DECISION SUPPORT DOCUMENT[1]

Current practice and potential impact of in-field separation strategies for GM and non-GM maize

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Abstract

The aim of this document is to summarise and interpret the current body of knowledge regarding the potential impact of the in-field spatial and temporal separation between environmentally released genetically modified (GM) and non-GM maize. Here separation distance refers to the physical distance between plots containing GM and non-GM maize and temporal separation refers to the deliberate disruption of flowering synchrony between adjacent fields. Distance and time represent barriers to cross-fertilisation and are therefore employed as primary containment measures to ensure that GM traits, when released into the environment, are not unintentionally spread to non-GM crops. Several countries have proposed or are legally imposing largely differing minimum separation distances to minimise cross-fertilisation. However, there is a need for specific criteria to define specific, science-based separation strategies for South Africa. Recent studies have suggested that the agricultural context, the intended use of the harvested maize and the specific landscape and environmental conditions should be considered when defining specific separation strategies for each unique situation. Based on the current literature we propose the use of only a few, specific separation strategies based on the applicable risk management system and the fate of the neighbouring non-GM products. Dedicated research is needed to confirm if these proposed strategies are appropriate for South African agricultural conditions.

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Introduction

The adoption of genetically modified (GM) crops in agriculture has internationally given rise to concerns regarding unwanted GM-inputs in non-GM crop products. Given the increasing commercial cultivation of GM maize and the development and subsequent field testing of new GM traits, there is a need to define measures to prevent the unintentional spread of GM material to non-GM production systems and wild relatives in the environment surrounding GM crops. Two different scenarios where these measures can be considered include pre-commercialisation field trials of new traits and coexistence cropping systems (systems in which GM and non-GM crops are cultivated).

In the case of field trials, the main issue is risk management and the containment of the GM trait, the GM crop and the GM product that is being evaluated under field conditions. On the other hand, in coexistence cropping systems, the primary aim is to allow farmers to make a practical choice between conventional, organic or GM crop production systems to deliver products in compliance with a particular set of regulations, e.g. the legislation on labelling and/or content standards of the EU (European Commission, 2003). Because these crops have already been approved for commercialisation the primary issue is not containment or crop/product safety but rather the production and marketing of a product that complies with the criteria of a particular market.

Adventitious presence of GM material in non-GM crops/products can arise from seed impurities, cross-pollination/ -fertilisation, volunteers from previous GM cultures and harvesting, transporting and storage practices on farms. Apart from good agricultural/farming practices, specific provisions should be made to ensure that out-crossing between GM and conventional or organic maize is minimised to ensure successful coexistence. Although cross-fertilisation naturally takes place between different maize varieties grown in adjacent fields, there is no genetic exchange between maize and other plant species in Europe or South Africa since its wild relatives (the teosinte-complex) are limited to parts of Mexico and Guatamala (OECD, 2003). Given that maize is a cross-pollinating species and its pollen is transported by wind, cross-fertilisation could be a major cause of the spread of GM material to non-GM crops. This can be negated by the in-field separation of crops by space and/or time, the planting of buffer crops as pollen barriers and/or the location of sites in relation to prevailing wind direction (Ingram, 2000).

In this document we specifically review current international practices and the use of spatial and temporal separation or “isolation” of GM and non-GM maize fields as a means to minimise cross-fertilisation. This information is then used to propose specific separation strategies for GM maize in South Africa and identify gaps in the current body of knowledge that could be addressed in future research projects.

Cross-pollination/-fertilisation in maize

Maize is a cross pollinating crop and pollination relies primarily on the wind dispersal of pollen. The plant is diclinous (an angiosperm with unisexual flowers) and more specifically monoecious, i.e. have separate male and female flowers on the same plant. Maize is also protandrous, with pollen being shed before the silks are receptive, but as there is as much as 5% overlap some self pollination may occur (OECD 2003; OGTR 2008). Maize pollen is relatively heavy and the majority is deposited within a short distance from the emitter plant, i.e. 98% of the pollen in conventional maize crops is deposited within 25 m of the emitter field and practically 100% within 100 m. Furthermore, 99% of the cross-pollination that occurs outside the emitter field takes place within 18 to 20 m of the emitter field borders (Brookes et al., 2004).

A multitude of studies have been performed world-wide to determine cross-pollination rates and maximum out-crossing distances for maize. These studies differed with respect to the arrangement of the fields (e.g. size, shape and size relation between donor and recipient fields), tested varieties (e.g. 'colour' maize or GM-maize), detection system (e.g. DNA content and colour maize), size of measured area (e.g. one plant, part of field or whole field), production situation (e.g. agricultural production or seed production) and the climatic and weather conditions. All the studies, however, showed a characteristic rapid decrease of cross-fertilisation (vertical gene flow) rates with increasing distance from the pollen emitter plants (Fig 1; Henry et al., 2003; Brookes et al., 2004; Ma et al., 2004; Devos et al., 2008; Reuter et al., 2008; Sanvido et al., 2008).

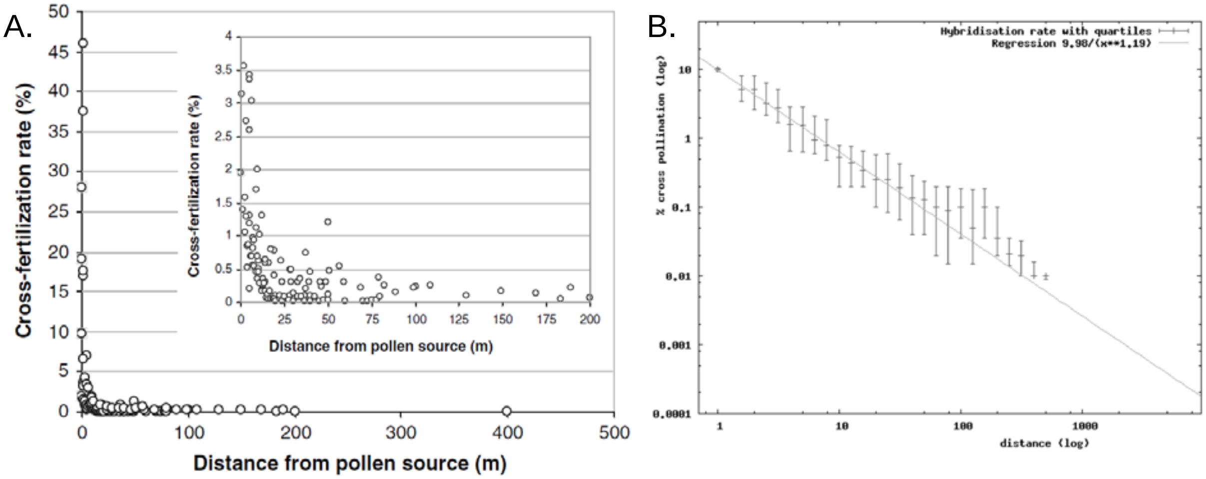

Figure 1. (A) Comparison of the cross-fertilisation rates from nine (9) different studies (from Sanvido et al., 2008). (B) Consolidated distant dependent cross-pollination rates from ten (10) different studies (from Reuter et al., 2008).

