COGEM REPORT

Coexistence in agriculture
Mixing, outcrossing and isolation distances

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Netherlands Commission on Genetic Modification (COGEM)
COGEM's task is to advise the government at their request or independently about the risks of using genetically modified organisms (GMOs) and to inform the government about ethical and societal issues associated with genetic modification. COGEM's task is described in the Environmental Management Act.

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Summary

Coexistence in agriculture refers to a situation where different forms of agriculture exist side by side – for example, conventional farming or organic agriculture and agriculture using genetically modified (GM) crops, without one excluding the other.

As a result of mixing and outcrossing, the production of GM crops could result in the contamination of conventional or organic crops and the products derived from these. Such a mixing of products may result in economic losses for the growers involved as well as the processing industry, as it limits their potential markets. Furthermore, if the production of conventional, organic or GM crops becomes impossible, the customers' freedom of choice could be limited.

Although coexistence is not an environmental safety problem and therefore strictly speaking not part of COGEM's remit, COGEM believes that in view of its expertise and the controversies surrounding this subject, it is worthwhile issuing a further report about the technical aspects of production, as announced in a previously published report on coexistence.

COGEM points out that when GM crops are produced side by side to conventional and organic (non-GM) crops, a certain amount of mixing between non-GM products and GMOs in the food chain is inevitable. In order to prevent reciprocal claims of liability, agreements must be reached about the measures and separation criteria to be used. However, it is important that these agreements relate to everyday practice and are made with the consent of the parties involved. This seems the only way to guarantee the coexistence of different forms of agriculture and, accordingly, the consumer's freedom of choice.

By providing insights into the effects of farm management measures on mixing, COGEM hopes to contribute to a consensus being reached. In this report, COGEM has taken the frameworks set by the EU as a starting point. However, it should be borne in mind that the threshold value of 0.9% set by the EU for adventitious mixing with GMOs in the final product and the proposed values of 0.3% and 0.5% for seed are actual trading standards, and the extent to which these satisfy consumers and growers of non-GM products is not clear.

COGEM distinguishes four stages in the production phase that are important for mixing, namely sowing, growing, harvesting and the control of volunteers. Each stage may lead to unintended mixing and contribute to the final percentage of adventitious presence of GM components in the end product. The measures needed to limit this mixing will have to be examined for each stage per crop. The stages and the level of mixing in these stages cannot be viewed in separation, as they influence each other (see figure 1).

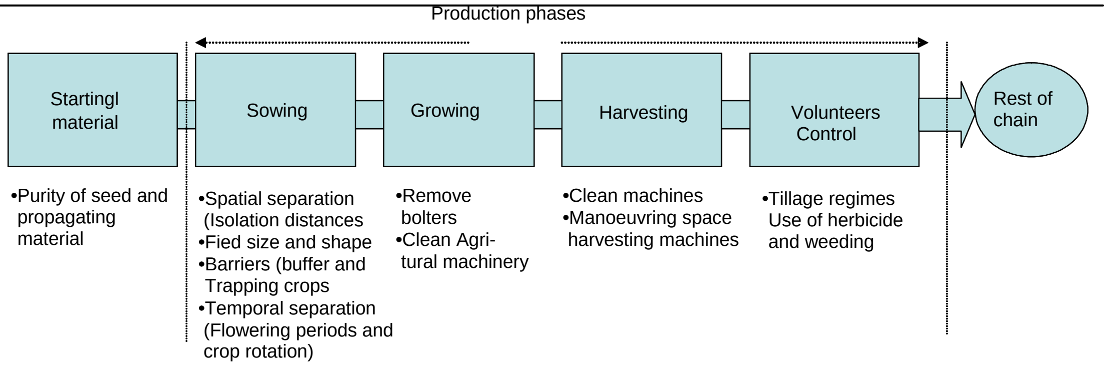

Figure 1 *Stages where mixing can occur and possible factors to prevent mixing.*

In this report, COGEM considers the production of oilseed rape, maize, beet and potato. However, the line adopted by COGEM can be extrapolated beyond these four crops. The effect of each stage can be determined for any crop, in a comparable manner, by considering each of the aforementioned stages in turn. After this the adequacy of the current farm management measures can be assessed and where necessary tightened. However, if there is a large degree of uncertainty for one of the stages with respect to mixing then taking far-reaching measures for one of the other elements would not appear to be worthwhile.

Oilseed rape

Based on the current data, COGEM observes that it is not yet possible to accurately determine isolation distances for the production of oilseed rape. As volunteers from seed banks plays an important role in the production of oilseed rape, the use of a good tillage regime is highly recommended. With respect to this COGEM notes that data on the persistence of seed banks under Dutch growing conditions are virtually non-existent and that further research in this area is urgently needed. COGEM also calls for further research into the possibility of planting buffer crops to prevent the spread of pollen.

Maize

Experiments have revealed that the majority of inbreeding occurs in the border rows of the acceptor field and that the presence of buffer crops can lead to considerably lower mixing percentages in the acceptor field. Inbreeding percentages far below the threshold values set by the EU can be achieved by growing buffer crops at isolation distances of 25 to 80 metres. The isolation distance chosen will have to be a compromise between practical feasibility, in which a production system may not be excluded, on the one hand and the social acceptability of contamination in final products on the other.

Potato

In COGEM's view it is not necessary to apply isolation distances for the production of potatoes to prevent effects of inbreeding. However, applying a manoeuvring space of several metres (about 2 to 3 metres) for the harvesting machine can prevent GM potatoes from an adjacent plot being harvested as well, or prevent GM potatoes that are being harvested from ending up on an adjacent non-GM plot.

If the regulations with respect to the control of potato blight and potato root eelworm are fully complied with, the effect of possible volunteers on mixing is negligible. Further, COGEM considers the probability and frequency of infection from propagating material to be minimal.

Sugar beet and fodder beet

In COGEM's view it is not necessary to set isolation distances for the production of genetically modified fodder beet and sugar beet under Dutch conditions in order to reduce mixing. However, applying a manoeuvring space of about 2 to 3 metres, as adviced in the case for potatoes, may prevent unwanted mixing. COGEM recommends that every grower of GM beets be legally required to remove bolters to prevent adventitious seeding.

COGEM points out that the discussion about measures for coexistence should not be restricted to spatial separation in the form of isolation distances. Another option is using asynchronous cropping plans to achieve temporal separation. On balance, this is probably a more effective means of preventing outcrossing than the setting of isolation distances. Agreements about the crops to be cultivated as well as the size and position of the plots or fields in which these are to be grown, can also be an effective means of reducing mixing. Tillage regimes, control of volunteers and seed banks and other farm management measures are also important elements for preventing mixing. However, all of these approaches require clear agreements and close cooperation between local growers.

1. Introduction

Coexistence in agriculture refers to the possibility of harmoniously cultivating genetically modified crops (GM) alongside conventional and organic crops. A central premise in this concept is that all possible forms of agriculture must be able to exist side by side without one excluding the other.

As a result of mixing and outcrossing, the production of GM crops could lead to the contamination of conventional or organic crops and the products derived from these. This admixing may result in economic losses for the growers concerned as well as the processing industry, as it limits their potential markets. Furthermore, the consumers' freedom of choice could be limited when the production of either conventional, organic or GM crops becomes impossible.

In November 2003 COGEM compiled a report about coexistence in agriculture[1]. In this report, COGEM noted that coexistence involves the production of products that have been approved following a complete risk analysis and therefore is not an environmental safety problem. Coexistence is an economic and ethical-societal problem which involves the consumers' freedom of choice and the right to existence of organic, conventional and GM agriculture. COGEM also noted that the policy with respect to coexistence should not just be limited to the production phase but must be extended to the entire chain, including the possibility to separate chains.

COGEM issues advice about the risks of genetic modification for humans and the environment. Although coexistence is not an environmental safety problem and therefore strictly speaking is not part of COGEM's remit, COGEM believes that in view of its expertise and the controversies surrounding this subject, it is worthwhile issuing a further report about the technical aspects of crop production.

If both conventional and GM crops are grown on a commercial basis, a complete separation of GM and conventional products or a zero tolerance for mixing will be virtually impossible. The EU has decided to set a statutory threshold value of 0.9% for traces of GMOs in the final product. This means that if traces of GMOS are in a proportion no higher then 0.9% the final product may be labelled as GMO-free, "(..) provided that these traces are adventitious or technically unavoidable "[2]. Adventitious mixings are those which occur due to human errors or unforeseeable situations. Reaching agreements or imposing measures will not be enough to completely prevent this chance of mixing.

This is different for the requirement "(..) technically unavoidable". Once the decision has been taken to permit coexistence, an 0.0% GM trace value in the final product becomes unfeasible, i.e. mixing cannot be technically avoided. Even if GM crops are not grown in the Netherlands, mixing elsewhere in the chain will frequently turn out to be unavoidable. However, measures can be imposed to minimise the level of mixing. For example, by implementing separate processing systems or setting isolation distances.

