German).[2,3]

If a crop and its wild relatives co-occur, gene flow (i.e. the transfer of genetic information between different individuals, populations, or species) and introgression (i.e. the permanent incorporation of genetic information from one set of differentiated populations into the gene pool of another) can occur.[4] Several papers describe gene flow from *B. napus* to wild relatives. The possible feral character of *B. napus* as well as its capability to transfer transgenes into wild relatives, entails consequences for the environmental risk assessment (ERA) and post- market environmental monitoring (PMEM) of both import and cultivation applications of GM *B. napus*.

In recent years, COGEM has published several research reports concerning the identification, distribution, hybridisation and spillage of *B. napus* (seeds) in the Netherlands.[5,6,7] The identification of *B. napus* and *B. rapa* has been re-examined and their distribution in the Netherlands has been recorded.[5,6]

Because of the updated insights in the occurrence of feral *B. napus* populations in the Netherlands and the growing knowledge concerning the putative flow of (trans)genes of *B. napus* into wild relatives, COGEM has reviewed the aspects relevant for the ERA and PMEM of GM *B. napus* events in the Netherlands. The aspects encompass, among other things, the biological characteristics of the crop and the currently commercially available GM *B. napus* events.

In this advisory report relevant aspects and (inter)national field observations on the distribution of (GM) *B. napus* and its close relative *B. rapa* are summarised. This information provides knowledge on the invasiveness and weediness potential of the currently available *B. napus* varieties and the possibly associated environmental risks of the introduction of GM *B. napus* events into the Dutch environment. The information also provides insight on aspects relevant to the PMEM of GM *B. napus* applications. At the end of the report, COGEM summarises its considerations concerning the ERA and PMEM of the currently commercially available GM *B. napus* events in the Netherlands. These considerations endorse previous COGEM advisory reports on GM *B. napus* market applications.[8,9,10,1112,13]

3 Crop characteristics

Since 4000 years *B. napus* is grown worldwide for its oil and protein content.[14,15] Seeds are crushed and solvent extracted for their oil content. [19] Depending on its components, the oil is used for industrial purposes or for human consumption.[19,20] The remainder of the crushed seeds is processed into ‘meal’, a high quality protein fraction which is mainly used for feed. *B. napus* cultivars can be differentiated on basis of their content in erucic acids and glucosinates, and in spring and winter varieties.

Old *B. napus* varieties are high in erucic acid and aliphatic glucosinolates, such as progoitrin, and were used for the production of lamp fuel and machine oil.[14,16] Due to the high erucic acid content, this oil is very stable at high temperatures in the presence of water and steam.[14] Although the seeds of *B. napus* have a high nutritional value because of their protein quality, old *B. napus* varieties are less useful as food for animals or humans. They have a bitter taste due to the high levels of glucosinolates.[14] Besides, high doses of both glucosinolates (especially progoitrin) and erucic acid are assumed to have detrimental effects on humans and animals.[17,18]

In the past plant breeders focussed their efforts on lowering both glucosinolate and erucic acid levels in *B. napus*, *B. rapa*, and *Brassica juncea* (brown or Indian mustard, ’sareptamosterd’ in Dutch). These three species are collectively known as ’rapeseed’.[19] Modern varieties of ’rapeseed’ are low in erucic acid (LEAR, low erucic acid rapeseed) and/or glucosinolates, depending on whether they are used for food and feed, or for industrial purposes such as the manufacture of biofuel (bio-diesel), paints, and lubricants. There has been a recent revival in interest in (super) high erucic acid rapeseed ((S)HEAR) varieties of *B. napus* and *B. rapa* due to their suitability for biofuel and industrial purposes. Such varieties are grown now in Canada, the United Kingdom (UK) and Germany.

The trademark ’canola’ (’Canadian oil low acid’) was introduced for oil derived from *B. napus, B. rapa,* and *B. juncea* containing low levels of both erucic acid and glucosinolates. These varieties are also known as ’double low’, ’double zero’ or ’00’ rapeseed.[19,20] The oil has a low iodine value, which means it is more stable and less prone to oxidation.[20] Canola meal and oil are regulated to have a maximum of 30 µmol/g glucosinolates, and to a maximum of 2% erucic acid by weight in the United States of America (USA) and 5% in the European Union (EU), with special regulations for infant food.[21,22] In 1985 canola was granted the generally regarded as safe (GRAS) status by the U.S. Food and Drug Administration.

B. napus cultivars are available in winter and spring varieties.[14,23] Winter *B. napus* is sown in the autumn, hibernates as a small plant, and is harvested the following summer. Winter *B. napus* can be subdivided in two types, depending on the need for vernalisation during the winter months for flower production. Spring *B. napus* is planted in the early spring and harvested in late summer.[14] In the Netherlands mainly winter *B. napus* varieties are cultivated, since yields of spring *B. napus* are too low.[23,24]

B. napus is extensively grown in Europe, Canada, Asia and Australia, and to a more limited extent in the USA. In 2009, the EU was the world's largest producer of LEAR varieties, followed by China, Canada, India and Australia.[19]

4 GM *B. napus* events

GM *B. napus* is predominantly cultivated in Canada and the USA, but is also grown in Australia and Chile.[19] No GM *B. napus* is cultivated in the EU.[25] The currently worldwide GM varieties with a regulatory approval harbour GM traits resulting in herbicide tolerance, male sterility and changes in fatty acid composition.[26,27,28]

Transgenes conferring herbicide tolerance or resistance include the *bar* gene (encoding phosphinothricin acetyltransferase (PAT), conferring glufosinate-ammonium resistance), the *nitrilase* gene (conferring oxynil resistance), the *cp4 epsps* and *goxv247* genes (conferring glyphosate tolerance and resistance respectively), and the *gat4621* gene (encoding GAT4621 N-acetyltransferase, conferring glyphosate resistance).