No data on maize pollen movement under South African climatic conditions have been published to date. In 2004, however, a study was conducted at the University of the Free State where the impact of pollen movement on the identity preservation (IP) of maize was investigated in two geographic locations, i.e. Delmas, Mpumalanga and Lichtenburg, North West Province (Chetty, 2004). Phenotypic evaluation of out-crossing revealed a high incidence (between 22.3 and 39.1%) of out-crossing between adjoining yellow and white maize rows, which decreased to 1% at a distance of 25 m and to 0.36% at a distance of 81.6 m (Chetty, 2004). These results were summarised in a poster presentation (Fig 2; Chetty and Viljoen, 2008).

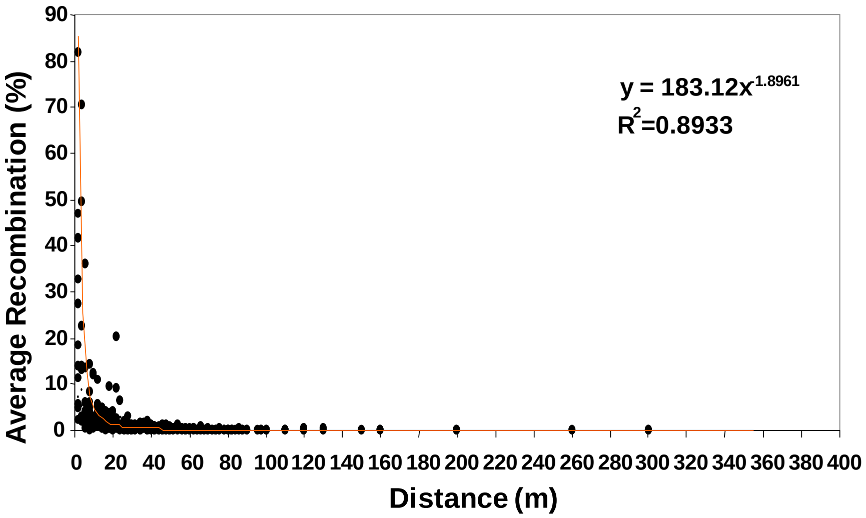

Figure 2. Average percentage recombination over distance in a South African maize pollination trial (from Chetty and Viljoen, 2008).

In France the MAPOD® gene flow model was used to estimate adventitious presence due to cross-pollination between GM and non-GM maize. This gene flow model accounts for factors such as the crop's characteristics, agricultural practices, climatic conditions and the spatial patterns of crop fields (Messean et al., 2006; Viaud et al., 2008). It was shown that cross-fertilisation between GM and non-GM fields varies considerably with landscape, field characteristics, varietal pollen characteristics and wind conditions. Nevertheless, by considering the worst-case scenario, e.g. small non-GM fields downwind of large GM fields, the 0.9% threshold could be ensured everywhere with simple rules like adequate separation distances. Viaud and co-workers (2008) suggested that the separation distances between GM and non-GM crop fields should be adapted to the local climatic context, especially prevailing wind conditions. Due to the great variability, a flexible decision support system taking into account key factors such as isolation distances, area of the non-GM field, flowering time-lag and even the amount of pollen produced by both GM and non-GM varieties, may be valuable to minimise overall segregation costs in those situations where isolation distances may be difficult to implement, e.g. clustered fields (Messean et al., 2006).

It is important to clearly distinguish between *cross-fertilisation* and *pollen movement* because pollen dispersal does not necessarily result in fertilisation. Successful cross-fertilisation depends on the foreign pollen being available at the right time, it being conveyed in the direction of the receiving plants and low levels of competing pollen produced by the receptor plant itself (Ingram, 2000). The main factors influencing cross-fertilisation rates include pollen viability and longevity, male fertility or sterility, synchrony in flowering between anthesis of the pollen donor and silking of the recipients, wind direction and velocity, weather conditions, size, shape and orientation of both pollen source and recipient field as well as the distance, topography and vegetation between pollen source and recipient field (Sanvido et al., 2008). In addition, the inheritance characteristics of the transgene will also influence the cross-fertilisation rate. In conventional maize studies, 100% of the emitted pollen is of relevance to cross-fertilisation levels. However, depending on the dominance pattern of the transgene and level of heterosis of the commercial GM hybrid, less than 100% of the emitter pollen will contain the GM trait. In the case of GM maize containing one trait, e.g. single copy Bt maize such as MON810, the plants are heterozygous and only 50% of the emitted pollen is of relevance because only 50% of the pollen contains the Bt gene (Brookes et al., 2004). It should be noted that the situation will be different for stacked genes that could be inherited separately from each other (Messéan et al., 2006).

The EU-funded project SIGMEA (Sustainable introduction of GM crops into European agriculture) has collated and analysed more than 20 European studies of gene flow in maize (Hüsken et al., 2007). Most of these studies have used the European Union positive labelling threshold of 0.9% (GM content) as benchmark for determining the maximum level of adventitious presence of GM material that is allowed in non-GM products. In a more recent publication, Sanvido and co-workers (2008) have summarised the methodology and findings of 18 studies that assessed the cross-fertilisation in maize in the context of coexistence of GM and non-GM crops worldwide. Modern hybrid varieties were used for all these studies. They reported that cross-fertilisation rates among open-pollinated varieties were distinctively higher than those reported for hybrid varieties due to the biology of maize flowering (Fig 3, Sanvido et al., 2008).