The EU directive on labelling is open to different interpretations. Although the term ‘adventitious’ seems to be unequivocal, the term ‘technically unavoidable’ is open to different interpretations. Whereas one party will interpret this as taking the most stringent measures, such as implementing isolation distances of several hundred metres, the other party will point out that when fields directly border each other it will be technically impossible to prevent mixing due to inbreeding. However, the EU legislation only regulates the labelling, etc. and not who must take measures to prevent mixing. Should this be the person who wants to keep his products GM-free or the person who wants to grow or process GM crops? In practice this could lead to growers issuing claims against each other if the products become contaminated and therefore not sellable or only sellable against a lower price. In order to prevent reciprocal claims of liability etc., agreements must be reached about the measures to be implemented. It is important that these agreements relate to everyday practice and are made with the consent of the parties involved. It is obvious that the GM grower will call for measures that will allow possible contaminations of the final product just under the EU threshold (0.9%) whereas organic farmers will assume contaminations under the detection limit of (0.1%). A compromise will have to be found in which all forms of production can exist and the producers’ and consumers’ freedom of choice continues to be guaranteed.

By providing insights into the effects of farm management measures on mixing, COGEM hopes to contribute to a consensus being reached. For this COGEM has taken the frameworks set by the EU as a starting point. One of the premises of these statutory measures is that no single form of agriculture can be excluded. Further the EU has created boundary conditions, including the setting of threshold values for admixing with GMOs. However, it should be borne in mind that the threshold value of 0.9% set by the EU for adventitious mixing with GMOs in the final product and the proposed values of 0.3% and 0.5% for seed are actual trading standards, and the extent to which these satisfy consumers and growers of non-GM products is not clear.

COGEM also points out that mixing can take place throughout the entire chain. The contribution of each step of the chain to the final level of mixing will need to be examined along with the measures necessary to reduce this. Meaning that permissible or unavoidable mixing in each stage or step of the chain needs to be subtracted from the final permitted mixing (0.9% or lower) in order to see what ‘space is left for mixing' for the other steps. Accordingly the intended measures (and the discussions concerning these) will need to focus on those steps where the greatest amount of mixing will occur.

In view of its expertise and field of activity, COGEM does not have any insight into the occurrence of possible mixing in the processing phase after the harvest. Therefore in this report it has limited itself to indicating the chances of mixing during the production phase including the use of harvesting machinery. COGEM distinguishes four stages in the production phase that are important for mixing, namely sowing, growing, harvesting and volunteer control. Five elements can be distinguished in this: the initial materials (seed and propagating material), field sizes and the location of fields (spatial planning), inbreeding (by pollen), volunteers from seed banks or tubers, etc, and finally harvesting machinery. There is a high degree of correlation between these factors and they sometimes require the same measures. The use of GM-contaminated initial seed has immediate consequences for the percentage of mixing in the final product. Seed may become contaminated due to inbreeding during seed production. The geographical location of both GM and non-GM fields will determine the degree of mixing in the final product. In determining this, consideration should be given to the distances between fields, the size of the fields and environmental factors such as overgrowth etc. The third element that can lead to mixing is the behaviour of pollen. To what extent does pollen flow take place and does this affect mixing? This is directly correlated with the positioning of the fields, etc. The fourth aspect concerns the presence of volunteers from seed banks or viable remains left in the field. The following question plays a role with respect to this: does seed remain on the field and to what extent will this result in volunteers and therefore mixing in the final product? Contaminated seed banks can be a direct consequence of previous inbreeding due to pollen dispersal. Volunteers control can be necessary after, prior to or during the production. Harvesting machinery can transport seed from GM to non-GM fields but also facilitate mixing of the different parties if material remains in the machinery after harvesting. Chapter 2 will consider this matter in general terms in greater depth and in chapter 3 the contribution of each element to the mixing during the production of specific crops will be considered.

This report only considers the coexistence of commercially grown GM, conventional or organic crops and the measures needed to realise this. For field trials with GM crops, farm management measures and isolation distances are set to minimise outcrossing in order to prevent possible environmental risks. In this respect, field trials and commercial production are not comparable situations, and therefore the measures such as isolation distances stated in this monitoring report cannot be applied to field trials.

This report considers outcrossing between cultivated crops and measures to limit this. Outcrossing to a wild relative is not considered in this monitoring report. The production of GMOs or their introduction to the environment is subject to a licence and is only permitted following an extensive risk analysis. During the authorisation procedure for production (commercial production or field trials), the risks for outcrossing to wild relatives and the possible consequences of this are exhaustively assessed within the framework of EU Directive 2001/18[7]. The precautionary principle is the basic premise of this regulation. This issue is only considered in this report in cases where wild relatives may act as a possible reservoir for mixing with non-GM crops as a result of outcrossing with GM crops.

It may be possible to draw some lessons from issues related to the production of so-called 'pharma crops' for the production of ‘normal’ GM crops. Pharma crops are GM crops in which pharmaceuticals are produced. The discussion concerning pharma crops differs from the discussion on the production of 'normal' GM crops because for pharma crops the main issue is safety. Commercial production will only be possible in the case of strict separation of chains and certification. Pharma crops have an extremely high added value as a result of which strict containment measures will be economically feasible. However the experience with chain separation and certification as well as the expertise which will be acquired with biological containment systems for growing pharma crops, can also be of direct benefit for realising the coexistence of the different production systems discussed in this report.

The monitoring report has been written on the basis of scientific literature and recently published reports on this subject[3,4,5,6].

2. Mixing

As previously stated, the mixing of agricultural products with GMOs can take place in many different ways throughout the entire production chain. Contaminated initial materials such as propagating material or seeds may lead to mixing just like the transport of seeds by the wind, animals or machinery, volunteers from seed banks, tubers left in the field, rootstocks and similar or storage off the field. During the production, mixing may occur due to outcrossing with nearby GM crops or with wild or feral relatives. Yet mixing can also occur during the harvesting, storage and processing phases, for example, due to the use of machinery or lorries that have not been thoroughly cleaned. The extent to which mixing may and will occur is mainly determined and influenced by the nature of the crop, but also by the size and nature of the production system, the farm management and harvesting methods used and the varieties grown.

Although many factors play an important role in the occurrence of mixing, the public discussion in the Netherlands and Europe often seems to focus on outcrossing via pollen and the setting of isolation distances. However there are many other possible measures that are just as effective or perhaps even more effective than the setting of isolation distances. Mixing can also be minimised by means of crop rotation, specific farm management measures such as soil tillage to prevent volunteers, carefully cleaning machinery and implementing different planting times to separate the flowering periods of crops.

In 2002 a report was published by the EU Joint Research Centre[4] in which a number of scenarios were described for the coexistence of GM and conventional crops. In this report, the effects of farm management measures, including isolation distances, is calculated on the basis of the final percentage of mixing in the harvested product. This was done by examining the production of oilseed rape (for seed production), maize (for animal feed) and potato (for consumption purposes) in both organic and conventional production systems.

Although these scenarios are mostly based on assumptions and predictions of computer models (GENESY[8]; MAPOD[4]) they still provide some insight into the effect of farm management measures. For example, soil tillage to control the emergence of volunteers was found to be a highly important means of preventing mixing in oilseed rape, but hardly has any effect in maize. For maize they observed that in addition to outcrossing, contaminated initial seed forms an important source of mixing. Measures in the processing phase could provide a possible solution to this problem. Interestingly, the scenarios developed indicated that it was only necessary to set isolation distances in a few cases. However it should be noted that not all of the measures proposed by the writers are feasible or applicable for the Dutch situation. Due to the Dutch climate it will be infeasible to realise a difference in flowering times within the growing season to prevent outcrossing between GM and non-GM crops. As full use is made of the growing season in the Dutch situation, it will not be possible to shift the planting period and with this the flowering period of maize. The crop is sown early spring, after the frost period and harvested late in the autumn. Shifting this period would have a negative effect on the yield. Separation of the flowering period is also not an alternative for oilseed rape due to the growing conditions. Furthermore, the onset of flowering is strongly influenced by weather conditions. That can lead to shifts in the flowering period, making the method unreliable. However by modifying the cropping plans used, the rotations could be harmonised so as to minimise the growing of similar crops in neighbouring fields.

Further the computer simulations revealed that small organic farms which produce their own oilseed rape seed soon experience problems. Firstly, due to the small fields the incidence of inbreeding is relatively higher than for farms with larger fields (see §2.1). Secondly, the transfer of possible contaminations is enhanced because the farm-saved seed can already be contaminated and on organic farms volunteers are less efficiently controlled. With this it should be noted that to the best of our knowledge oilseed rape is not grown organically in the Netherlands and no seed production takes place.

Although the aforementioned findings are mainly based on models, and experimental data to validate these is lacking, these findings nonetheless indicate that setting isolation distances is not always enough to prevent mixing. A good coexistence policy for growing crops (including the harvest) should consider the presence of pollen and seeds as well as volunteers. To a large extent these will determine the measures needed to prevent mixing.