Male sterile events have been developed by the integration of the *barnase* gene resulting in plants, which are unable to produce viable pollen. Cross-breeding of these male sterile lines with GM events in which the *barstar* gene is introduced, results in *B. napus* hybrids with restored fertility.

In GM *B. napus* events with an altered fatty acid composition, the introduced gene encodes a thioesterase enzyme that is active in the fatty acid biosynthetic pathway of the developing seed. As a result, triacylglycerides containing esterified lauric acid and, to a lesser extent, myristic acid accumulate at the expense of oleic acid and linoleic acid.

4.1 GM B. napus *authorisations in the EU*

In the EU five GM *B. napus* events (T45, Ms8, Rf3, Ms8xRf3, and GT73) are currently authorised for import and processing. No event has been authorised for cultivation.[25,26] T45 ('LibertyLink' or LL canola), MS8, RF3 and the stacked event Ms8xRf3 express the glufosinate ammonium resistance (*bar* gene). In addition, MS8 harbours the *barnase* ('male sterility') gene, Rf3 the *barstar* ('restorer fertility') gene, and Ms8xRf3 both genes. GT73 (RT73) expresses glyphosate tolerance and resistance genes (*cp4 epsps* and *goxv247* gene respectively).

Currently, three GM *B. napus* events have been submitted for regulatory approval (import and processing): MON88302 (glyphosate tolerance, *cp4 epsps* gene), 73496 (glyphosate resistance, *gat4621* gene) and Ms8xRf3xGT73 (glufosinate resistance, glyphosate tolerance and resistance, male sterility and fertility restorer; *bar*, *cp4 epsps*, *goxv247*, *barnase*, and *barstar* genes respectively).[29]

4.2 GM B. napus *authorisations outside the EU*

In addition to the aforementioned lines, authorised and commercialised lines outside the EU include HCN92 (also known as 'Topas 19/2 or LL canola; *bar* gene), Ms1 (*bar* and *barnase* gene), Rf1 and Rf2 (*bar* and *barstar* gene), Ms1xRf1, Ms1xRf2, PHY14, PHY35 and PHY36 (all *bar*, *barstar* and *barnase* genes), and GT200 (also known as MON89249, *cp4 epsps* gene).[26,28] Line HCN10 has been authorised but is not commercialised.[26] Additionally, three lines harbour events which have not been authorised under the current European legislations: OXY-235 (oxynil resistance, *nitrilase* gene), 23-18-17 and 23-198.[26,27,28] The latter two lines contain an altered fatty acid composition and have not been commercialised.[26]

The authorisations differ per country. The countries involved are: Canada, USA, Chile, Mexico, Australia, New Zealand, China, Japan, South Korea and South Africa.[27,28]

4.3 Field studies with GM B. napus

Worldwide multiple lines have been authorised for experimental releases of GM *B. napus* into the environment.[30,31] Besides traits such as herbicide tolerance, male sterility and modifications in fatty acid composition, GM traits in field trials include resistance to pests (fungal diseases and insects), tolerance to abiotic stresses (cold, heat, drought, salt) and alterations to the plant's metabolism.[30,31]

5 Biological characteristics of *B. napus*

B. napus is a member of the *Cruciferae* or *Brassicaceae* family which also includes *B. rapa* (synonym *B. campestris*)[32], *B. juncea*, *Brassica oleracea* (cabbage), *Brassica nigra* (black mustard), and *Brassica carinata* (Ethiopian mustard).[33] *B. napus* is a natural hybrid and originates from the interspecific hybridisation of *B. oleracea* and *B. rapa.*[33]

5.1 Genetics

The cells of the allotetraploid *B. napus* include the sum of the chromosomes of the parental species *B. oleracea* and *B. rapa*. The genome of *B. oleraceae* is genetically reported as CC and the number of chromosomes in the diploid cells is $2n = 18$. The genome of *B. rapa* is genetically reported as AA, and the diploid cells ($2n$) contain 20 chromosomes. The genome of *B. napus* is genetically reported as AACC and the cells contain $2n=2(9+10)= 38$ chromosomes.[33]

5.2 Reproduction

B. napus only reproduces sexually, and is a self-compatible species.[4] Self- and cross-pollination normally occur.[14,34] The effective cross-pollination rate is influenced by several factors, such as cultivar, pollen viability, distance, and insect activity. Cross-pollination rates of between 12% and 55 % have been reported.[35,36] In fields, the average rate of cross-pollination is 30%.

The pollen from *B. napus* can be transferred from plant to plant through physical contact between neighbouring plants, and by wind and insects.[15,36] The pollen is large (32 to 35 µm), heavy and sticky. Pollen deposition decreases rapidly with increasing distance from the source.[34,36] Long-distance pollination events are presumably mediated by insects such as honeybees and bumblebees, which are attracted to the flowers of *B. napus*.[4,15,36] The pollen viability varies with environmental conditions, particularly temperature and humidity. Under natural conditions, pollen viability gradually decreases over 3-5 days.[36,37]

5.3 Seed characteristics, dormancy, seed bank

The seeds of *B. napus* develop in a seedpod. The seeds are small, light and produced in large quantities.[15] Seed shed of matured seeds in the field can be up to 70%, particularly in hot and windy conditions. The seeds are dispersed by wind, water, animals and humans.[4]