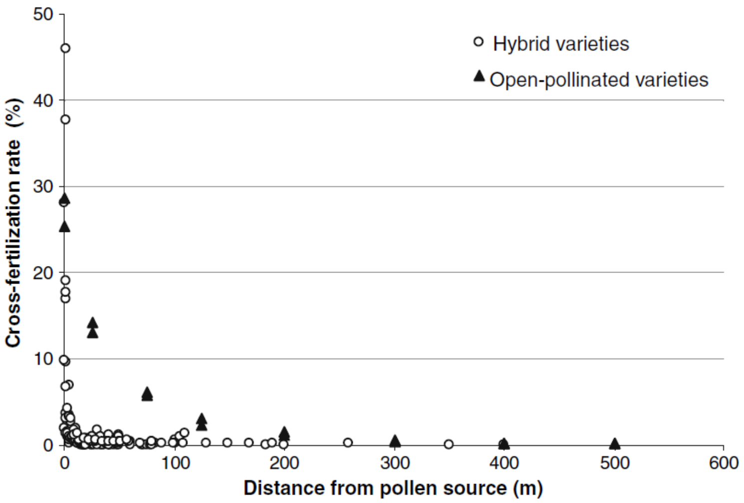

Figure 3. Difference between the cross-fertilisation rates of open-pollinating and modern hybrid maize varieties (from Sanvido et al., 2008).

In order to avoid self-fertilisation, open-pollinated varieties release their pollen from the tassels before the female flowers (silks) of the same plant are receptive - flowering is therefore not synchronized. The silks of individual plants are therefore fertilised by pollen from other maize plants in the same or neighbouring fields. However, a major breeding target for modern hybrid varieties is to optimise the synchrony between male and female flowering in order to reduce the time period in which female silks are receptive after male pollen shed. This reduces the probability of cross-fertilisation by pollen from neighbouring fields (Sanvido et al., 2008).

The potential impact of GM maize out-crossing varies for different products and under different conditions

GM maize is isolated in field trials or coexistence cropping systems to minimise gene flow to nearby non-GM maize fields. The extent of this gene flow and its potential impact does not only depend on the factors discussed above but also on the characteristics and intended purpose of the neighbouring, or receiving, non-GM crop. Moreover, (i) the receptiveness of the receiving crop for pollen from the GM maize may vary, (ii) the final percentage GM content in different end-products from the same field will differ, (iii) the acceptable levels of GM content for the same product in different markets vary and (iv) the potential risk associated with the GM content in the various end-products may differ.

Seed: Genetically pure hybrid maize seeds are intended for propagation purposes only and are the products of crossing two parental inbred maize lines with specifically selected traits. As such, reproductive isolation of seed fields is required to ensure genetic purity of the hybrid progeny. Fields for the production of conventional hybrid seed usually contain rows of pollen-producing male plants alternated with rows of sterile or detasseled female plants acting as pollen receptors. Depending on the planting pattern, only 20 to 25% of the plants in a field are pollen parents (Ireland et al., 2006). As a consequence, they are highly receptive not only to the pollen from the directly adjacent male plants but also to adventitious pollen carried from neighbouring fields by the wind. Because of inbred depression, the inbred pollen parents normally have relatively small tassels that produce less pollen than other maize and very often this pollen production lacks timing synchrony with female plant maturity (Brookes et al., 2004).

In order to ensure the high degree of genetic purity required for hybrid seeds, i.e. 99.5% for basic seeds and 99.0% for certified seeds (OECD, 2008), strict growing conditions and managing practices have been established over many years and are routinely maintained in the industry (Ireland et al., 2006; Messéan et al., 2006, Goldschagg, 2009). Isolation standards typically include a large minimum distance, e.g. 200 m, from all potential pollen sources, and a recommended number of border rows of the inbred male parent for a given isolation distance (Brookes et al., 2004; OECD, 2008).

Grain: In contrast, conventional maize grain is grown for direct use of its dry seed as food, feed or for processing and fields contain 100% fertile parent plants. The amount of pollen present and its competitiveness are therefore much higher than in seed production fields and the influence of adventitious pollen from neighbouring (GM) fields is much smaller. Maintaining a particular degree of purity in a grain maize field therefore requires less strict measures than those used for seed production (Brookes et al., 2004). Sweet corn production could also be regarded as grain production with the only difference being that the cobs are harvested before the seeds have had a chance to ripen.

Whole plant: In the event of out-crossing only the newly produced maize kernels are affected by this cross-fertilisation and not the rest of the plant. Furthermore, the introgression of the transgenes will only affect specific kernels on the cob (Ingram, 2000). While only the kernels and cobs are harvested for grain and sweet corn production the whole plant, including its vegetative parts, is harvested and used directly for animal feed in the form of silage/fodder. By weight, grain normally represents only 20-40% of the whole crop, which therefore translates into a proportional reduction in the overall percentage GM content (w/w) in silage/fodder. Compared to grain production systems the separation distances for non-GM receptor maize intended for feed/silage/fodder can therefore be reduced without impacting on the prescribed thresholds (Messéan et al., 2006).

The GM content of products is quantified as a percentage by weight (w/w) and specific but varying minimum thresholds for GM content have been set by various regulatory authorities to delineate non-GM products. Almost all traded agricultural commodities accept some degree of adventitious presence of unwanted material and for several crops maximum acceptable thresholds have been set for positive labelling of food and feed products containing or derived from GM crops. Countries which have mandatory labelling policies for GM products include Australia, Brazil, China, the European Union, Indonesia, Israel, Japan, New Zealand, Russia, Saudi Arabia, South Africa, South Korea, Switzerland, Taiwan and Thailand. However, the percentage of GM presence which requires labelling differs. The European Union, Israel, Russia and Switzerland require mandatory labelling if foodstuffs contain 0.9% or more GM traces. In Australia, New Zealand, Brazil, China and Saudi Arabia the threshold is 1%. In South Korea it is 3% and in Indonesia, Japan, Taiwan and Thailand the threshold is 5% (Gruère, 2006). It is important to note that these thresholds are for approved GM events and that many countries have a zero tolerance for unapproved events. In addition, specific market segments such as the organic industry have set more stringent thresholds in order to comply with current EC regulations. According to these regulations the use of GMOs or products produced by or from GMOs is prohibited (EU Organic standards, 2008). Separation distances are therefore increased accordingly to reduce the frequency of cross-fertilisation.

Finally, under certain conditions low levels of out-crossing could have an impact on the long term sustainability of a particular GM trait. The spread of insect resistance genes in open pollinated varieties (OPVs) where the grains are kept for seed, e.g. small scale farming, could for example impact negatively on resistance management practices. Moreover, the presence of the resistance gene in only some of the individuals in a field or sub-lethal protein levels in subsequent generations could increase the chances of resistance development. In this case it is not a safety or a market related issue but rather about the sustainability of the particular trait/technology. It is therefore not only important to consider the possible impacts of GM maize on non-GM maize but also *visa versa*.

Because the reasons for minimising gene flow in GM field trials are different this will be discussed in detail under a separate heading later in the document (see p11).