In practice, DNA analysis will be used to determine the percentage of mixing in the final product. Meaning that in the final product, the number of copies of the transgene compared to the number of copies of the non-transgenic genome will be determined., The proportion of paternal and maternal DNA in seed is often not equal. For example, the endosperm in the maize kernel contains three copies of the genome, two of which originate from the maternal plant and one from the paternal plant. The embryo contains one maternal and one paternal copy of the genome. If a non-GM maternal plant is fertilised by GM pollen, only that part originating from the paternal plant will contribute to the percentage of mixing in the final product. In the case of a processed product such as maize meal, bearing in mind that the majority of the kernel consists of endosperm, this percentage is about 33%. Therefore the percentage of incrossing is often not the same as the percentage of mixing in the final product.

2.1 Pollen

Outcrossing and the formation of hybrids between related species is a natural phenomenon. Hybridisation takes place between wild species, between agricultural crops, and between agricultural crops and wild relatives. Research into hybridisation between agricultural crops and wild relatives has revealed that 22 of the 25 most important crops hybridise with wild relatives[9]. Little is known about the frequency with which this phenomenon occurs. This also applies to the hybridisation frequency between agricultural crops. However, research in England on hybridisation between *Brassica napus* (oilseed rape) and *B. rapa* (weedy oilseed rape) has revealed that hybrids are formed each year[10].

With the arrival of GM crops, there is an increasing need to know the frequency with which hybridisation and/or outcrossing occurs between agricultural crops. The correct and consistent use of the term outcrossing is also important. Frequently outcrossing is used when in fact incrossing is meant. In this report the term outcrossing is used when pollen flow originates from the 'donor field' (source). However, the term incrossing is used if the 'receiving' or 'acceptor' field is taken as a starting point.

Important factors for outcrossing

The degree of outcrossing (and incrossing) due to pollen flow is determined, for example, by the type of crop, the distance over which the pollen can travel, the viability of the pollen over time and the size of the pollen cloud in relation to competing pollen clouds. Factors such as size, shape and position of the fields also play an important role. The type of crop must also be considered. The degree of cross-fertilisation varies widely between crops, from self-pollination to complete cross-pollination.

- *Pollen flow*

The distance that pollen can travel is primarily determined by the mode of dispersal, wind or insects. Some plants even make use of both wind and insects. A large number of insect species serve as vectors for pollen transport. There are considerable differences between these insects. Some species, such as bumblebees, only travel relatively short distances whilst other insects travel over large distances.

In the case of wind dispersal, both the size and weight of the pollen grains are important. The local geography and weather conditions will also have a considerable effect on the distances travelled by the pollen. In very hilly areas, wind-mediated pollen flow will be less effective than in flat areas. The presence of bushes, trees, etc. can strongly hinder dispersal and large bodies of water can also act as a barrier. Strong winds transports pollen over considerable distances. On the other hand rain can capture pollen, resulting in deposition. Also wet surfaces are 'stickier' than dry surfaces and therefore capture more pollen.

Pollen flow is usually represented by a leptokurtic curve. The quantity of pollen deposited initially decreases exponentially over distance and then tails off. Accordingly most of the pollen produced is found within a short distance of the plant from which it originates[11,12].

As plants produce vast amounts of pollen, considerable quantities of pollen grains can be transported over longer distances. Various publications report pollen being transported over distances of several hundred kilometres. For example, pollen grains have been found above the Atlantic Ocean and the North Sea[13]. Therefore pollen can clearly be transported to higher aerial strata where it is carried by the prevailing winds[14].

- *Pollen viability*

The distance over which pollen is transported is just one of the factors that determines the degree of incrossing. Another important factor is the viability of the pollen. Pollen is sensitive for dehydration, high temperatures and damage caused by ultraviolet light. The viability of the pollen decreases over time and this results in a reduced competitive strength, or in the loss of the ability to form a pollen tube. The viability or longevity of pollen differs per crop and is determined, for example, by the thickness of the pollen grain wall, the degree to which the pollen grain is dehydrated or the presence of sucrose in the cytoplasm. This depends on the plants dispersal strategy. For some plant species the pollen grains are dehydrated and the metabolism is inactive, whereas other sorts of pollen are dispersed in a metabolically active and partially dehydrated state[15,16]. After the anther has split open the pollen grains are either immediately dispersed or remain in the anther until favourable dispersal conditions arise.

- *Pollen cloud*

A third important factor for both outcrossing and incrossing is the size of the ‘pollen discharge’ or ‘pollen cloud’. The degree of outcrossing is determined by the size and position of the ‘donor’ field (source) with respect to the receiving field (acceptor) and the size of the pollen cloud compared to other fields. These elements determine the competition between the pollen produced in the field and the pollen which enters the field. A field that produces a large pollen cloud will facilitate outcrossing. But if this same field is the acceptor field, the large pollen cloud will hinder incrossing. Incrossing will predominantly occur in smaller field, or field producing relative small pollen clouds since the influx of pollen will be high and the competition between pollen low. This means that experiments with small trial plots (as donor) will systematically underestimate the percentage of outcrossing. On the other hand many experiments in the past were carried out using sterile buffer plants. In these experiments small trial plots were surrounded by sterile plants which did not produce any pollen. The amount of incrossing was subsequently measured by determining the degree of fertilisation. However, this led to an overestimation of the occurrence of outcrossing because the competing pollen cloud from the receptor plants was missing.

The shape of the donor field and the acceptor field will also influence the degree to which outcrossing and incrossing occur. Generally speaking the majority of pollen transfer will take place in the field margins. Relatively speaking, a circle has the smallest amount of field margin and will therefore have the lowest chance of pollen transfer. In more practical terms, long, narrow fields will have more margin than square fields with the same surface area and therefore more pollen transfer.

As well as size and shape, the relative position of the fields is important. More pollen transfer will be observed between long, narrow fields lying next to each other than between the same two fields located behind each other. However in such cases it should also be noted that differences in the prevailing wind direction and so forth have a considerable effect.

Measures

There are various ways of limiting incrossing due to pollen flow. For example, spatial separation (e.g. isolation distances) and temporal separation methods can be used. Although the setting of isolation distances has received most attention, methods such as establishing barriers, making use of different flowering periods, or an asynchronous cropping plan may be effective in preventing the effects of pollen flow.

- *Difference in flowering periods*

The Dutch climate does not permit the introduction of measures to induce differences in flowering time for crops, like oilseed rape and maize, without severe yield losses. The Dutch growing season is too short for such measures. However it is possible to implement an asynchronous crop rotation. Agreements can be made not to grow crops at the same time. This will require a harmonisation of the crop rotation plans of growers of GM crops and growers of non-GM crops.

- *Barriers*

Wind-mediated pollen flow can be reduced by placing a barrier between the donor field and the acceptor field. This barrier can be a buffer zone sown with non-GM plants of the same crop or other crops, hedges, trees, etc.

Buffer crops or catch crops can be used to trap pollen and also to discourage insects from visiting the donor field. Research has revealed that the presence of a trapping crop that is not pollinated by insects, discourages insects from moving between the two fields[17].

- *Isolation distances*

In the Netherlands, methods to limit the consequences of pollen dispersal will mainly focus on isolation distances.

In recent years a number of reports about pollen flow have been published, written by both proponents and opponents of the production of GM crops. The authors differ widely concerning the isolation distances which should be implemented to limit outcrossing. For example, the distances proposed vary from 0 metres[4] to 500 metres[18] for the production of potato and from 10 metres[19] to 6000 metres[18] for the production of oilseed rape. These differences are partly due to the different threshold values used for mixing, which vary from zero to several percent.

The setting of isolation distances to limit incrossing is neither new nor specific to the production of GM crops, but has been used in seed production and in breeding since a long time. Statutory isolation distances are enforced for seed production to guarantee the purity of the seed consignments. These provide a good insight into the effectiveness of using isolation distances. The isolation distances seem to be predominantly based on experiential data. However, they are not directly applicable to the setting of isolation distances for growing crops. The threshold values for purity differ and furthermore, seed production has its own problems, such as the production of hybrid seed.

For the production so-called ‘waxy’ maize and ‘high erucic acid content’ (HEAR) oilseed rape, isolation distances are used to prevent mixing with crops intended for consumption. Erucic acid is a toxic substance which naturally occurs in oilseed rape, for example. The EU has set a threshold level of 2% for the presence of erucic acid in crops intended for consumption. Oilseed rape with a high erucic acid content (50%) is grown for industrial processing. However the isolation distances imposed provide little guidance because the erucic acid content and not the percentage of incrossing is the critical factor and heterozygotes produce smaller amounts of erucic acid. Furthermore, the threshold value is considerably higher than for mixing with GMOs. A similar situation applies to so-called ‘waxy’ maize and normal maize. Normal maize contains about 75% amylopectin and 25% amylose. ‘Waxy' maize contains almost 100% amylopectin and is used for industrial purposes in the starch industry. However the thresholds for mixing with ‘waxy’ maize are also far higher than those for the legally permitted mixing with GMOs.

Sometimes farmers also implement isolation distances for sweetcorn to prevent crossing with silage maize. No reliable data are available, but the distances used probably vary between 80[20] and 200 metres[19].

A number of other factors should also not be forgotten when determining isolation distances. Isolation distances partly depend on the geographical and climatological conditions and for some crops the presence of a buffer crop can reduce the isolation distance required.