Generally, seeds germinate immediately if the conditions for germination are met. *B. napus* seeds display little or no primary dormancy.[38] However, seed contact with the soil is critical for germination.[14] If conditions are not favourable, germination can be delayed and secondary dormancy may be induced by a combination of several factors such as darkness, moisture level, oxygen level, osmotic stress and temperature.[4,38,39] *B. napus* varieties differ in their germination rate and in their ability to develop secondary dormancy, suggesting a genetic background for these traits.[39,40]

Several publications on the persistence of *B. napus* seeds in the seed bank have been published. One of these publications describes a mean loss of 60% of the seeds in the first few months. The subsequent decline in seed number was observed to be much slower, with a mean decline of 20% per year.[41] Another publication reports that only 1.5% of *B. napus* seeds survived the first year, and 0.2% survived the second year.[42] In general, under normal agricultural conditions *B. napus* seeds can persist for over four years. However, *B. napus* seedlings have also been observed after a dormancy period of ten years.[41,43] Immediate burial of seeds appears to be important for seed bank development, and delayed tillage and no tillage greatly decreases (or minimises) the number of seeds in the seed bank.[41,44,45,46] Counter-intuitively, it has been reported that the soil seed bank was smaller in a delayed tillage treatment than in a no tillage treatment. In a no tillage system, the straw cover possibly provided conditions suitable for inducing dormancy.[44]

From these data, it can be concluded that the seed-bank of *B. napus* has quite a rapid turnover. However, a small portion of *B. napus* seeds may remain viable for several years under certain conditions, depending on factors such as soil moisture content and the carbon and nitrogen percentage of the soil.[4,47]

5.4 B. napus populations outside the agricultural area

B. napus is generally regarded as a ruderal species and one of the first to colonise disturbed habitats such as field margins, road verges, paths, ditches, railways, building sites, ports, waste ground and seed handling, storage and processing facilities.[4,34] However, in undisturbed natural habitats it generally lacks the ability to form stable populations due to the absence of competition-free germination sites. Furthermore, seedlings are effected by fungal diseases, pests (birds, molluscs, insects) and abiotic stress. Normally, local feral populations become extinct over a period of years.[4,34]

In general, the persistence or recurrence of a spontaneous *B. napus* population is variously attributed to seed shed by resident feral adult plants, recruitment from the soil seed bank, or replenishment with fresh seed spills from agricultural fields and transport.[4,34] Several studies conclude from existence of genetically highly diverse populations that feral *B. napus* populations may persist via self-recruitment. However, feral *B. napus* populations are generally found along transport routes and field margins, suggesting that seed-loss during transportation is a major cause in the rise of feral populations.

Studies on feral populations of *B. napus* worldwide provide insight into its persistence and invasiveness potential. In the next section, these studies are briefly discussed per country or geographical area, except for the Netherlands. The distribution of Dutch *B. napus* populations is discussed in Chapter 6.

COGEM points out that *B. napus* can easily be confused with its closest relative *B. rapa* because these two species closely resemble each other. *B. napus* and *B. rapa* plants only can be distinguished by careful examination of their external features such as their inflorescence and the morphological characteristics of leaves, stem and seeds.[3,5] Counting the chromosome numbers in the cells provides an unambiguous identification but is laborious and costly.

5.4.1 Presence of feral conventional and GM B. napus *populations worldwide*

In Germany and Austria *B. napus* populations have been observed in road verges and along railways, and to a lesser extent on construction sites, fallow lands, riverbanks and field margins. The populations were genotypically highly diverse and partly corresponded to commercial varieties. Population sizes varied from a few individuals to more than 1,000.[48,49] In the German study the identification of *B. napus* was based on external morphological characteristics and chromosome counting.[48] For the Austrian study, populations were genotyped and compared with a set of *B. napus* and *B. rapa* cultivars with a known genotype.[49]

In France *B. napus* populations have been observed along main roads within a radius of 5 to 8 km from a grain silo.[50] Population sizes ranged from one to more than 100 individuals. Another study in the same farmland area indicated that about half of the feral populations derived from seeds that had fallen the previous year at harvest or during sowing. The other feral populations resulted from seeds that remained in the soil for more than one year or, in smaller proportions, from seeds produced within the feral population the previous year.[51] The plants were identified either at distance (from a slow-moving van)[50] or from the biochemical characteristics of collected seeds (erucic acid and glucosinolate content, enzyme profiling),[51] but these methods do not unambiguously prove that these plants were *B. napus*.

In a study of land adjoining railway lines in Switzerland, the presence of *B. napus* was reported in 73% of the investigated locations.[52] Population sizes varied from one plant to around 250 plants. The import and cultivation of GM *B. napus* is not allowed in Switzerland, except for imports with a GM *B. napus* content up to a threshold of 0.5%. However, in populations located at railway stations that are important entry points of imported commodities, in total 50 GM plants were found. Three populations consisted almost entirely of plants expressing the *cp4 epsps* glyphosate tolerance gene. Herbicides including glyphosate are regularly applied to railways to keep them free of weeds. Two populations survived spraying with glyphosate. The authors suggest that these populations had multiple reproduction cycles. No data are available on the method used to identify of the plants but since GM *B. rapa* is not imported in the EU, the GM herbicide tolerant plants were almost certainly *B. napus*.[53]

In Japan *B. napus* used to be grown extensively, but current demand is met almost entirely by imports from Canada. While three systems of herbicide-tolerance (glyphosate, glufosinate and bromoxynil) have been approved for cultivation, import and distribution as food, GM *B. napus* is not commercially cultivated.[54] During a three-year survey (2005-2007) in a district where *B. napus* is not cultivated, *B. napus* plants were found on the sides of a road leading from a port to an inland facility for processing *B. napus* seeds.[55] Population sizes varied from one to several hundreds of individuals. The proportion of GM herbicide tolerant *B. napus* plants amounted 1.6%, 0.2 %, and 1.8% per subsequently surveyed year (35, 8, and 5 plants, respectively). Another three-year survey (2006-2008) describes the frequent occurrence of GM glyphosate and glufosinate tolerant *B. napus* in the area of eight Japanese ports. Plants were observed on roadsides and riverbanks along routes used to transport *B. napus* seeds. The riverbanks were located under bridges. The proportion of GM herbicide tolerant *B.*

napus plants amounted 12%, 18% and 58% per subsequently surveyed year.[56] Both Japanese studies do not provide data on the application of herbicides for weed control along roadsides, which could have selected for the herbicide tolerant plants. The identification of *B. napus* was based on morphological characteristics and chromosome counting.