Temporal separation of GM and non-GM crops as a mechanism to minimise cross fertilisation

The asynchrony in flowering between male and female flowers in the same field that was discussed above should be distinguished from asynchrony in planting dates and flowering between different maize fields of different farmers to minimize cross-fertilisation. This deliberate disruption of flowering synchrony between plots or fields, with the goal of enforcing genetic isolation, is called “temporal separation or isolation”. Temporal separation has not been as extensively quantified as spatial separation as a mechanism to minimise cross-fertilisation. Messeguer et al. (2006) studied cross-fertilisation between *Bt* and conventional maize in real coexistence situations in Spain and established an estimated cross-pollination (ECP) index, which can accurately predict the percentage GM content in neighbouring fields. Moreover, they clearly showed that the two main factors that determine cross-pollination are coincidence of flowering and the distances between pollen donor and receptor fields.

Separation of flowering times can also be achieved by providing a choice of varieties, some flowering earlier than others. It is easier to fulfil this requirement by choice of variety than by sowing on different dates because climate, in particular, could affect this practice, limiting the days available for sowing or synchronising flowering time - periods of high or low temperature (Messéan et al., 2006). Nonetheless, according to the ECP index, even in the case of fully coincident flowering, a physical separation distance of approximately 20 m should be enough to keep the adventitious presence of GM maize due to pollen flow below the critical threshold of 0.9% for the total yield of these fields (Messeguer et al., 2006).

The viability of including a coexistence strategy on the basis of the non-coincidence of flowering in coexistence regulations was also investigated by Palaudelmas and co-workers (2008). They showed that in temperate areas small delays in flowering produced substantial decreases in cross-pollination rates and suggested that it should be possible to use the knowledge of specific agronomical practices and climatic conditions to improve coexistence through temporal separation of flowering dates, on the basis of appropriate delays in sowing dates. They warned, however, that this strategy should be combined with spatial separation in cooler areas, because only a few days of temporary separation are possible without important yield losses and this may result in significant, but not sufficient, reduction in pollen-mediated gene flow (Palaudelmas et al., 2008).

Current international practices regarding the spatial separation for GM maize cultivation

As mentioned above, the spatial separation between GM and non-GM maize fields and its impacts on cross-fertilisation have been extensively investigated. It is widely accepted as an effective measure to reduce GM-inputs into non-GM crops and that the required distance should be based on the percentage of tolerated impurities. In a recent study to determine appropriate separation distances for GM maize cultivation, Sanvido and co-workers (2008) suggested separation distances of only 20 m for silage and 50 m for grain maize. They arrived at this conclusion based on statistical data analyses of maize acreage and aerial photograph assessment of a typical agricultural landscape in Switzerland by means of Geographic Information Systems (GIS) to consider the agricultural context of maize cultivation in the analysis of cross-fertilisation. They also showed that the potential for coexistence cropping systems is strongly dependent on the prevalent landscape structures (Sanvido et al., 2008).

Devos and co-workers (2008) arrived at a similar conclusion and added that it would be optimal if a certain degree of flexibility is built into *ex ante* coexistence regulations to take different regional situations into account (Devos et al., 2008). They also developed a method to search for optimal field locations for GM maize in order to minimise the proportions of non-GM maize fields and farmers that have at least one non-GM maize field occurring within the separation perimeters (Devos et al., 2008). Although locally adapted coexistence strategies for each maize-growing area in South Africa might lead to optimal coexistence practices, it may be impractical for small scale growers and very difficult to manage and police for regulators.

Practically however, the question of adequate separation distances is still a subject of controversy and largely varying distances have been proposed in different countries. In Table 1 and Fig 4 the minimum separation distances between GM and non-GM maize crops proposed or legally imposed by different competent authorities are summarised. All these distances are based on local field trial data and are currently being used or proposed for commercial coexistence cropping systems.

Table 1. Minimum recommended or legally imposed separation distances between GM and non-GM maize in different countries

Country	Separation distance and additional measures	Reference
Brazil	100 m with no pollen trap border or 20 m with a pollen trap border of at least 10 rows of conventional maize plants of similar size and vegetative cycle to the GM maize	CTNBio, Normative Resolution No. 04 of August 16th, 2007
Canada and USA	200 m between GM and conventional maize Insect resistance (IRM) and herbicide tolerant management plans[a]	http://www.inspection.gc.ca/english/plaveg/bio/dt/term/2007/zeamaye.shtml
Czech Republic	70 m between GM and conventional maize 200 m between GM and organic maize	Devos et al., 2008
Denmark	200 m conventional cultivation 200 m organic cultivation and seed production	http://www.agrsci.dk/gmcc-03/Co_exist_rapport.pdf http://www.gmo-safety.eu/en/archive/2004/274.docu.html Devos et al., 2008
France	50 m between GM and conventional maize	Devos et al., 2008
Germany	150 m between GM and conventional maize 300 m between GM and organic maize	Devos et al., 2008 www.gmo-safety.eu
Hungary	400 m between GM and conventional maize 800 m between GM and maize for seed production and organic maize	Devos et al., 2008
Ireland	50 m between GM and conventional maize 75 m between GM and organic maize	Devos et al., 2008
Luxembourg	800 m between GM and conventional maize 800 m between GM and maize for seed production and organic maize	Devos et al., 2008
Poland	200 m between GM and conventional maize 300 m between GM maize and organic maize	Devos et al., 2008
Portugal	200 m between GM and conventional maize 300 m between GM maize and organic maize	Devos et al., 2008
Slovakia	200 m between GM and conventional maize 300 m between GM maize and organic maize	Devos et al., 2008
South Africa	200m between seed maize (certified) and any source of contaminating pollen	OECD, 2008
Spain	50 m between GM and conventional maize 50 m between GM and organic maize 300 m between GM and maize for seed production	http://ec.europa.eu/agriculture/events/vienna2006/presentations/ortega_en.pdf Devos et al., 2008
Sweden	50 m between GM and conventional or organic grain maize[b]	Devos et al., 2008

	15 m between GM and conventional or organic forage maize	
Switzerland	50 m for grain maize 20 m for silage	Sanvido et al. 2008, http://agrisite.de/doc/ge_img/pollen-swiss.pdf
The Netherlands	25 m between GM and conventional maize 250 m between GM and maize for seed production and organic maize	Devos et al., 2008
UK	200 m between GM and sweet corn 110 m between GM and conventional grain maize 80 m between GM and conventional forage maize[c]	Henry et al., 2003; Brookes, 2004; Devos et al., 2008
Wales	420 m seed crops and sweet corn 290 m for maize silage	www.defra.gov.uk/environment/acre/advice/advice12.htm

[a] In Canada and the USA farmers planting insect resistant (Bt) crops are required to implement IRM to contribute to minimizing the possibility of target pests developing resistance (guidelines on separation distances, refuges when planting over 5 hectares of Bt maize and insecticide usage). Farmers of herbicide tolerant crops (GM and non-GM) are provided with advice on managing volunteers in succeeding crops (integrated weed management system, crop rotation, rotation of herbicides and timing of herbicide applications, timing of tillage, use of certified seed)

[b] Isolation distance doubles when GM maize varieties contain more than one transgene.