COGEM believes that the total percentage of incrossing, and therefore mixing, over the entire field must be considered when setting isolation distances. An incrossing percentage of 1% at 100 metres does not mean that the isolation distance will have to be greater than 100 metres. After all the incrossing must be viewed over the entire field. If the incrossing percentage at 200 metres has fallen to 0.1 % then, as the percentage decreases exponentially, the total amount of incrossing and mixing on the field will be considerably lower than 1%.

2.2 Seed

For seeds, there are two possible situations with respect to mixing and the measures to be taken. The seed itself can contribute to mixing, or other plants which arise from spilt seed or seed left in the field can also result in mixing. The first situation can arise with the use of initial material that contains a given percentage of GM seed or if, as a result of harvesting machinery not being cleaned properly, GM seed becomes mixed with non-GM seed. In both cases the final product will contain a percentage of GM seed due to mixing.

Whether spilt seed or seed that remains in the field will contribute to the eventual GM percentage in the final product will depend on many factors. Is the seed capable of germinating? How long does it remain in the ground? And is it then still capable of germinating? The answers to these and other questions will differ per crop and even per variety. In addition to this factors such as soil tillage and weather conditions will have a considerable effect.

Seed

The European Commission is in the process of setting a threshold value which seed must satisfy to be labelled as GMO-free. In a 'draft' version of the directive from the EC for marketing seed (July 2004)[21] the following percentages are stated: 0.3% for seed originating from cross-pollinating crops and 0.5% for self-pollinators. For both oilseed rape and maize values of 0.3 % have been set. Although seed production for these crops does not take place in the Netherlands, the percentage of mixing in the initial seed does of course affect the permitted percentage of mixing in the other phases of production, harvesting and processing. In order to remain under the threshold value of 0.9% set for the final product, the total percentage of mixing in the other phases must be lower than 0.9%. How much lower will not only be determined by the percentage of GM contamination in the seed but also by the fitness effect of the modification and the proportion of the seed that contributes to the percentage of genetically modified DNA in the final product. Before a good coexistence policy can be implemented, the crops have to be identified for which the initial seed stock may be an important source of mixing.

Seed banks

Removing all of the seeds from the field after harvesting is unfeasible. A certain amount of seed will always remain on the field. This can be large numbers. If these seeds are capable of surviving in the ground over a longer period of time, in so-called seed banks, and can germinate again with the same crop in subsequent plantings, mixing can result. Seed banks can still form a source of contamination many years later. As large numbers of seeds can be left in the field it is plausible that these could substantially contribute to mixing in the final product[22,23].

The persistence of seed banks is determined by a considerable number of factors. Large differences in persistence have been found between crops, but also between cultivars or varieties of the same crop. In addition to this, post-harvesting procedures, weather conditions and the type of soil are important. Soil tillage increases the rate at which seed disappears from the seed bank. On fallow ground where there is no disruption to the soil, seeds can remain present for more than 10 years[23] whereas on cultivated fields this is just four years. Also rain soon after the harvest ensures that a large proportion of the remaining seed germinates instead of ending up in the seed bank. Finally seeds can survive for longer in heavy soils than light soils[23].

As well as being left on the field after harvesting, seed can also be transported to other fields and this can give rise to mixing in subsequent plantings. Insects, the wind and machinery can transport seeds. However, harvesting machinery is responsible for most of the adventitious transport of GM seed. For example, in the first 200 metres of a field sown with winter barley, oilseed rape seedlings were found which originated from a field two kilometres away where the same harvesting machines had been used[25].

When separate harvesting machinery is not used for the harvesting of GM and non-GM crops such as oilseed rape, transport or mixing via harvesting machinery is likely to occur. Cleaning the machines can reduce the number of seeds but the methods currently employed will not remove all of the seeds present. It is also likely that if the same machinery is used, GM seed left behind will mix with the non-GM seed thus contributing to the percentage of mixing in the final product.

Seed loss during the harvest can result in volunteers in the following crop or in field margins and verges. Of course the seeds of these plants will not be harvested with the following crop and become part of the final product, but if they flower at the same time, outcrossing of pollen to nearby fields could take place.

The extent to which seeds are capable of becoming feral in field margins and verges will depend upon the extent to which these can compete with weed seeds and survive under non-cultivated conditions. It is still questionable whether the presence of a relatively small amount of plants in verges and field margins can actually contribute to mixing (see §2.1 about field size and pollen clouds).

Measures

The field situations described are often applicable for crops grown for seed. The prevention of seed setting to prevent mixing is therefore not relevant. The measures to be taken need to focus on controlling seed banks by implementing a soil tillage regime, cleaning harvesting machinery, rotating crops, etc. However for crops not grown for seed, the prevention of seed setting can be an important measure for preventing mixing and outcrossing. For example, in the Dutch beet growing sector the removal of bolters (adventitious seed setting) can prevent seed spreading and ending up in seed banks. Beet is a biennial crop. During the first year the beet forms a tuber and in the second year flowering and seed setting take place. Stressful conditions, in particular lots of cold weather at the start of the growing season, can cause the plant to switch to flowering and seed setting during the first year. Failure to remove these bolters on time can result in 2000 to 4000 seeds being released per plant, some of which will end up in the seed bank. Research has revealed that the seed bank can contain thousands of seeds and that volunteers can emerge from the seed bank many years after the crop was grown, despite repeated tillage of the soil.

2.3. Viable components

On the field remaining viable material, like tubers and rootstocks, can give rise to volunteers which can result in subsequent plantings becoming contaminated. Just as for seeds, the quantities involved can be large. For example after harvesting, 1 to 2 tonnes of small potatoes per hectare can remain on the field. As this is initial material, the control of volunteers is important.

3. Crops

At present no GM crops are grown on a commercial basis in the Netherlands. However, a number of field experiments are being carried out. With the exception of starch potatoes, the large-scale production of GM crops in the Netherlands is not expected to take place in the near future[26]. There is considerable public resistance within the EU towards GM crops and food, and the market possibilities seem to be limited. Worldwide only a limited number of GM crops are grown on a large scale: soya, maize, cotton and oilseed rape. Soya and cotton cannot be grown under the Dutch climate. One of the most important characteristics incorporated into GM maize, resistance against the corn borer, is not currently relevant for Dutch agriculture.

The following sections provide more detailed information about a number of crops for which the issue of coexistence might play a role in the future.

3.1 Oilseed rape

Crop and production

Oilseed rape (*Brassica napus*) belongs to the family *Brassicaceae* (*Cruciferae*) and the genus *Brassica* which also includes cabbage (*B. oleracea*), turnip/weedy rape (*B. rapa*) and black mustard (*B. nigra*). Oilseed rape probably arose as a consequence of natural hybridisation between *B. oleracea* and *B. rapa*[27]. Oilseed rape and black mustard are part of the Dutch flora. It is not clear whether oilseed rape has become completely naturalised in the Netherlands or that these plants continually arise from spilt seed[27]. In the Netherlands rape is found as a cultured plant on and around fields and as a feral plant on waste ground.

A distinction is made between two different types *B. napus*: kohlrabi and fodder rape on the one hand and oilseed rape on the other. Oilseed rape is further subdivided into winter and summer oilseed rape. Winter oilseed rape gives a high yield but needs a mild winter to flower. Summer oilseed rape gives a low yield but is not dependent on a mild winter for its flowering. Due to the low yield, summer oilseed rape is scarcely grown in the Netherlands[28]. Oilseed rape is a relatively minor crop in the Netherlands with a total acreage of about 600 ha and a harvest of 2000 tonnes[29]. To the best of our knowledge no organic oilseed rape is grown in the Netherlands[30].

Most GM oilseed rape is grown in Canada and the United States. In Canada 73% of the oilseed rape grown is genetically modified. The main characteristics incorporated are herbicide tolerance and changes in the composition and levels of fatty acids. Although field trials have been carried out within Europe, the marketing of GM oilseed rape is still not permitted.

No seed production takes place in the Netherlands. Most seed is supplied by German seed companies. Seeds are produced by crossing male sterile plants and female plants to obtain F1 hybrids. This results in an increased risk of contamination with GM pollen. A complicating factor is that in some varieties the male fertility of the F1 hybrids is often not completely restored ('varietal association cultivars'). This means that in commercially grown varieties, male sterile plants that do not produce pollen often occur. This reduces the pollen cloud produced by the crop. In some crop systems no more than 20% of the plants are fertile. Due to the smaller pollen clouds there is an increased chance of incrossing with pollen from other oilseed rape fields.

Pollen flow

Oilseed rape is self-compatible, although outcrossing percentages of 5 to 55% have been reported[31]. The large differences observed might be due to the presence of male sterile plants in the field. On the other hand this variation might be due to differences in the experimental design and natural variation in geographical and climatological conditions.