In 2012 a survey conducted along a 10 km stretch of a highway in a GM free farming zone in Western Australia reported a widespread population of over 60% GM glyphosate tolerant *B. napus* individuals.[57] This highway is a major transport route for GM *B. napus*. In 2011 a truck spilled a large amount of GM *B. napus*, which was cleaned up. The authors suggest that glyphosate tolerant *B. napus* was probably actively selected for because glyphosate is used to control weeds on this highway.[57] No data are available on the method used for identifying the *B. napus* plants, but GM *B. rapa* is not imported into Australia.[53]

In North Dakota, the dominant canola growing region of the USA, a recent survey reported that 80% of the roadside *B. napus* populations included GM herbicide tolerant *B. napus*.[58] In California GM glyphosate tolerant plants were found along county roads leading to a farm growing GM glyphosate and glufosinate tolerant *B. napus*.[59] For both studies, no data are available on the method used for identifying the *B. napus* plants, but GM *B. rapa* is not imported or commercially grown in the USA.[53]

Canada is the largest exporter of *B. napus*. GM herbicide tolerant varieties are grown in 87% of the cultivation area.[19] In Western-Canada, GM herbicide tolerant *B. napus* was found at frequencies of 93% to 100% along roadsides and field margins in a *B. napus* producing region.[60] No data are available on the method used for identifying the *B. napus* plants. Cultivation of herbicide tolerant GM *B. rapa* is authorised in Canada, but not for commercial purposes.[26,53] As the study was conducted in a region where *B. napus* is grown, it is most likely that feral populations consist of *B. napus*.

In summary, in several countries and geographic regions feral *B. napus* populations are closely associated with spillage of *B. napus* seeds along transport routes or previous cultivation spots. Population sizes generally vary from a few individuals to several hundreds of plants. GM herbicide tolerant *B. napus* is able to establish feral populations. GM herbicide tolerant *B. napus* plants have been found in locations where herbicides were applied for weed control, suggesting an active selection for herbicide tolerant plants and indicating the inadvertent presence of GM seeds in conventional batches.

5.5 Hybridisation with closely related species

Controlled pollination experiments (laboratory, greenhouse and field conditions) have shown that *B. napus* can fertilise species from several allied genera. These include *B. rapa*, *B. oleracea*, *B. carinata*, *B. juncea*, perennial wall rocket (*Diplotaxis tenuifolia*), shortpod mustard (*Hirschfeldia incana*), wild radish (*Raphanus raphanistrum*) and charlock (*Sinapis arvensis*).[4,2,15,61,62,63]

Hybridisation under natural conditions is influenced by many factors, including climatic conditions, selective advantage, insect movements, the direction of the cross (i.e. which species is the pollen donor and which is the pollen recipient) and chromosome numbers.[4,15]

Most interspecific hybrids with *B. napus* have a severely reduced fertility (very low pollen viability and seed production).[4,62,63,64] Exceptions are hybrids obtained from crosses between *B. napus* and *B. rapa*, and to a lesser extent *B. napus* x *B. juncea* and *B. napus x B. oleracea* hybrids.[4,63,64] *B. napus* x *B. rapa* hybrids are observed in nature where *B. napus* and *B. rapa* populations co-occur, and have been reported in Canada[63,65], the USA[4], Japan[54,56], New Zealand[4], the Czech Republic[4], Denmark[66], the UK[67,68,69] and the Netherlands[5,6]. Naturally occurring *B. napus* x *B. juncea* have incidentally been reported in Canada and Japan, and *B. napus* x *B. oleracea* hybrids have incidentally been reported in the UK.[4]

5.6 Gene flow

Crop (trans)genes can be transmitted by pollen to other populations of the same crop and wild relatives. If volunteers arise, their pollen can fertilise neighbouring conspecific plants (intraspecific gene flow) or sexually compatible close relatives (interspecific gene flow). The seeds of the receiving plant will harbour the transferred gene. If the resulting hybrid seeds are viable and germinate, the progeny will harbour the gene as well, although the offspring may be sterile or less viable than the parent plants. If successive generations are obtained by back-crossing, the transferred gene is permanently incorporated within the gene pool.[4]

5.6.1 Intraspecific (trans)gene flow, stacking of herbicide tolerance traits in B. napus

Stacking of GM traits in *B. napus* has been described in several countries. In Japan non-GM *B. napus* maternal plants along transport routes of *B. napus* seeds were found to have produced GM herbicide tolerant seeds. The plants grew in mixed non-GM and GM *B. napus* populations on roadsides and riverbanks.[56] The same study reported maternal plants harbouring one herbicide tolerant trait (either glyphosate or glufosinate tolerance) while producing seeds with both traits. In a previous study, the researchers observed *B. napus* plants at the same location that were both glyphosate and glufosinate tolerant.[70] Stacked *B. napus* lines harbouring both herbicide tolerance traits are not imported into Japan.[54,26] Different seed companies import either glyphosate or glufosinate tolerant events. The authors suggest that pollen-mediated intraspecific transgene flow and stacking of herbicide tolerance traits within the escaped *B. napus* populations was most likely.