[c] Recommended by SCIMAC (Supply Chain Initiative on Modified Agricultural crops)

For coexistence systems, the great majority (>80%) of countries as listed in Table 1 propose separation distances of between 25 and 200 meters between GM and conventional grain maize in order to maintain a threshold of approximately 0.9 % (Fig 4). For organic and seed production, separation distances between 201 and 300 meters are most commonly used and for silage production, distances below 100 meters are usually imposed.

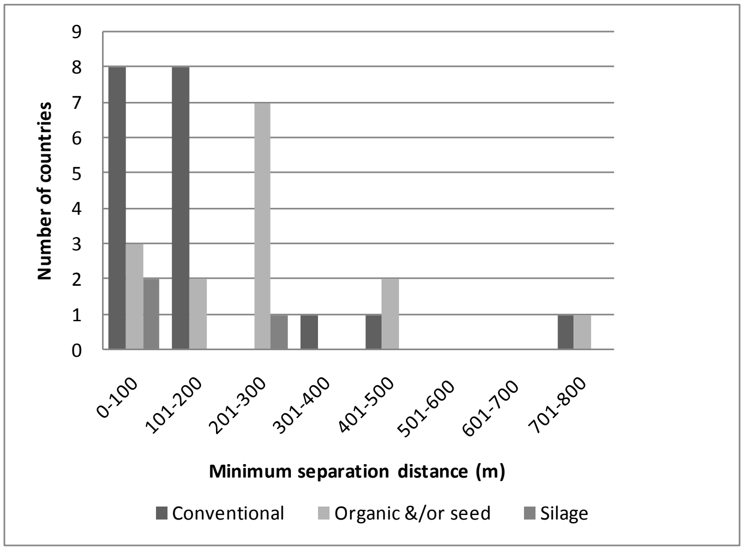

Figure 4. Distribution of the minimum separation distances proposed or imposed by different countries.

Pre-commercialisation, confined GM maize field trials

Because the risk assessment process has not been concluded at the time when new GM cultivars are evaluated in field trials, containment of the novel trait(s) is a crucial objective. In contrast to coexistence cropping systems where the aim is to keep gene flow (GM content of the non-GM products) below a pre-determined value based on the desired properties of the product from the receiving field, the aim here is to prevent gene flow. This is done through an integrated risk management program, incorporating various complementary strategies aimed at both the physical and biological restriction of transgene movement. These strategies include the limitation of seed movement during planting, harvesting, transportation and storage, the prevention of cross-fertilisation (gene flow) with wild relatives and/or neighbouring non-GM maize and the post-harvest management of volunteer plants.

Strict, specific terms and conditions are therefore imposed for confined GM maize field trials by regulatory authorities. Although these conditions are different for different countries they generally include conditions for the spatial and/or temporal (reproductive) separation of the GM maize from neighbouring non-GM maize fields, harvesting, storage practices and post-trial management of volunteers. In most countries where GM maize field trials are performed, the permits are issued for trials that take place over several growing seasons on the same trial site. This not only minimises the potential for pollen dispersal from more than one site but also facilitates the implementation of rigid isolation perimeters, the monitoring of gene flow and the management of volunteer plants at the conclusion of the trial (Devos et al., 2007). Although maize has lost its ability to survive in the wild due to its long process of domestication and cannot persist as weeds (OECD, 2003), all regulatory permits require that the trial site is monitored for volunteer plants for a specific time period after the final harvest to ensure the effective containment of the transgenes. In addition, a single trial site also helps ensure the robust statistical evaluation of research results.

Below are four representative examples of GM maize field trials that have been approved in different countries during the last two years. The trials in Australia, Canada and Hungary have been approved for 3 to 5 growing seasons on the same trial site, while the South African one was approved for one season only. These examples were chosen to illustrate the different specific requirements that each of these countries impose in terms of minimising cross-fertilisation and post-trial monitoring.

Table 2. Summary of the attributes and regulatory requirements for recent GM maize field trials in four different countries.

Example 1: Australia
(http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir086-3/$FILE/dir086appsum.pdf)
GM plants: DIR 086/08, limited release of eleven maize lines to investigate gene function.
Trial site: 750m^2/ 0.15 ha.
Duration: 5 growing seasons on the same site.

- Maize not used for food, feed or processing
- Restricting access to site
- Detassel GM maize to prevent pollen production (no minimum separation distance specified)
- Pollinating GM maize under controlled conditions with non-GM pollen
- Harvesting maize seed by hand
- Cleaning site after harvest
- Monitoring trial site for 8 months after completion of trial and destroying volunteers
- Transporting seed according to guidelines

Example 2: Canada
(http://www.inspection.gc.ca/english/plaveg/bio/dt/term/2007/zeamaye)
GM plants: DD2005-55, maize with insect and herbicide resistance.
Duration: 5 seasons on same plot.

- 200m separation distance
- IRM (Insect resistance management plan)
- Transporting seed according to guideline
- Clean machinery and equipment at trial site
- Site must be marked and monitored
- GM not used for food or feed
- Weekly monitoring of trial site and related species removed
- The trial site, including a minimum 10 m zone around the trial site must not be used to grow maize for one year following harvest of the trial
- All details logged

Example 3: Hungary (EU)
(http://gmoinfo.jrc.ec.europa.eu/gmp_report.aspx?CurNot=B/HU/07/05)
GM plants: B/HU/07/05, field trial program with genetically modified maize variety resistant to certain Coleopteran insects (Event MIR604 with *mcry3A*).
Duration: 3.5 years on the same site.
The purpose of the release was to investigate the potential effects of Event MIR604 maize cultivation on key non-target arthropods.