Oilseed rape is mainly pollinated by insects (bees and bumblebees) but the wind and contact with neighbouring plants can also play a role. Oilseed rape pollen is relatively large (32-33 µm), heavy and sticky. Bees collect large quantities of pollen grains. Moreover, bees can take pollen again from the hive, thus transporting them even further[32]. Oilseed rape pollen can also be transported by the wind. However, data in the literature on the importance of wind pollination and the distances over which this can take place are contradictory[5]. This is probably because it is impossible to completely exclude insect pollination in the experimental design. However, it is clear that the wind-mediated flow of oilseed rape pollen decreases exponentially with distance to the field.

Pollen viability

Less is known about the viability of oilseed rape pollen. Under field conditions, there is a strong decrease in the viability of the pollen grains after 4 days[33]. Under *in vitro* conditions pollen grains can retain their viability for a period of 24 hours to 7 days[5]. Yet nothing is known about the viability of pollen grains on the bodies of bees or other insects or about the viability in the beehive. The microclimate which pollen grains are subjected to in the beehive or on the bee are probably very different from those on the plant. For *Cucumis melo* it has been reported that the viability of same-age pollen grains was lower on honeybees than on the anthers of the plant[34].

Outcrossing and isolation distances

A large number of studies have been carried out into the outcrossing of *B. napus* under field conditions. These have clearly demonstrated the importance of field size and field shape. A few of these studies are here described. A more extensive and detailed description of these studies can be found in several previously published reports[3,5,30].

A study by Scheffler *et al.*[35] investigated incrossing between two oilseed rape fields, each of 400 m^2. At a distance of 200 and 400 metres incrossing percentages of 0.02% and 0.004% respectively were observed. During experiments using small trial (receptor) fields (46 m^2) considerably higher percentages were found compared to large fields (65 ha). Even at a distance of 366 metres an incrossing percentage of 0.6% was still observed[36].

Comparable results were found for GM oilseed rape. In an experiment where the GM oilseed rape field (a circle with a diameter of 9 metres) was planted in the middle of the acceptor non-GM oilseed rape field, low incrossing percentages were found. At a distance of 12 metres from the GM field an incrossing percentage of 0.2% was found[37]. Yet in a Canadian experiment on incrossing using large (> 16 ha) bordering oilseed rape fields this same percentage was found at 250m[38].

In all of these experiments pollen flow seemed to satisfy the leptokurtic curve. Pollen flow and thus incrossing often takes place close to the source plant and then decreases exponentially before tailing off at greater distances from the source. Data about the flow of pollen between commercial oilseed rape fields in Australia, with sizes varying from 25 to 100 hectare, gave a different picture[39]. Instead of an exponential decrease in the quantity of pollen with increasing distance to the source, a more variable dispersal pattern was found. From this the authors concluded that laboratory experiments and small-scale field trials cannot automatically be translated to large-scale commercial production. Outcrossing is a complex process in which many factors play a role, including environmental factors, varieties, insect behaviour and plant density.

To determine the effectivity of so-called trapping crops field experiments with canola were conducted in Canada. Four GM fields of 30 x 60 metre were encircled with hedges of 15 to 30 metre of conventional canola. Although 80% of all incrossing took place within the first 10 metres of the field edge, a percentage of 0.02% was still found at 30 metres. These results show that although it can not be prevented field margins can significantly reduce incrossing.

Determining isolation distances

In a review for the English Department for Environment, Food and Rural Affairs by Ingram[19] a number of recommendations about isolation distances were made. Assuming field sizes of two hectares it was stated that isolation distances of 1.5 metres, 10 metres and 100 metres were sufficient to limit incrossing between conventional varieties and hybrids with complete fertility to 1%, 0.5% and 0.1% respectively. If the ‘acceptor’ field contains ‘varietal association’ hybrids an isolation distance of 100 metres is recommended to limit incrossing to 1%.

In a meta-study, Damgaard and Kjellson[41] analysed the published data on outcrossing between oilseed rape fields to calculate which isolation distances and other management measures had to be implemented to realise an incrossing percentage of less than 0.3%. They calculate that when the acceptor oilseed rape fields are very small (maximum width of 50 metres), an isolation distance of 200 metres must be implemented. For acceptor fields with a width of 100 metres this distance could be reduced to 50 metres. Where ‘varietal association cultivars’ (in which male sterility occurs) are cultivated in the acceptor field, larger isolation distances will probably have to be implemented. According to their calculations the most effective means of preventing outcrossing is increasing the size of the acceptor field to a width of 200 to 300 metres (=4-9 ha). This last recommendation is supported by the outcomes of the ‘GENESYS’ computer model. In this model increasing the width of the acceptor field was also proposed as an effective means of preventing incrossing[42].

Other factors for mixing on the field

Seeds are an important risk factor for the transport of GM oilseed rape. In particular, volunteers from seed banks can substantially contribute to mixing in the final product[22,23]. Oilseed rape seeds are small and are produced in large quantities. Under normal growing conditions the seeds can survive in the soil for at least 4 years. A longevity of more than 10 years has been observed if soil tillage does not take place. The longevity of the seed is also strongly dependent on the cultivar.

The seed bank can form a source of mixing in the field, even if no GM oilseed rape has been grown on the field concerned. This is because part of the seed bank can consist of GM seeds produced during a previous incrossing.

Seeds can easily be transported by the wind or by machinery. Large quantities of seed are lost during the harvesting of oilseed rape and remain on the field. A loss of 5000 to 10,000 seeds per m^2 is not unusual[23]. Also large quantities of seeds can remain in the machinery with the result that seed is adventitiously transported to other fields. In harvesting machinery quantities of up to 3.9 kilogram (= ca. 1 million seeds) have been encountered[25]. Seed loss off the field can also lead to mixing. Oilseed rape populations can become established in roadside verges and can give rise to mixing on the field due to outcrossing. However these percentages will often be marginal due to the relatively small pollen cloud.

The extent to which the seed bank or verge populations contribute to incrossing is strongly dependent on the farm management measures and cultural control measures implemented. The control of volunteers, soil tillage, and the nature of the crop rotation have a considerable influence on this.

Wild relatives

Outcrossing of oilseed rape with wild relatives is possible. One of the conditions for spontaneous outcrossing of *B. napus* with wild relatives is a partially overlapping flowering period. It is known that under field conditions, oilseed rape can outcross at low percentages with four wild relatives: *B. rapa* (weedy oilseed rape), *Brassica juncea* (brown mustard), *Hischfeldia incana* (grey mustard) and *Raphanus raphanistrum* (wild radish). The hybrids formed have a reduced fitness and a reduced sterility. For the crossing between *B. napus* and *B. rapa* it has been demonstrated that this hybrid can more or less permanently establish itself. This has never been demonstrated for other hybrids. Outcrossing with wild relatives can be a source of mixing. However in view of the low percentage at which it occurs, outcrossing with wild relatives does not seem to make a significant contribution to mixing in the field.

Discussion

Most researchers and regulatory authorities do not generally consider oilseed rape to be a crop with a particularly high risk of outcrossing[5,6,19].

From a meta-analysis of published data on outcrossing between oilseed rape fields, Damgaard and Kjellson[41] concluded that an isolation distance of 50 metres was sufficient to keep incrossing below 0.3%. For very small fields (less than 50 metres) a distance of 200 metres would have to be implemented to achieve this threshold value. However this meta-analysis only considers outcrossing. The possible contributions of the seed bank and contaminated seed to the final mixing in the field were not investigated.

Outcrossing is just one of the factors that can result in mixing or contamination of the crop. For oilseed rape, seed loss, volunteers and the adventitious transport of seeds are possibly more important factors than outcrossing. Coexistence is only possible if strict measures are taken to control volunteers and the adventitious transport of seeds. Preventing the adventitious transport of seeds requires far-reaching measures for the cleaning of machinery (such as harvesting machinery, tractors and lorries). Training and certification of personnel and contract workers will form a necessary part of this.

An article was recently published in which, with the help of the GENESYS computer model, it was calculated that an isolation distance of 200 metres must be implemented to realise a mixing of 0.9% under French growing conditions[42]. In this model the contribution of the seed bank as an extra source of contamination played a strong role. This model also took crop rotation and soil tillage into account. It should not be forgotten that this result was obtained from a computer model with all of its associated limitations. The calculations are based on assumptions and how realistic these are is not always clear. The extent to which the model has been validated with results from long-term experiments over several years is also unclear. Further the authors state that the model underestimates outcrossing for rotations with spring crops and outcrossing as a function of distance. They have used a correction factor to compensate for this. The outcomes of this model are specific for the data entered for the region concerned and cannot simply be extrapolated. It is not clear to what extent

Dutch growing conditions and measures are compatible with those in France.

No clear picture can be obtained from the internationally published literature concerning the actual contribution of the seed bank to mixing in the field. The little data available with respect to volunteers has often been published as grey literature in conference proceedings and the like without stating the exact experimental design. When the importance of the seed bank is determined, it is important to consider whether the characteristic inserted provides a selective advantage.

Concluding remarks

Based on the current data, COGEM observes that it is not yet possible to accurately determine isolation distances for the production of oilseed rape. In general a isolation distance of 50 metres seems to be sufficient to remain under the threshold value of 0.9% mixing[41]. With this it should be borne in mind that only the part of the genomic DNA originating from the paternal plant contributes to the percentage of mixing in the final product.