In the USA both glyphosate and glufosinate tolerant GM *B. napus* varieties are cultivated. A systematic roadside survey reported populations of GM glyphosate and glufosinate tolerant *B. napus* along road verges.[58] The populations were large and widespread. The methods used for weed control were mowing or herbicide treatment. In 0.7% of the surveyed sites, plants harboured stacked herbicide tolerant trait combinations. The observation indicates that stacking of herbicide tolerance traits occurred spontaneously in the field because the traits originate from different seed companies.

In Western-Canada, unintentional gene stacking of herbicide tolerance traits in *B. napus* volunteers resulting from intraspecific pollen flow is common in cultivation areas.[71] Over time, *B. napus* volunteers in escaped populations with multiple herbicide tolerant tolerance traits have been reported in adjacent fields. The distances between agricultural fields and escaped populations amounted 550 m.

5.6.2 Interspecific (trans)gene flow from B. napus *to* B. rapa

Since *B. napus* and *B. rapa* have different chromosome numbers, their offspring have intermediate chromosome numbers. If *B. napus* and its close relative *B. rapa* hybridise, the first generation of hybrids (F1, genome AAC) possesses $2n = 29$ chromosomes. Ten pairs of chromosomes belong to the A set ($2n = 20$), derived from the *B. napus* and *B. rapa* genome, and nine unpaired chromosomes belong to the C set (derived from the *B. napus* genome). F1 hybrids can produce pollen and seed, although pollen viability and percentage seed set are typically lower than that of the parental species.[4] If an F1 AAC hybrid crosses with another F1 AAC hybrid, or back-crosses to *B. napus* (AACC) or *B. rapa* (AA), the A-genome chromosomes in the offspring are paired whereas a variable number of C-chromosomes is passed on.[72] First generation *B. rapa* backcrosses (BC1) will have an A-chromosome number of $2n = 20$ and a C-chromosome number varying from 0 to 9.[73] The A-chromosomes originate partly from the parental *B. napus* plant. During backcrossing, crossover and recombination can occur between the A or C-chromosomes of *B. napus*, and the A-chromosomes of *B. rapa*.[74,75] Repeated backcrossing with *B. rapa* results in offspring with decreasing C-chromosome numbers and leads to a plant with a *B. rapa* genotype. However, the AA-genome originates partly from the *B. napus* parent plant.[76,77]

Stable introgression through the formation of BC generations depends on F1 hybrid fitness, i.e. growth vigour, fertility, and ability to set viable seed. First generation *B. napus* x *B. rapa* hybrids are often genetically unstable and have reduced fertility, features that can be overcome in subsequent back-cross generations.[4] Hybridisation frequencies are highly variable as they are influenced by multiple factors. Factors that are assumed to be essential for the initiation of the introgression process include the occurrence of mixed populations, the proportion of the species involved, and the efficiency of the weed (and herbivore) control.[66] (Trans)gene flow of *B. napus* into *B. rapa* under naturally occurring conditions has been observed in the field and under experimental conditions for several geographical regions.

In the UK the presence of F1 *B. napus* x *B. rapa* hybrids (2n= 29) have been reported in natural riverside *B. rapa* populations adjacent to *B. napus* fields. The observed frequencies varied from 0.3% to 1.6%.[67] The study did not provide data on the presence or absence of second generation hybrids or backcrosses. Another British study estimates by calculation that across the UK 49,000 *B. napus* x *B. rapa* hybrids are formed annually in waterside *B. rapa* populations.[69]

In Denmark weedy *B. rapa* populations within *B. napus* fields displaying 3% and 60% hybridisation rates have been documented.[78] Two other studies of a single field in Denmark reported F1 hybrids (2n= 29) in a mixed weedy population of *B. napus* and *B. rapa*.[66,75] Backcrosses of F1 hybrids and *B. rapa* were also found. The offspring contained intermediate numbers of C-chromosomes in mitotic cells, variously 1, 3, 5 and 6 C-chromosomes. The plants grew as weeds in an organically grown field with pea, clover and barley. As the change from conventional to organic cultivation took place some years before, the *B. napus* plants were probably remnants from the time when the field was grown conventionally. Some *B. rapa*-like plants (genome AA) were found to contain C-genome fragments and chloroplast DNA of *B. napus*.[75]

In a study of hybridisation between *B. napus* and *B. rapa* in the Netherlands, F1 hybrids (2n=29) were found in *B. rapa* populations close to crops or feral populations of *B. napus*.[6] The proportion of the observed F1 hybrids (three populations) varied from 11% to 23%. The researchers did not find any wild *B. rapa* plants with other numbers of C-chromosomes. They argue that if F1 hybrids are formed, the progeny arising from crosses between these hybrids and *B. rapa* are probably unfit. This would limit the establishment of hybrids between *B. napus* and wild *B. rapa* to the first generation.

A Chinese field experiment with GM herbicide tolerant *B. napus* varieties and several *B. rapa* cultivars showed that some of the interspecific embryos developed into viable seeds. About 10–70% of the interspecific hybrid embryos were aborted in the course of development.[79] In contrast, in Canadian field experiments GM hybrids of the first generation (2n= 29) between a herbicide tolerant *B. napus* variety and *B. rapa* were observed at frequencies of 7%.[63] In the same study, rates of spontaneous hybridisation between *B. napus* and two different populations of *B. rapa* varied markedly under field conditions, at 0.023% (a commercial maize field with herbicide tolerant *B. napus* volunteers and weedy *B. rapa*) and 13.6% (a feral *B. rapa* population located in the margin of a commercial herbicide tolerant *B. napus* field).[63] All F1 hybrids had reduced pollen viability (55%). According to the authors, the observed differences in hybridisation rates are probably explained by differences in the degree of isolation of *B. rapa* plants from con-specific plants and *B. napus* plants.[63] Over the years, both herbicide tolerant *B. napus* x *B. rapa* F1 (2n= 29) and backcross hybrid generations were observed.[65] Hybrids were found over a 6-year period, in the absence of herbicide selection pressure. Stable incorporation of the herbicide tolerance transgene was observed in one *B. rapa* plant (genome AA). In green-house experiments, offspring were produced harbouring the herbicide tolerant trait and displaying high pollen viability (over 90%).