- Field trials conducted in areas where maize is not cultivated for the production of seeds
- Separation distance to closest maize field 500m;
- The trials to be surrounded by a border of no less than 6 rows of conventional maize plants that will serve as pollen trap and that will reduce edge effects.
- Release plot will be visited regularly for observations and to ensure that the appropriate action is taken to control weeds and diseases.
- The grain produced in the field trials will be harvested and destroyed.
- The season following year a crop other than maize will be planted in the field, to ensure that any maize volunteers are easily identified and destroyed. During the following season the trial site will be monitored for detection of maize volunteers. Any volunteer plants will be destroyed.

Example 4: South Africa
17/3 (4/07/156)
GM plants: Trial release of maize event Syngenta GA21
Duration: permit valid for one growing season only

- Trial site isolated from any other maize by a minimum distance of 400 m
- If trial is to be planted closer than 400 m, but at least 50 m to the nearest non-GM maize, the tassels and tillers must be removed
- If GM maize is planted within 500 m of other maize, it needs to be planted at least 4 weeks prior to or 4 weeks after such maize
- Five meter zone around trial site free of any vegetation
- After completion of the trial, the trial site should be monitored during the next growing season to ensure no volunteer maize appear
- The site must be left fallow for one year and volunteers should be checked at regular basis

It is important to emphasise the integrated nature of the risk management strategies for limiting gene flow during field trials. Because it is nearly impossible to reduce any single mechanism of gene flow to zero in practice, this redundancy ensures that possible gene flow is maintained within acceptable limits, i.e. approaching zero overall.

Conclusions and recommendations

The spread/flow of genes from GMOs is managed for two distinct reasons - to maintain the GM content of particular non-GM products below a specified threshold in commercial production systems or to contain the transgene(s) as a biosafety precaution during field trials. Cross fertilisation is the gene spreading mechanism that causes most concern to maize growers because it is the most difficult to control and depends on a multitude of factors including the characteristics of the respective maize fields, the specific varieties involved, climatic and geographical conditions. As a result the outcomes and recommendations of field and modelling studies that investigated the separation of GM and non-GM crops are highly variable. Different countries have proposed largely differing separation distances for maize in coexistence cropping systems, but most countries are currently using minimum separation distances of 25 to 200 m to ensure that the 0.9% labelling threshold of the EU is adhered to for the harvest as a whole.

South Africa does not currently enforce any coexistence cropping regulations and has recently increased the general separation distance for GM maize field trails from 400 m (based on the OECD basic hybrid seed requirements) to 650 m (EC decision). In support of science-based, efficient and cost-effective separation strategies between GM and non-GM maize we discuss the impact of spatial separation on cross pollination and integrate this into a risk assessment matrix to support the recommended in-field separation strategies we propose in Table 3.

To better understand how spatial separation impacts on cross fertilisation rates all the available data (from 21 different studies) were integrated to generate a simple equation (i.e. model) that could be used to determine the average expected cross fertilisation rate at a particular distance. As discussed earlier cross fertilisation rates are highly variable because they are influenced by many field specific parameters. It is therefore important to realise that this average does not necessarily describe a particular situation – it is actually impossible to predict an absolute accurate cross fertilisation rate even for the same field because the environmental conditions will be different in different seasons.

Despite this inherent uncertainty, integrating all the available data provides some valuable insights that could help define separation strategies for GM and non-GM maize (Fig 5; Annexure 1 contains the raw data).

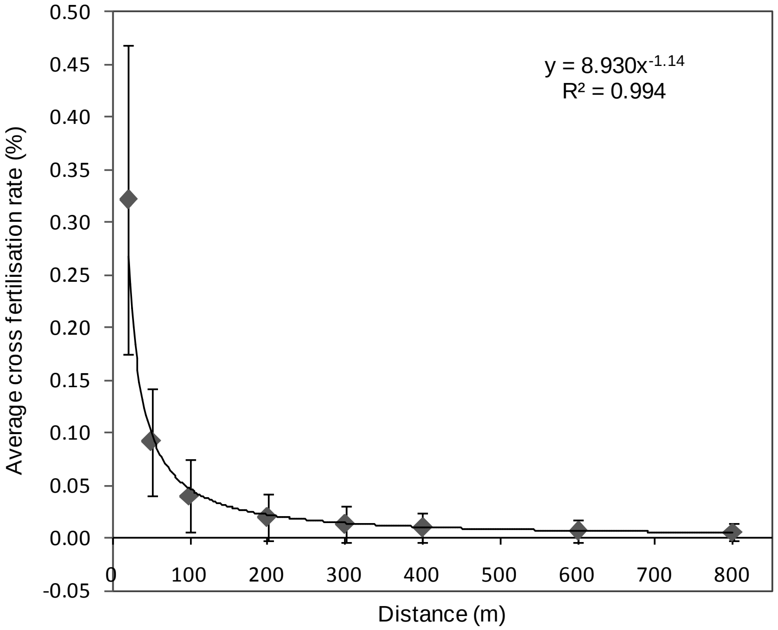

Figure 5. Average cross fertilisation rates in maize expressed as a function of the distance between the cross fertilising plants (also see Annexure 1).

- The average relationship between cross fertilisation rate (y) and distance (x) can be described by the equation $y = 8.930x^{-1.14}$ (for grain the cross fertilisation rate can be equated to GM content).
- In absolute terms the variability (indicated by standard deviation bars in Fig 5) in cross fertilisation rates (GM content) decreases over distance, e.g. at 20 and 400 m respectively the average GM content varies by 0.146 and 0.014%. This means that although cross fertilisation data is variable it can still be used with high levels of confidence at certain distances to ensure compliance with predetermined thresholds.
- The average cross fertilisation rate reaches the technical detection threshold (0.01%; Botha and Viljoen, 2009) at approximately 400 m, which means any data beyond this will either be theoretical or based on a specific experiment that does not take the whole harvest into account, i.e. very small sample sizes.
- Although the rate of cross fertilisation declines rapidly over distance, calculating an absolute zero rate is impossible, e.g. at 1,000 m the theoretical average GM content will still be 0.003%.
- Increased separation distances has a smaller impact at larger distances, e.g. while a 200 m increase between 100 and 300 m reduces cross fertilisation by 0.034% the same distance reduces it by only a tenth of that (0.003%) between 400 and 600 m.

Based on the literature and data presented here, we therefore propose the following:[2]

1) The reasons and goals for separating pre- and post-release GM maize fields from non-GM fields are different and they should therefore be considered separately.

2) In <u>pre-commercialisation field trials</u> appropriate spatial, physical and/or temporal separations strategies should be used as part of an integrated risk management program to prevent gene flow. Moreover, the extent of these containment measures should be based on both the possible fate and potential impact of the transgene if it "escapes", i.e. the outcome of a likelihood x consequence risk assessment matrix (Annexure 2). GM trails that are deemed to pose a moderate to high risk should then obviously be contained more stringently than trails with a low to negligible risk (Table 3).