However the percentage of incrossing will be affected by the presence of male sterile plants (‘varietal association cultivars) in the donor or acceptor field as well as the presence and persistence of the seed bank. Almost no empirical data is available about the contribution of seed banks to mixing. Taking these factors in account, it can be reasonably concluded that isolation distances of at least 50 metres or more must be implemented.

In addition to isolation distances, farm management measures will be needed to prevent mixing. Implementing a good tillage regime will reduce the formation of seed banks and is therefore highly recommended. Not ploughing a field immediately after harvesting ensures that much of the remaining seed will germinate in the same season and not end up in the seed bank. With this it should be noted that there are almost no data concerning the persistence of seed banks under Dutch growing conditions and that further research on this is therefore needed as a matter of urgency. This applies just as much for the contribution of mixing from oilseed rape populations in verges and field margins. It is not clear how much oilseed rape seed is present in verges and field margins, what the origin of the seed is, and to what extent this feral oilseed rape seed affects the production of oilseed rape. More research data is needed to clarify this. COGEM notes that if GM oilseed rape is grown in a field, the production of conventional oilseed rape should strongly be advised against for the next couple of years due to the emergence of volunteers from seed banks. A GM-free cropping interval of more than 10 years should be considered.

COGEM also calls for further research into the possibility of creating a buffer zone of conventional oilseed rape around the GM oilseed rape field in order to limit incrossing and therefore mixing.

COGEM also points out that as more data become available, either about incrossing and mixing under Dutch growing conditions or from large-scale studies such as the European SIGMEA project[43], it might be possible to further specify the isolation distances to be implemented.

3.2 Maize

Crop and production

Maize (*Zea mays* L.) belongs to the family *Gramineae* (grass family) and as an agricultural crop it originated from Central America. Maize is grown throughout the world and is one of the most important food crops. The only known wild relative of maize is Mexican teosinte (*Z. mexicana*), which is indigenous in Central America.

In the Netherlands most of the maize grown is silage maize. This is grown as animal feed for use on the farm[28]. During the harvest the aboveground parts are chopped, and then the material is ensilaged to be used as animal feed at a later stage. Production and processing is often done by contract workers[28]. Sweetcorn and grain maize are grown in the Netherlands on a small-scale. The cob is harvested for human consumption, industrial processing or animal feed. About 205,000 ha of silage maize is grown in the Netherlands. In recent years the acreage of sweetcorn grown in the Netherlands has increased. In 2001, 27,000 ha were grown with a harvest of 239.000 tonnes[29].

No seed production takes place in the Netherlands. Seed is mostly obtained from France, Hungary and South American countries. F1 hybrids are mostly grown in the Netherlands. These hybrids are produced by crossing male sterile plants from one variety with fertile plants from the other variety. Due to the use of sterile plants there is an increased chance of adventitious incrossing with undesirable (such as genetically modified) varieties. In view of the area over which GM maize is grown, contamination of seed could be possible, particularly in consignments from South America.

A number of articles have been published about pollen flow and outcrossing in maize. These have included studies on pollen transfer, pollen viability and the percentage of outcrossing between fields.

Pollen flow

Maize has a single-sex inflorescence. The male inflorescence is present on the top of the plants whereas the female inflorescences (largely hidden) are in the axils of the middlemost petioles. Maize is a cross-pollinator but self-pollination can occur in low percentages (5%). All of the agriculturally grown varieties seem to be able to cross with each other.

Maize is mainly a wind pollinator. Insect pollination scarcely plays a role in maize, yet it cannot be completely excluded. Although bees seem to collect the pollen of maize they do not seem to visit the female flowers. Maize pollen grains are relatively large (90-125 µm) and heavy (0,25 µg) and therefore the distances over which the grains are transported are small[44,45]. Experiments have demonstrated that 90% of the pollen produced falls within five metres of the field edge and 98% between 25 to 50 metres of the edge[5,46]. The prevailing wind direction often has a strong effect on the dispersal pattern and the size and shape of the fields also appears to have a considerable influence.

Pollen viability

There are considerable differences between the published data on the longevity or viability of maize pollen. According to some publications this is 30 minutes whereas others state a longevity of nine days[47]. The viability of the pollen seems to be determined by the relative humidity and temperature. In general it can be stated that pollen remains viable the longest at a high relative humidity and low temperatures[48,49,50].

Outcrossing and isolation distances

A number of researchers have investigated outcrossing under field conditions. In the experimental designs often two different maize varieties are used which have seeds that are easy to distinguish on the basis of morphological characteristics. There are often considerable differences in the various published data. As much of the older data has been published in the grey literature (such as bulletins and conference proceedings) the experimental design is often not clear. One explanation for the large differences is that in several cases, the wind direction does not appear to have been considered[51]. In maize, outcrossing mostly just takes place on the leeward side [53,54]. In many other experiments very small trial plots were chosen or even just several plants. Therefore these results cannot be used to make predictions for commercial growing conditions.

In a 3-year experiment, Jones and Brooks[55] examined outcrossing between relatively large maize fields, in which the receptor fields were situated at various distances (0-500 m) from the source field. The space between the fields was filled up with a low-growing grain species which was harvested before the maize plants set pollen. In such a field situation, filling up the intervening space with a low-growing crop or absence of a crop, is called 'open space'.

Jones and Brooks found incrossing percentages of 28.6% for a field at 0 metres from the donor field, 1.2% for a field 200 metres away and 0.20% for a field 500 metres away. Their results also revealed that outcrossing mainly occurs at the edge of the field. In the case of receptor fields situated at various distances (0 to 500 m) from the donor field, the percentage of incrossing was determined for the first 25 rows. About 50% of the total number of incrossings in these rows took place in rows 1 to 5 (= ca. the first 5 metres of the field). It should be noted that the distance between the rows was greater than under normal growing conditions.

The presence of trees or plants between the fields can also reduce the occurrence of outcrossing. Jones and Brooks[56] reported a 50% decrease in outcrossing due to the presence of a row of trees with undergrowth. However this row of trees, comparable to a isolation distance of 75 metres without a barrier, was not as effective as planting the margin with maize plants the size of the row of trees.

Within the framework of ‘farm scale evaluations’ (FSE) a large-scale study has been carried out into the outcrossing of silage maize[53]. A total of 55 fields were tested for outcrossing over a period of three years. In this study, one half of a field was always sown with GM maize and the other half with conventional maize. The fields were located throughout England in areas where both a lot and a little maize was grown. Mixing by outcrossing was determined by analysing the DNA in the maize cob.

The research revealed that incrossing strongly decreased over the first 20 metres, after which the decrease tailed off to about 125 metres. The authors concluded that, in general, a isolation distance of 24.4 metres was sufficient to remain within the threshold value of 0.9% for mixing. Further they stated that in the majority of cases a isolation distance of 80 metres (as proposed by SCIMAC) was sufficient to keep the percentage of mixing below 0.3%. With this it should be noted that the result and data from the FSE study as described in the DEFRA report do not always seem to completely concur with the conclusions drawn by the authors. COGEM has therefore mainly based its opinion on the data presented.

Other factors for mixing on the field

In Europe maize has no wild relatives and in the Netherlands the circumstances for maize are unfavourable for it to become feral. In Europe volunteers from maize plants rarely have an agricultural significance and in the Netherlands they do not occur at all.

For maize a distinction must be made between silage maze, sweetcorn or grain maize. In the case of silage maize, all of the aboveground parts are harvested. Incrossing is only relevant for the cob, and this only constitutes part of the total product. Cob development and size is strongly dependent on the weather conditions during the growing season, the time of harvesting and the cultivar. According to Ingram[19] at most 50% (by weight) of the harvest of silage maize in England consists of maize cobs. It is likely that this figure will be about the same in the Netherlands.

In the case of sweetcorn and grain maize, the cob is harvested. Incrossing has a direct effect on the harvested product and will lead to a higher mixing for the final product than is the case for silage maize. This does not mean that incrossing leads to an equivalent amount of mixing in the final product for sweetcorn or grain maize. If incrossing occurs in a processed product of maize kernels such as maize meal, this will only contain about 1/3 'GM DNA'. The endosperm in the maize grain contains two copies of the genome from the maternal plant and one copy of the genome from the paternal plant (the incrossed part).

Discussion

The results from Jones and Brooks (1950) reveal that the degree to which incrossing strongly occurs, tails off at an isolation distance of 400 metres. Increasing this distance will not contribute to a lower level of incrossing. Yet data from the FSE study shows that the tailing off occurs at about 125 metres. The differences in distances can be accounted for by differences in the experimental design. For the experiments of Jones and Brooks, the fields were located at some distance from each other and the intervening space was not planted with a crop. This was not the case for the FSE study which assumed fields located next to each other. When the conclusion was drawn in the FSE study that tailing off takes place at 125 metre, it must be remembered that from the perspective of the donor field, the previous 125 metres acts as a buffer crop. Jones and Brooks demonstrated that when the open space is filled up with a barrier, the distance over which pollen flow occurred decreased. With this they observed that filling up the open space with maize will lead to a greater decrease in pollen flow than filling up the space with trees.