In Japan, mixed populations of *B. juncea*, *B. rapa*, and *B. napus* have become established throughout the country, particularly along rivers and dry riverbeds.[54] On a riverbank located under the bridge of an existing *B. napus* transport route, seeds were obtained from *B. napus* within a mixed weedy population of *B. rapa*, *B. napus* and GM *B. napus*.[56] Seedlings showed an F1 genotype (2n= 29) with a glyphosate resistance phenotype suggesting the occurrence of hybridisation between herbicide tolerant *B. napus* and feral *B. rapa*. The study does not provide data on the application of herbicides for weed control along roadsides, which could have selected for the herbicide tolerant plants.

In summarising the aforementioned data (sections *5.6.1* and *5.6.2*) it can be concluded that transgene flow from GM *B. napus* to wild relatives and stacking of transgenes in *B. napus* may occur under natural conditions. *B. napus* x *B. rapa* hybrids and back-crosses have been observed in the field. The stable incorporation of a herbicide tolerance transgene originating from GM *B. napus* into *B. rapa* has been reported under natural conditions. The frequency of (trans)gene flow varies and depends on several conditions.

6 *B. napus* in the Netherlands

In the Netherlands, *B. napus* has been grown as a crop since the early Middle Ages, especially in the north and east of the country.[5,15] *B. napus* seeds are also imported. Almost all imported *B. napus* seed is used for oil production.[7] A small part of the imported seed is used in pet food, particularly for birds and rodents. The estimated seed loss during transport ranges from 0.1% to 3.0%.[7]

Although *B. napus* has established itself, the assumption that *B. napus* is widespread in the Netherlands[80] was refuted by the results of a research project recently commissioned by COGEM.[5] The researchers found that in the Netherlands *B. napus* is often confused with *B. rapa*. The distribution of *B. napus* populations was investigated using a newly developed morphological identification key, validated by counting chromosome numbers.[5] The results of this study show that outside agricultural fields *B. napus* populations are present across the Netherlands, but in general only a small number of plants (25 or less) are found per location. They are mainly found on highly disturbed soil close to locations where the crop is cultivated or where seeds are spilled during transshipment and along transport routes. Populations of *B. rapa* are much larger and attain high densities along railways, ditches and road-side verges.

The results in general correspond with several international studies previously mentioned in section *5.4.1,* which conclude that feral *B. napus* populations are closely associated with spillage of *B. napus* seeds along transport routes or previous cultivation sites. However, population sizes reported in these studies are somewhat larger and generally vary from a few individuals to several hundreds of plants. It is not clear why population sizes of feral *B. napus* populations in the Netherlands differ from population sizes observed in other geographical regions.

A subset of the feral *B. napus* populations reported in the afore-mentioned Dutch study, have been investigated in the framework of the Dutch ERGO programme.[81] These *B. napus* populations contained higher aliphatic glucosinolate levels than modern canola varieties. Feral populations are expected to have low glucosinolate levels because 'double low' canola has been widely grown at a large scale in the Netherlands since the early 1980s. The results of the ERGO project suggest that several of the Dutch feral *B. napus* populations originate from more ancient *B. napus* cultivars. It has been shown that a higher glucosinolate content in the leaves of *B. napus* reduces the extent of grazing by generalist pests such as molluscs and birds.[16,82] As a consequence, modern canola varieties might be more sensitive to slug damage and therefore, might have a lower fitness under natural conditions.[82]

It is not known how much of the *B. napus* imported into the Netherlands is genetically modified.[7] In an experiment to find out whether GM seeds occur in seed mixtures commercially available in the Netherlands, 841 *B. napus* seedlings were treated with glyphosate.[5] None of them was tolerant to the herbicide, suggesting that few GM *B. napus* plants have been introduced into the Dutch environment. In a pilot study conducted by the Dutch customs in 2012, seeds imported from Argentina, Australia, Chile and Russia were monitored for the presence of GM varieties. Tested seeds showed a non-quantifiably low content of GM *B. napus* line GT73 originating from Australia.[83]

The following wild relatives of *B. napus* are present in the Netherlands: *B. rapa*, *B. nigra*, *B. juncea*, and *B. oleracea*.[3] Other closely related species that occur are *D. tenuifolia*, *E. gallicum*, *H. incana*, *R. raphanistrum* and *S. arvensis*.[3]

As mentioned before only *B. rapa*, and to a lesser extent *B. juncea* and *B. oleracea*, are able to hybridise with *B. napus*. *B. juncea* and *B. oleracea* are very rare in the Netherlands.[3] *B. brassica* x *B. rapa* hybrids have incidentally been reported in the Netherlands, as described in section *5.6.2*. Therefore, in the Dutch environment interspecific cross-hybridisation is most likely to occur with *B. rapa*. Future research might explain why only hybrids of the first generation have been found in the Netherlands.

