Genetically modified maize: pollen movement and crop coexistence

Ву

Graham Brookes & Peter Barfoot (PG Economics Ltd, UK)

Enric Melé & Joaquima Messeguer (Institut de Recerca I Tecnologia Agroalimentàries, Spain)

Florence Bénétrix, Daniel Bloc & Xavier Foueillassar (Arvalis, France)

Adeline Fabié & Cedric Poeydomenge (Maiz Europ, France)

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1 Introduction

Genetically modified (GM) maize was planted on a total of 15.5 million hectares in 2003 (11% of the global maize area). In the EU, GM maize¹ is the only GM crop currently grown commercially. In Spain, the main location where the crop has been planted since 1998, it accounted for about 7% of the total maize area in 2003 (31,000 hectares). Estimates for 2004 plantings are 60,000 hectares².

Against this background, one of the main subjects of current debate about the use of GM crops such as maize relates to the economic and market implications of GM and non GM crops being grown in close proximity (ie, co-existing). Within this co-existence debate, it is often claimed that GM and conventional (including organic) crops cannot co-exist without causing significant economic harm/losses to conventional and organic growers.

This paper³ examines these issues, with specific reference to maize and the extent to which adventitious presence of GM maize may be detected in non GM maize crops through maize pollen movement and gene flow.

The paper, after this introduction is structured as follows:

- Section 2: What is co-existence and the role of pollen movement and gene flow;
- Section 3: Adventitious presence arising from cross-pollination in maize: a review of literature and experience;
- Section 4: Conclusions.

2 GM pollen and crop co-existence

2.1 What is co-existence?

Co-existence as an issue relates to 'the economic consequences of adventitious presence of material from one crop in another and the principle that farmers should be able to cultivate freely the agricultural crops they choose, be it GM crops, conventional or organic crops'⁴. The issue is, therefore, not about product/crop safety⁵, but relates solely to the production and marketing of crops approved for use.

Adventitious⁶ presence of GM crops in non-GM crops becomes an issue where consumers demand products that do not contain, or are not derived from GM crops.

The main legal requirement in the EU of relevance to the planting of GM maize is the labelling requirements for products containing or derived from GMOs⁷. These set the

¹ Insect resistant (Bt)

² Source: Spanish maize growers association (AGPME)

³ The authors acknowledge that Agricultural Biotechnology in Europe (ABE) have given their support to the study. The material presented in this paper is, however the independent views of the authors

⁴ Source: European Commission 2003

⁵ Commercially grown GM crops having obtained full regulatory approval for variety purity, use in livestock feed, human health and safety for the environment. The issue of environmental liability (sometimes confused with economic liability) is addressed through the regulatory approval process

⁶ Or sometimes referred to as technically unavoidable

⁷ Regulation EC 1829/2003 on GM food and feed

adventitious presence threshold for positive labelling of food and feed products containing or derived from GM crops at 0.9%.

2.2 How can adventitious presence occur?

Adventitious presence of unwanted material can arise for a variety of reasons. These include, seed impurities, cross-pollination, volunteers (self sown plants derived from seed from a previous crop), and may be linked to seed planting equipment and practices, harvesting and storage practices on-farm, transport, storage and processing post farm gate.

Recognising this, almost all traded agricultural commodities accept some degree of adventitious presence of unwanted material and hence have thresholds set for the presence of unwanted material. For example, in most cereals, the maximum threshold for the presence of unwanted material (eg, plant material, weeds, dirt, stones, seeds of other crop species) commonly used is 2%.

In the European context, the labelling threshold of 0.9% for positive GM labelling has become the main benchmark for determining the maximum level of adventitious presence of GM material that is allowed in non GM products⁸. This threshold is considerably more onerous than, for example, Japan or Korea where the threshold is 5% and 3% respectively.

2.3 Adventitious presence in maize

As indicated above, adventitious presence of GM material in non GM crops can occur for a variety of reasons. In the case of maize there are three main potential routes through which adventitious presence may occur.

2.3.1 Cross-pollination

The importance of pollen movement (and the principles behind separation distances) and possibilities of adventitious presence occurring due to cross-pollination are well researched and documented (Ingram 2000):

- Pollen availability and transmission. The chances of pollen from a GM crop pollinating with a non GM crop (ie, introgressing) is a function of the availability and viability of pollen emitted from the GM crop and its delivery to the stigma of a non GM plant. Availability of pollen from one variety to another varies due to planting date and agronomic conditions. The delivery of pollen is influenced by factors such as wind speed and direction, presence of insect vectors to deliver the pollen, distance, rainfall and barriers to pollen movement (eg, barrier rows, trees, hedges and topography);
- Degree of cross-pollination. The efficiency of pollen from a GM plant cross-pollinating with the non GM plant when it lands on the stigma depends upon a combinations of factors; timing of flowering of the receptor (non GM) crop needs to coincide with the GM crop; the GM pollen must still be viable for fertilisation; and the foreign pollen has to compete with fresher pollen produced by the non GM plant itself and/or pollen from other non GM plants in the vicinity; Factors affecting gene expression in the receptor plant. After cross-pollination the