In addition to this several researchers have demonstrated that the majority of pollen transfer and therefore incrossing occurs in the first few rows of the receptor field. Stanley and Horn saw that 90% of the pollen ended up in the first 5 metres. Jones and Brooks found percentages of 50 to 70%. These lower values can be logically explained by the fact that Jones and Brooks did not examine the amount of pollen but the degree of incrossing. Not every pollen grain dispersed will result in fertilisation.

To achieve the lowest possible level of mixing on the field (= equivalent to the detection limit of about 0.1%) an isolation distance of 400 metres needs to be implemented assuming an open space (fallow or planted with a low-lying crop) between the acceptor and donor fields. When the open space is filled with a buffer crop (that is non-GM maize) a distance of 125 metres would seem to be sufficient.

If the parties concerned agree that a percentage of mixing is permitted, these distances can be reduced. Looking at the data from Brooks and Jones, a distance of about 125 metres is sufficient to keep incrossing below the percentage of 0.9% in the final product, and a distance of about 200 metres is necessary to keep it below 0.3%. Assuming that the distance between the source and receiver is not filled up with a buffer crop. These distances have been corrected for the contribution of the paternal genome to the final product (= about 33% of the endosperm). Shorter distances can suffice if the first rows of the acceptor (non-GM) fields are removed prior to the harvest. As previously stated the majority of incrossing takes place in the first few rows. An alternative is to plant the border rows of the GM field with non-GM maize that is eventually harvested with the GM field. However, there are no data to support this approach.

The use of buffer zones also results in smaller distances. FSE research has demonstrated that if buffer crops are used, a distance of about 25 metres is sufficient for an incrossing percentage of 0.9% and a distance of 80 metres for 0.3%. With this it should be noted that these percentages apply for rows at the distances stated and not for the field. As incrossing decreases as the distance from the source increases, the average percentage of incrossing and mixing on the field will be considerably lower than this value. Of course the deeper the acceptor field with respect to the source the lower this value will be.

Reasoning from the premise that for a distance of 125 metres or more a percentage comparable to the detection limit of 0.1% is present and a linear decrease applies for outcrossing over a distance of 25 metres to 125 metres, then for an isolation distance of 25 metres and a field depth of 100 metres an average mixing percentage of 0.35% will be found. For a distance of 80 metres and a field depth of 100 metres this value will be reduced to about 0.15%. For this to be true a buffer crop must be present. For acceptor fields deeper than 100 metres these values will decrease further still. Yet for narrow fields, for which the depth of the field and the isolation distance is below 125 metres, the field dilution will contribute to a much smaller extent. The aforementioned calculations are based on a linear relationship. It is most likely that the rate of decrease is more pronounced, as a result of which lower percentages are likely.

Concluding remarks

Maize is considered to be a crop with a high outcrossing potential[5,6,19]. For mixing on the field, incrossing is the most important factor. Volunteers and plants becoming feral do not contribute to mixing under Dutch conditions. Contaminated seeds can be another source for mixing.

By implementing an isolation distance of 400 metres for an open space, or 125 metres for a buffer crop, mixing on the field can be kept as low as possible. In practice such distances would, however, limit growers in their choice to grow GM crops, as production under these circumstances is not economically feasible. This seems to be in conflict with the rule that for coexistence all possible forms of agriculture must be able to exist side to side without one excluding the other.

Experiments have revealed that the majority of incrossing occurs in the border rows of the receptor field and that the presence of buffer crops can lead to considerably lower mixing percentages in the receptor field. This means that incrossing percentages far below the threshold values set by the EU can be achieved by growing buffer crops at more practically feasible isolation distances of 25 to 80 metres. The isolation distance chosen will have to be a compromise between practical and economical feasibility, in which a production system may not be excluded on the one hand, and the social acceptability of contamination in final products on the other. Also the possible contribution of contaminated seed to mixing on the field will need to be taken into account.

None of the isolation distances stated, not even those that result in a level of mixing on the field that is as low as possible, can exclude so-called hotspots being encountered. The FSE study revealed that unfavourable circumstances, such as wind turbulence due to storms, can lead to unexpectedly high levels of localised incrossing in fields at greater distances. The occurrence of hotspots is probably also strongly influenced by local geographic conditions. Although these conditions in the Netherlands are very different from those in England, it is likely that hotspots will also occur in the Netherlands. Therefore the parties involved will need to make agreements about liability.

In the aforementioned cases a spatial separation of crops has been assumed. COGEM points out that temporal separation is another option to prevent mixing. This will require a harmonisation of the crop rotation plans of growers of GM crops and growers of non-GM crops. This will need to take place following thorough consultations between the growers concerned.

3.3 Potato

Crop and production

The potato plant (*Solanum tuberosum*) belongs to the family *Solanaceae* which also includes tomato, egg plant, tobacco and pepper. Potato is an agricultural crop which originates from South America. *S. tuberosum* is divided into two subspecies: *tuberosum* and *andigena*. Only the subspecies *tuberosum* is grown in Europe.

The potato is not part of the Dutch flora, although it has a number of wild relatives: the black nightshade, (*S. nigrum* subsp. *nigrum*), the garden nightshade (*S. nigrum* subsp. *schultesii*), woody nightshade (*S. dulcamara*), hairy nightshade (*S. physalifolium*) and cut-leaved nightshade (*S. triflorum)*. However outcrossing to these relatives is not possible.

Potato is an economically important crop in the Netherlands. About 160,000 ha of potatoes are grown[28]. Seed potatoes, consumption potatoes and starch potatoes are grown in the Netherlands. These three crops differ with respect to how they are cultivated, stored and processed. Seventy percent of all seed potatoes grown in the Netherlands are exported. Starch potatoes are processed in the Netherlands. Most of the consumption potatoes (70%) are processed into chips and chip products. A large proportion of these chips are exported.

The production of GM consumption potatoes seems to have had little success and these are no longer grown in the United States. It is therefore unlikely that the production of GM consumption potatoes will take place in the Netherlands in the near future. However it is likely that GM starch potatoes will soon be grown on a commercial scale.

Outcrossing

Potatoes are vegetatively propagated via the tubers. Potatoes are usually grown from seed potatoes. These seed potatoes are produced by specialised companies. In many cases the first stage of the production process is *in-vitro* propagation. Due to a certification and monitoring system for the production of seed potatoes, the chance of adventitious mixing between varieties is effectively excluded. In the Netherlands, locally-produced seed potatoes are mainly used.

Crossing between varieties and seed production by seed only play a role in the breeding process. Potato is primarily a self-pollinator. The percentage of cross-pollination varies between 0 and 20%[52]. Pollen can be transported by both the wind and insects. Many, but not all, of the potato varieties grown in Europe are sterile. Some of the varieties grown in the Netherlands are capable of forming fertile flowers and crossing with other varieties in the field. Pollen flow seems to be limited to within 5 to 10 metres from the field edge[4].

Very little data is available about the frequency of outcrossing between potatoes in the field. Last season, the company AVEBE commissioned research into outcrossing between GM starch potatoes and non-GM potatoes in a field experiment. A total of 5459 seedlings (seeds) from the border rows of the trial field were tested. The provisional results indicate that at a distance of 1.5 metres, the incrossing percentage was 0.7%. At 5 metres from the GM field no more incrossing could be demonstrated. These unvalidated, data concur with the previously mentioned data concerning the transfer of pollen grains.

Crossing between GM potatoes and conventional varieties does not have any consequences for the product immediately harvested. Only if the seed formed is capable of growing into an adult plant and reproducing by means of tubers, can mixing due to the emergence of volunteers occur in a later harvest. However, the plants formed by incrossing are small and weak and are not capable of surviving in a subsequent crop or producing tubers. Crop rotation, the cold Dutch winters and the statutory control of volunteers mean that in practice there are sufficient barriers to prevent survival. Potato tubers are cold-sensitive and do not normally survive the winter in the Netherlands. Only in mild winters are tubers capable of surviving and germinating the next year. Tubers (or parts thereof) that remain on the field can therefore form a problem. However due to the statutory control of *Phytophthora infestans*, any possible potato volunteers are removed. To date no feralisation of potato plants has been observed in the Netherlands.

Concluding remarks

The risk of adventitious mixing with GM potatoes appears to be mainly present in the transport or storage of the crop or further in the chain during processing. In the Netherlands, the control of volunteers is compulsory for the control of potato blight (*Phytophthora infestans*) and potato cyst nematode disease (*Globodera rostochiensis* and *G. pallida*). If these requirements are strictly adhered to, the contribution of possible volunteers to mixing is negligible. Further, COGEM considers the probability and frequency of infection of propagating material to be minimal.

In view of the minimal risk of incrossing and the minimal risk of consequences should incrossing occur, the implementation of isolation distances for the production of potatoes in the Netherlands to prevent the consequences of incrossing does not seem to be necessary. However the implementation of a manoeuvring space of several metres (about 2 to 3 metres) can prevent GM potatoes accidentally being harvested with a neighbouring plot.