   Gene flow implies not only the primary transfer of genes (cross fertilisation) but also the establishment of that gene/trait in a population. Although the natural receiving environment is routinely considered during gene flow risk assessments the details of possible receiving agricultural systems should also be considered. The nature of receiving fields could impact greatly on the possible persistence (or lack thereof) of a transgene in the particular system and should be considered accordingly when separation strategies are established (Annexure 2 and Table 3). Relative high cross fertilisation rates between different <u>contained</u> field trials with low to negligible risk traits, for example, should not be a concern because all possible hybrid seeds will be destroyed through the other management practices (i.e. gene flow stays highly unlikely). Conversely, relative low cross fertilisation rates could still establish the transgene in an agricultural system if the receiving seeds are used for propagation or breeding. Similarly, multi-seasonal field trials on a particular GM line should be limited to a single site (the same environment) to reduce possible exposure, allow the efficient integration of risk management strategies and enable accurate assessment of the crop.

   The integrated nature of a risk management program is crucial because it is practically impossible to reduce the risk of gene flow through any single strategy to "zero". Conversely, employing several containment strategies has an "accumulative" effect (redundancy) that helps ensure the effective containment of a transgene.

3) In <u>coexistence cropping systems</u> the main aim is to ensure an inclusive production system where the GM content of products (expressed as a % in weight) from closely situated non-GM fields are kept below the relevant thresholds. Separation distances should therefore be based on these thresholds for the non-GM crop/product as a whole. From the data presented above (Fig 5) it is clear that spatial separation alone will be able to ensure compliance, to these relative high threshold values, e.g. 0.9% as set by the EU, in most coexistence cropping systems. In only a few particular cases it might be necessary to include other separation strategies (Table 3).

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[2] *Although specific and objective data can be obtained to, for example, describe the correlation between cross fertilisation rates and distance, the choice of how to integrate this into a risk management program is still <u>subjective</u>. I.e., what does "safe" mean? A 1:10,000 or 1:1,000,000 or "NO" chance of an event occurring...? It is therefore important to realise that the <u>principles</u> described here are always applicable and should be applied consistently even if a more "liberal" or "conservative" safety stance is assumed.*

The only non-market related exceptions where more stringent separation strategies should be employed are instances where gene flow should be managed to ensure the sustainability of a particular GM trait, e.g. insect resistance in open pollinated varieties where grain is kept as seed (Table 3).

If the need ever arises to introduce a formal coexistence cropping system into South Africa only a few, specific, but generally applicable in-field separation strategies could be implemented to ensure a simple, yet efficient management system for the regulation of gene flow (Table 3).

Because temporal separation strategies are severely limited by a multitude of practical considerations it should not be included as a standard biological containment strategy in large production systems before its viability has been established under local conditions.

Table 3. Possible in-field separation strategies between GM and non-GM maize released into the environment in South Africa. Proposed separation distances are based on the estimated risk associated with a particular transgene and the fate of possible hybrid seeds in the receiving field.

Situation	**Proposed separation distance**	**Notes**
Pre-commercialisation field trials		
Transgenes with “negligible” estimated risk	100 m	From maize fields where grain is destined to be destroyed, e.g. commercial grain production. Based on this separation distance only it will result in a theoretical cross fertilisation rate of ~0.05% or 5 seeds in 10,000 at this minimum distance. Combined with the dilution effect of the whole receiving field and the fact that possible hybrid seeds/plants will be destroyed (assume 99.999% efficient) gene flow will be unlikely (≤0.00005%, i.e. less than 1 in 2 million).
Transgenes with “low” estimated risk	400 m	From maize fields where grain is destined to be destroyed, e.g. commercial grain production. Based on this separation distance only it will result in a theoretical cross fertilisation rate of ~0.01% or 1 seed in 10,000 at this minimum distance. Combined with the dilution effect of the whole receiving field and the fact that possible hybrid seeds/plants will be destroyed (assume 99.999% efficient) gene flow will be highly unlikely (≤0.00001%, i.e. less than 1 in 10 million).
Transgenes with “moderate” to “high” estimated risk	1000 m	From maize fields where grain is destined to be destroyed, e.g. commercial grain production. Based on this separation distance only it will result in a theoretical cross fertilisation rate of ~0.003% or 3 seeds in 100,000 at this minimum distance. Combined with the dilution effect of the whole receiving field and the fact that possible hybrid seeds/plants will be destroyed (assume 99.999% efficient) gene flow will be highly unlikely (≤0.000003%, i.e. less than 1 in 33 million). Could also be combined with temporal separation (especially varietal), border rows and other risk management practices depending on the potential risks associated with the particular transgene.

Distance between two different contained field trails	10m	Because both sites will be subjected to integrated risk management programs to limit gene flow, spatial separation to limit cross fertilisation is not crucial. In this case spatial separation should rather be based on the requirements of the planned trials/experiments.
Distance from any breeding programs or open pollinated varieties where the grain is reused as seed, e.g. subsistence farming	1000 m	Based on this separation distance only it will result in a theoretical cross fertilisation rate of ~0.003% or 3 seeds in 100,000 at this minimum distance. Should also be combined with temporal separation (especially varietal), border rows and other risk management practices irrespective of the potential risks associated with the particular transgene. If the separation distance is >1 km but <3 km the OPV population should also be monitored for the presence of the transgene.
Coexistence cropping systems, i.e. post-general release		
Silage production	50 m	No temporal or other physical separation required. Should result in a theoretical GM content (TGMC) of ~0.031% (w/w).[1]
Grain production	200 m	No temporal separation required. Under extreme conditions, e.g. small non-GM and large GM fields, border rows could be incorporated in GM field. TGMC of ~0.021% (w/w).
Seed production	300 m	No temporal separation required. Border rows as required by seed producer. TGMC of ~0.013% (w/w).
Organic production	300 m	No temporal separation required. Under extreme conditions, e.g. small organic and large GM fields, border rows could be incorporated in GM field. TGMC of ~0.013% (w/w).
Open pollinated varieties where gene flow could impact on the long term viability of the GM trait, e.g. insect resistance.	400 m	No temporal or other physical separation required. TGMC of ~0.010% (w/w).

[1] TGMC was calculated using the average value of the regression analyses of 21 different studies looking at the relationship between cross-fertilisation and distance only (Fig 5 and Annexure 1).