In conclusion, Dutch feral *B. napus* populations are small and not as widespread as has previously been assumed. Plants are observed on highly disturbed soil close to locations where the crop is cultivated or where seeds are spilled during transshipment and along transport routes. In undisturbed areas, plants are unable to survive for more than a few generations. *B. napus* x *B. rapa* hybrids of the first generation have been observed incidentally.

7 Points to consider for the environmental risk assessment and post-market environmental monitoring of GM *B. napus*

7.1 Environmental risk assessment

In the EU, authoristion of a GM crop is subject to the outcome of an ERA. The purpose of the ERA is to assess whether the introduction of the GM plant into the environment would have adverse effects on human and animal health and on the environment. Relevant points to consider are, amongst other things, the capability of the GM crop to form feral populations, and its weediness and outcrossing potential. Several biological characteristics are taken into account such as vegetative and reproductive growth, seed germination, dormancy, seed bank formation, persistence, fitness (susceptibility to insects, diseases and abiotic stresses) and hybridisation with closely related species.

7.1.1 (GM) B. napus *forms feral populations but its weediness potential is low*

The persistence or recurrence of a *B. napus* population is variously attributed to seed shed by resident feral adult plants, recruitment from the soil seed bank, or replenishment with fresh seed spills from agricultural fields and transport. As discussed in section *5.3*, *B. napus* seeds generally display little dormancy. However, the occurrence of seedlings after a dormancy period of 10 years has been reported. The seed bank of *B. napus* has quite a rapid turnover but a small portion of *B. napus* seeds may remain viable for over four years. *B. napus* is able to form volunteers in disturbed environments near roadsides, railways and handling areas. Spillage of seeds during harvest, transport and transshipment has led to the establishment of feral *B. napus* populations (reported in both the Netherlands and other countries). Population sizes vary from a few individuals to several hundreds of plants. If herbicide tolerant GM *B. napus* is imported or cultivated, herbicide tolerant populations can occur along transport routes in proportions from less than 1% to 100%. Herbicide use actively selects for herbicide tolerant plants.

In conclusion, there is little evidence that *B. napus* is invasive, although feral *B. napus* populations can persist for several years. Feral populations occur along distribution routes and transshipment areas.

7.1.2 Transgene flow from (GM) B. napus *to wild relatives occurs under natural conditions*

B. napus can cross-pollinate with wild relatives. If *B. napus* populations become established, closely related species can be fertilised, in particular *B. rapa.* Both intraspecific and interspecific flows of (trans)genes have been reported under naturally occurring conditions. Unintentional stacking of herbicide tolerance traits in *B. napus* has been observed for *B. napus* cultivation areas, which are sprayed with herbicides (Canada, USA). Incidental stacking of herbicide tolerance traits in *B. napus* plants located on a riverbank along a transport route has been reported in Japan. *B. napus* x *B. rapa* hybrids of the first generation have been observed at locations where *B. napus* and *B. rapa* populations co-occur. Hybridisation rates were variable (0.3 % to 60%) which can be explained, among other things, by differences in the degree of isolation between *B. rapa* plants, conspecific plants and *B. napus* plants. Back-cross progeny and the stable incorporation of an herbicide tolerance transgene into a *B. rapa* plant have been observed in Canada.

In conclusion, stacking of transgenes into feral *B. napus* and introgression of transgenes into feral *B. rapa* may occur under natural conditions.

7.1.3 Herbicide use actively selects for GM herbicide tolerant B. *napus*

Currently, the GM traits in *B. napus* events authorised for import and processing in the EU include tolerance and resistance to herbicides containing glyphosate, tolerance to herbicides containing glufosinate ammonium and both male sterility and the restoration of male sterility.

Herbicide tolerance can confer a selective advantage when the herbicide is applied. In the Netherlands, the policy of the road maintenance authority is to use non-chemical methods to control weeds on road verges. However, along railways glyphosate and to a lesser extent glufosinate applications are the most commonly used methods of weed control.[84]

COGEM points out that spilled herbicide tolerant GM *B. napus* seeds will have a selective advantage over other plants if herbicides are used for weed control. On the long term GM herbicide tolerant *B. napus* populations may occur. Subsequently, there is a chance that cross-fertilisation may lead to the stacking of several transgenes in single *B. napus* plants. As mentioned above, a few studies have described stacking of herbicide tolerance traits under naturally occurring conditions. Additionally, if *B. rapa* populations exist in close proximity of feral *B. napus* plants, *B. napus* x *B. rapa* F1 hybrids may be produced by hybridisation. Occasionally, F1-hybrids have been observed in the Netherlands.[6] *B. rapa* is more common than *B. napus* and is found in similar areas, such as disturbed habitats in road verges and waste grounds.[5] In the long term, gene flow of GM traits could lead to the incorporation of one or more transgenes into the gene pool of *B. rapa* populations.

In summary, spilled herbicide tolerant GM *B. napus* seeds will have a selective advantage over other plants if herbicides are used for weed control. In the long term, the prolonged use of herbicides may lead to the establishment of feral herbicide tolerant *B. napus* harbouring GM traits, including plants with stacked events, or fersl herbicide tolerant *B. rapa* harbouring GM traits.

7.1.4 *The currently authorised GM* B. napus *events in the EU do not pose an environmental risk*

Under natural conditions, the currently authorised GM *B. napus* events are unlikely to lead to an increased fitness or a selective advantage. Therefore, these traits will not lead to an environmental adverse effect.