3.4 Sugar beet and fodder beet

Crop and production

Sugar beet, fodder beet, red beetroot and seakale beet all belong to one species, *Beta vulgaris* (family *Chenopodiacae*). Sugar beet is grown on a large-scale in the Netherlands. About 107,000 ha are grown with a harvest of almost 6 million tonnes of beet (Eurostat, 2002). In 2002, about 300 hectares of organic sugar beet was grown[28]. Less than 1000 hectares of fodder beet is grown with a harvest of 49,000 ton. Red beetroot and seakale beet are vegetable crops, grown in the Netherlands on a small scale.

Beet is a biannual crop and the plant flowers and set seed in the second year. However, in beet production, the root or beet is harvested at the end of the first growing season. Sugar beet is processed in the factory to produce sugar. By-products of this, mainly beet pulp, are process into animal feed. Fodder beet is used as animal feed.

Beet seed is mostly produced in France or Italy. No seed production for sugar beet and fodder beet takes place in the Netherlands and the crop is always harvested after the first growing season. However so called bolters are found in the crop. These are plants which flower and set seed during the first year. Bolters are a consequence of cold conditions (vernalisation) during the growing season or contaminations in the seed formed by crosses between sugar beet and a wild relative, sea beet. Sea beet is indigenous to Europe and is found in areas in France where seed production takes place.

The majority of growers remove bolters by hand to prevent the viable seed from setting and causing problems in subsequent years. If bolters are not removed, weed beets may establish. Beet seed is capable of surviving in the soil for a long period of time and germinating and maturing under favourable farm management measures. In the Netherlands, fields with large populations of weed beets have arisen in this manner and these populations are difficult to control.

If these volunteers are also capable of successfully competing in other crops they can disrupt the crop rotation and the associated disease control.

Outcrossing

Beet is mainly wind pollinated, although insect pollination can also occur[5,57]. Pollen can be transported by the wind over considerable distances.

Relatively little is known about pollen viability. Pollen which is stored cool can remain viable for a period of 50 days. However, pollen can lose its viability in one day if it becomes damp during dew formation[51].

The species, *B. vulgaris,* contains several different types of beet, such as sugar beet, fodder beet, red beetroot, seakale beet, etc. All of these subspecies can cross with each other. Beets can also outcross with the wild relative sea beet, *B. vulgaris* ssp. *maritima*. Sea beet inhabits coastal regions of Europe and can also be found sporadically in the Netherlands. Outcrossing with sea beet needs to be borne in mind during seed production, as it results in annual hybrids which lead to bolters and flowering in the crop.

For beet seed production, the field margins are sometimes planted with hemp (*Cannabis sativa*) to limit outcrossing. However, outcrossing can take place over considerable distances and the planting of buffer crops such as hemp cannot prevent this. In experiments with male sterile plants and a field surrounded by hemp plants, hybridisation was still observed 200 metres outside of the buffer zone[58].

Much greater isolation distances are adhered to in seed production in order to guarantee the seed purity. For the production of so-called basic seed, an isolation distance of 500 metres from related subspecies and 1000 metres for other subspecies of *B. vulgaris*[59] applies in Europe.

Concluding remarks

Outcrossing does not play a significant role in the production of beets in the Netherlands. Beets are mostly harvested before the plant reaches the reproductive stage. No seed production for sugar and fodder beet takes place in the Netherlands. Seed production on a very limited scale only takes place for the vegetable forms of beet, such as red beetroot. Mixing might occur on the field if the so-called bolters outcross. This source of mixing can be prevented by removing the bolters.

The setting of isolation distances for sugar beet and fodder beet production to prevent incrossing is not necessary, as in principle the crop does not flower and the final product (beet) is not a crossing product either. However, applying a manoeuvring space of several metres (about 2 to 3 metres) for the harvesting machine prevents GM beets from an adjacent plot being harvested as well or prevent GM beets that are being harvested from ending up on an adjacent plot.

In case a lot of weed (feral) beets are present on the field, GM beets should not be grown on this field. Removing all of the bolters will be difficult due to the large number of bolters from the weed beets on the field. If the bolters are not completely removed the chance of outcrossing is present. If the GM beet contains a herbicide tolerance then the production of GM beet could be a means of controlling the weed beet. Spraying with the herbicide concerned can control the weed beets whilst the GM beet continues to grow. Yet with this it must be noted that an effective removal of bolters is exceptionally important from an agronomic viewpoint. Weed beets can form a significant problem and make it impossible to grow sugar beet on some fields. At present, spraying with herbicides is the only method for controlling weed beets. Outcrossing of a herbicide tolerance to the weed beet is therefore undesirable, especially as the number of herbicides and tolerance genes is limited and in recent years hardly any new herbicides have been developed.

3.5 Mixing in different stages of the production

As stated in the introduction, COGEM distinguishes four phases in the production which are important for mixing. In each stage mixing can occur and contribute to the final percentage of mixing in the final product. The measures needed to limit this mixing will have to be examined for each stage per crop. The stages and the mixing that occurs as a result of these cannot be viewed in separation, as they influence each other.

The previous sections have shown that the importance of each element can differ per crop. As a consequence of this the measures needed to prevent mixing differ per crop. Whereas for maize and oilseed rape the implementation of isolation distances is necessary to prevent the consequences of pollen flow, such a measure is not relevant for beet and potatoes. A similar story applies to volunteers and the measures to control these. For potato and even more importantly oilseed rape, measures to prevent volunteers will contribute to a reduced chance of mixing. For maize such measures to prevent mixing are not important because it is known that seed left behind in the field does not result in volunteers under Dutch conditions.

In this monitoring report, COGEM has limited itself to four crops. However, the line adopted by COGEM can be extrapolated beyond these. By considering each of the five aforementioned elements separately the effect of each element on mixing can be assessed in a similar manner for any crop. After this the adequacy of the current farm management measures can be assessed and where necessary tightened. However, if there is a large degree of uncertainty for one of the stages with respect to mixing then taking far-reaching measures for one of the other elements would not appear to be worthwhile. An example of this is oilseed rape. For oilseed rape there is too much uncertainty about the role of the seed bank under Dutch conditions. As a result of this it is not possible to make a meaningful statement about the isolation distances which need to be implemented to prevent incrossing due to pollen flow.

4. Concluding remarks

- It seems inevitable that a certain amount of mixing will occur between non-GM products and GMOs. This mixing can occur throughout the entire production chain, from the seed to the processing of final products. Therefore measures to be taken must focus on reducing mixing to below the threshold value set for the final product.
- COGEM distinguishes four stages in the production phase that are important for mixing, namely sowing, growing, harvesting and the control of volunteers.
- The effect of each element on mixing should be determined for any crop by considering each of the aforementioned stages separately.
- The measures needed to limit this will have to be examined per crop.
- Mixing in the field may be prevented by implementing a number of measures including farm management measures such as soil tillage, separate flowering times, control of volunteers, planting field margins and the setting of isolation distances.
- COGEM notes a lack of data concerning the persistence of the seed bank during the production of oilseed rape under Dutch growing conditions and a lack of data about the importance of 'wild' oilseed rape in verges and field margins. Further research is needed.
- COGEM points out that for genetically modified maize, setting isolation distances of 25 to 80 metres and using buffer crops will keep contamination under the thresholds set by the EU.
- In COGEM's opinion, incrossing does not play a role in possible mixing during the production of GM potatoes and GM beets. However, using a manoeuvring space of several metres (about 2 to 3 metres) for the harvesting machine prevents GM potatoes or GM beets from an adjacent plot also being harvested or GM potatoes or GM beets from ending up on an adjacent plot during harvesting.
- COGEM recommends that the growers of GM beet should be legally required to remove bolters before flowering and setting of seed.
- The discussion about measures for coexistence must not be restricted to the setting isolation distances. Spatial separation and temporal separation can be used as well. For example by using asynchronous cropping plans.
- Agreements with respect to spatial planning, soil tillage regimes and farm management measures are also necessary.
- Reaching such agreements will require consultation and cooperation between local growers.

- COGEM observes that data are needed on outcrossing and mixing over a period of several years under Dutch conditions. Such data are necessary to provide a better scientific basis for measures to reduce mixing.

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Appendix 1: Elements for mixing during crop production and possible measures to prevent mixing.

	Initial material	Outcrossing by pollen transfer			Volunteers		Harvest	
		Regulating growing period	Regulating use of land					
Element	purity of seeds and propagating material		size and shape of fields	position of fields	seeds	plant parts	adventitious transport by machines (also during production)	mixing in harvesting machine
Oilseed rape*	x	x	x	x	x		x	x
Maize	x	x	x	x	x		x	x
Potato	x	x				x	x	x
Beet	x	x			x	x	x	x
Possible measure	production and purchase of 'clean' material	separating flowering periods		isolation distances	removing bolters		cleaning machines	
		cropping plan		barriers, catch crops or buffer crops	controlling volunteers (using herbicides, weeding) soil tillage measures		manoeuvring space for machines	

Ad *: The relevant elements per crop are shown. Not all elements have the same effect on the final level of mixing.