Establishing specific separation strategies based on the above principles would facilitate the easy incorporation of possible future activities such as the use of maize as bio-factories for pharmaceutical and/or industrial compounds. Dedicated research to support these proposed strategies in the South African agricultural context is of crucial importance.

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Annexure 1: GM content calculator

Ref 1		Ref 2		Ref 3		Ref 4		
Sanvido et al, 2008		Reuter et al, 2008		Chetty and Viljoen, 2008		Messeguer et al, 2006		
%GM=3.193*(SD^-0.78)		**%GM=9.98/(SD^1.19).**		**%GM=153.12*(SD^-1.8961)**		**%GM=(FS/((SD/10)^2))*0.069**		
Sep Dist (m)	**GM content (%)**	**Sep Dist (m)**	**GM content (%)**	**Sep Dist (m)**	**GM content (%)**	**Sep Dist (m)**	**Flower sync (days)**	**GM content (%)**
20	0.3086	20	0.2824	20	0.5226	20	10	0.1725
50	0.1510	50	0.0949	50	0.0920	50	10	0.0276
100	0.0879	100	0.0416	100	0.0247	100	10	0.0069
200	0.0512	200	0.0182	200	0.0066	200	10	0.0017
300	0.0373	300	0.0113	300	0.0031	300	10	0.0008
400	0.0298	400	0.0080	400	0.0018	400	10	0.0004
600	0.0217	600	0.0049	600	0.0008	600	10	0.0002
800	0.0174	800	0.0035	800	0.0005	800	10	0.0001

Theoretical Average GM Content (%)		
Grain all	**StDev**	**Silage at 30% (w/w)**
0.3215	0.1464	0.0965
0.0914	0.0505	0.0274
0.0403	0.0348	0.0121
0.0195	0.0223	0.0058
0.0131	0.0168	0.0039
0.0100	0.0136	0.0030
0.0069	0.0101	0.0021
0.0054	0.0081	0.0016

Predicted TGMC[#] based on		
%GM=8.93*(SD^-1.14)		
Sep Dist (m)	**TGMC[#] (%)**	**% gain between 2 consecutive distances**
20	0.2935	
50	0.1033	0.1903
100	0.0469	0.0564
200	0.0213	0.0256
300	0.0134	0.0079
400	0.0096	0.0037
500	0.0075	0.0022
600	0.0061	0.0014
800	0.0044	0.0017
1000	0.0034	0.0010

[#] Theoretical GM content for grain

Annexure 2: Risk assessment matrix

To determine the extent of in-field containment measures employed during a field trail the potential risk associated with gene flow should be estimated. This risk estimate is the product of the likelihood of gene flow occurring and the potential consequence of that and can be based on a risk assessment matrix (Fig 6 and Table 4).

		RISK ESTIMATE			
LIKELIHOOD ASSESSMENT	**Highly likely**	Low	Moderate	High	High
	Likely	Low	Low	Moderate	High
	Unlikely	Negligible	Low	Moderate	Moderate
	Highly unlikely	Negligible	Negligible	Low	Moderate
		Marginal	**Minor**	**Intermediate**	**Major**
		CONSEQUENCE ASSESSMENT			

Figure 6. Risk assessment matrix (from Risk Analysis Framework, OGTR, Australia).

Table 4. Scales for assessing likelihood and consequence assessments (adapted from Risk Analysis Framework, OGTR, Australia).

Likelihood	**Likelihood assessment definition**
Highly unlikely	May only occur in very rare circumstances ($\leq 10^{-4}$% or less and equal to 1 in a million)
Unlikely	Could occur in some circumstances ($\leq 10^{-2}$% or less and equal to 1 in 10,000)
Likely	Could occur in many circumstances (<1% or less than 1 in 100)
Highly likely	Is expected to occur in most circumstances (≥1% or greater and equal to 1 in 100)
Consequence*	**Consequence assessment definition (related to human health and environment)**
Marginal	No or minimal adverse health effects or damage/disruption to the environment
Minor	Adverse but limited and reversible health effects or damage/disruption to the environment that is reversible and limited in time, space and numbers affected
Intermediate	Adverse, widespread and not readily reversible health effects or widespread damage/disruption to the environment that is of limited severity and reversible
Major	Adverse, severe, widespread and irreversible health effects or extensive damage/disruption to whole natural ecosystems, communities or species that persists over time and is not readily reversible

*In SA economic considerations should be formally incorporated into the likely consequence.

When considering the likelihood of an event/risk the whole process/system should be considered and not only one of the likely mechanisms. Gene flow, for example, implies not only the primary transfer of genes (fertilisation) but also the establishment of that gene/trait in a population. Gene flow from a GM crop to sexually compatible wild relatives in its close proximity would therefore probably be "highly likely" (if no genetic use restriction technologies [GURTs] are used) because the resulting wild individuals might be able to establish themselves in the environment and proliferate (especially if the new trait imparts a selective advantage). Although maize does not have wild relatives in South Africa gene flow to "other maize" could still be considered a risk and should therefore be evaluated. If the whole system is considered again it is clear that it is not only the characteristics of the GMO, but also the nature/fate of the receiving maize that impact on the likelihood of gene flow.

Example 1: When a nearby receiving field is well managed as a contained unit, e.g. another GM trail, the likelihood of gene flow is still "highly unlikely" even though cross fertilisation rates might be high because of a small spatial separation.

Example 2: If non-GM grain maize (e.g. commercial field) is fertilised by GM pollen the resulting hybrid seed(s) are likely to be destroyed through processing or if it germinates as a volunteer through general field management practices. Combined with low cross fertilisation rates (ensured for example through adequate separation strategies) and the inability of maize to establish itself in the wild, gene flow will therefore be "highly unlikely" in this case. *(Note that this should be distinguished from the GM content of the receiving grain.)*

Example 3 (a worst case scenario): The only way in which an unwanted gene can "establish" itself in maize in South Africa is if it is unintentionally incorporated into a commercial breeding program where it will be proliferated through consecutive generations or a commercial hybrid. Because of the likelihood of this potential mechanism of gene flow the other risk management strategies in an integrated program should be improved accordingly, e.g. larger separation distances between GM and breeding trails, to ensure the overall likelihood of gene flow is still highly unlikely.

The possible consequences of a particular gene/trait in a particular organism/crop in a particular environment are fairly fixed and are therefore designed to be within acceptable limits early in a research and development program. Changing the likelihood of an event occurring is therefore the only practical mechanism through which potential risks associated with a field trail can be modulated. From the examples discussed above it is clear that the risk assessment matrix could be used as an iterative tool to help establish and integrate the different strategies of a risk management program.