7.2 *Post-market environmental monitoring*

Holders of GM crop authorisations are required to monitor for the occurrence of adverse effects from the import and/or cultivation of the GM crop.[85] PMEM consists of two parts: ‘case-specific monitoring’ and ‘general surveillance’. Case-specific monitoring is designed to confirm that any hypotheses regarding the occurrence and impact of potentially adverse effects of the GM crop or its use in the ERA are correct. Case-specific monitoring is therefore only necessary when the ERA identifies a potentially adverse effect to the environment. As stated in the EFSA guidance on the PMEM of GM plants (*Legislative Background*), general surveillance is instituted 'in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market'.[85] General surveillance is *the* instrument 'to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the ERA'.[85] If an adverse effect is identified, remedial measures can be taken. To summarise, general surveillance is used to identify any unexpected, (in)direct, (possibly adverse) effects of GM populations on the environment.

7.2.1 *Case-specific monitoring of the currently authorised GM* B. napus *events in the EU is not necessary*

Taking into consideration the afore-mentioned considerations regarding ERA (section *7.1.4*) and general surveillance (section *7.2*), in COGEM’s view case-specific monitoring of the currently commercially available GM *B. napus* events is not necessary because the corresponding ERAs have not identified potentially adverse effects on the environment.

7.2.2 *General surveillance of GM* B. napus *must include handling areas, transport routes, and in specific cases feral* B. rapa *populations*

The preparation and implementation of a general surveillance plan is compulsory for all applications of GM crops. In 2010, COGEM remarked that general surveillance for crops that have outcrossing potential, such as *B. napus*, should generally cover handling areas and distribution routes.[86]

In the Netherlands *B. napus* populations with GM plants can arise at roadsides, railways and handling areas where spillage of GM *B. napus* seeds occurs during transshipment and transport. As pointed out before, spilled herbicide tolerant GM *B. napus* seeds will have a selective advantage over other plants if herbicides are used for weed control (section *7.1.3*). In the long term, prolonged use of the herbicide may lead to the establishment of feral herbicide tolerant GM *B. napus*, including plants with stacked events, or feral herbicide tolerant *B. rapa* harbouring GM traits. It cannot be excluded that a possible combination of GM traits and/or a possible interaction between gene products, expressed by the genetic modification, may result in a potentially adverse effect. In the view of COGEM, general surveillance is *the* instrument to identify such (in)direct, unanticipated, delayed, potentially adverse environmental effects. Therefore, in the general surveillance plan monitoring of *B. napus* transport routes (including roadsides and railway beddings) and transshipment areas should be included.[*] If GM *B. napus* is observed, *B. rapa* populations in the vicinity of the observed GM *B. napus* population will have to be included in the general surveillance plan as well. Since glyphosate application is the most common method for weed control along railways in the Netherlands, railway companies and/or companies in charge of the maintenance of railways should be involved by the authorisation holder in monitoring for the occurrence of GM *B. napus* and *B. rapa* with GM traits along railways.

7.2.3 *Additional remark*

Interest is reviving in the older *B. napus* varieties suitable for industrial purposes. Some of these varieties contain higher amounts of glucosinolates and erucic acid in comparison with the currently commercially available modern canola (LEAR) varieties. Although it is generally considered that the high palatability of modern *B. napus* varieties to generalist pests, such as molluscs and birds, is due to its low glucosinolate content, its low erucic acid content may play an additional role. COGEM notes that a shift in the use of *B. napus* cultivars to more HEAR varieties could affect the establishment of the plant and consequently could cause a change in its feral behaviour.

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[*] COGEM points out that new import applications of stacked GM *B. napus* events, which have been obtained by conventional crossbreeding and whose parental lines already have been approved in the EU, also have to be assessed for a possible interaction between transgenes and their products because such an interaction could lead to a potentially adverse environmental effect. As stated in an opinion, COGEM is of the opinion that it is of utmost importance to involve qualified existing monitoring networks in order to detect all possible unanticipated effects.[86]

8 Conclusions

COGEM concludes that:

- Spillage of *B. napus* seeds can lead to feral *B. napus* populations. Feral populations occur along transport routes, such as railways, and along previous cultivation sites. If a population arises from spilled herbicide tolerant GM *B. napus* seeds, the use of corresponding herbicides provides a selective advantage.
- *B. napus* can hybridise with wild conspecifics and transgenes may be transferred. Eventually, several transgenes can come together in one plant (stacking). Stacking of transgenes in *B. napus* has been reported in the literature.
- Interspecific hybridisation between *B. napus* and *B. rapa* occurs under natural conditions and has been reported in the Netherlands and elsewhere. From the literature, it is known that backcrosses between these hybrids and *B. rapa* occur in the field. Introgression of *B. napus* transgenes into *B. rapa* has been reported under natural conditions.
- Based on the environmental risk assessments, the GM *B. napus* events currently authorised in the EU do not pose a risk in themselves to the environment in the Netherlands.

Based on these conclusions, COGEM is of the opinion that the following points should be considered regarding the ERA and post-market environmental monitoring of GM *B. napus* import authorisations:

- Case-specific monitoring on the currently commercially available GM events is not necessary as no environmental risks have been defined for these events.
- Cross-fertilisation of *B. napus* volunteers harbouring GM traits from separate events could lead to stacking of transgenes in one plant. A possible combination of these GM traits or a possibly unanticipated interaction between products of these transgenes could result in an (in)direct, unexpected, delayed, potentially adverse, environmental effect. Therefore, monitoring of *B. napus* volunteers harbouring GM traits along transport routes or transshipment areas is of the utmost importance to enable the identification of such effects.
- The likelihood of the occurrence of (stacked) GM *B. napus* plants is the greatest in handling areas and transport routes such as railways. General surveillance should focus on these areas.
- When GM *B. napus* plants occur, even in short-lived populations transgene flow to *B. rapa* is possible. If GM *B. napus* is identified, any *B. rapa* populations in the vicinity should be monitored for the presence of transgenes and therefore, should be included in the general surveillance plan as well.

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