Factors affecting gene expression in the receptor plant. After cross-pollination the genetic material is incorporated into the seed and may influence the characteristics of the resulting seed crop. It does not impact on the integrity of the parts of the non GM

⁸ Some operators, in some markets, may operate to tighter thresholds but the 0.9% represents the only current legally enforceable threshold

plant, other than the seed (eg, in the case of maize, any introgression of GM material will show up only in specific kernels of the cob and will not be present in the rest of the plant);

Inheritance considerations. Depending on the characteristics of the gene (dominance pattern and level of heterosis of the commercial hybrid) less than 100% of the emitter (GM plant derived) pollen will contain the GM trait. Therefore a part of the pollen produced will **not** contain the GM trait (eg, for Mon 810 insect resistant (Bt) maize varieties, only 50% of the emitted pollen contains the Bt trait).

As indicated above, the chance of cross-pollination occurring depends upon the availability and viability of maize pollen to introgress with the receptor maize plant. Maize pollen is released in very large quantities, between 4.5 and 25 million pollen grains per plant (Paterniani & Stort, 1974) over a typical 5-8 day period. It remains viable under natural conditions for about 24 hours, although this can fall to only a few hours in hot, dry weather or, extend to up to 9 days in cooler, humid conditions (Emberlin, 1999). Compared with other crop species that rely on the wind to disperse pollen across large distances, maize pollen grains are relatively large (90-125 μ m) and heavy, with a high terminal velocity resulting in higher comparative deposition (ie, it falls to the ground rapidly in a limited area and does not travel far⁹). Maize produces pollen before silk emergence ensuring that the majority of maize plants cross-pollinate with a neighbouring maize plant. However, an overlap between pollen shedding and silk emergence can occur and up to 5% self-pollination may occur.

The extent to which cross-pollination in maize crops causes problems of adventitious presence of unwanted material (together with measures to minimise this occurring) are discussed further in section 3 below.

2.3.2 Seed mediated pollen flow (ie, volunteers)

Volunteers (self sown plants derived from seed of a previous crop) are not an important media for gene flow in maize. The probability of a volunteer maize crop appearing in subsequent (maize) crops and then contributing to gene flow via cross pollination from the volunteer to a maize crop is very low due to the inability of the maize plant to shed seed naturally, a limited dormancy period, the common use of mechanical pre-planting soil preparation practices and the inability of maize seed to survive low winter temperatures that often occur in many (continental) maize growing regions. Even in regions where winter temperatures do not usually fall to low enough levels to kill off volunteers (eg, Mediterranean countries), they are not considered to be problematic for growers.

2.3.3 Other possible sources of adventitious presence in maize

Seed purity can affect the levels of adventitious presence, indeed the higher the purity level, the lower the 'knock-on' level in the final product¹⁰. A few instances have arisen in recent years where adventitious presence of GM material has been found in some non GM maize seed. In 2000, for example, some maize seed lots imported into France from North America were found to have low levels of GMO presence (under 0.2%).

On and off farm storage and handling of seed and crops post harvest also represent possible opportunities for adventitious presence of GM material being found in non GM maize crops, especially if crops from GM and non GM growing farms are dried, cleaned and stored in central (often co-operative) facilities. This has, however not been a problem in countries

⁹ Defra (2003) Review and knowledge of the potential impacts of GMOs on organic agriculture

¹⁰ In the absence of EU legislation for labelling of seed for GM adventitious presence, the EU seed industry operates to a threshold of 0.5% (ie, non GM maize seed will have less than 0.5% GM adventitious presence)

where GM maize is currently grown (eg, the USA, Spain). It is also not expected to be an important source of possible adventitious presence occurring across other parts of the EU if GM maize is more widely grown because of increasing farm-level experience of undertaking practices to minimise adventitious ad-mixing of arable crops (eg, by keeping crops and specific varieties segregated) in order to meet buyer (contractual) conditions, to derive price premia attached to produce meeting specific quality standards (eg, the supply of waxy maize to the starch industry) and/or as members of quality assurance schemes.

3 Adventitious presence in maize from crosspollination: review of literature and experience

3.1 General studies of pollen flow and cross-pollination in maize

A review of literature¹¹ into the <u>general</u> dynamics of pollen flow and cross-pollination in maize shows the following key points:

a) Pollen dispersal

Most maize pollen falls within 5 metres of the field edge (Sears and Stanley-Horn, 2000, Pleasants *et al*, 1999). In the Sears and Stanley-Horn study of seven different Bt maize fields 84% to 92% of pollen fell within 5 metres and between 96% to 99% of pollen remained within a 25-50 metre radius of the maize fields (Figure 1). All pollen was deposited within 100 metres. Other studies have also analysed the influence of size and shapes of fields, wind speeds and direction, and environmental conditions (Klein et al, 1998). Large rectangular fields result in pollen travelling further than small circular fields due to the higher concentration of pollen in the atmosphere at a given time. Also, the "depth of a field" and the direction of the wind is far more important than total area planted.



Figure 1: Cumulative % of pollen deposition of various distances from 7 Bt maize fields in Ontario

Source: Sears & Stanley-Horn, 2000

¹¹ Many of the references cited in this section are drawn from the literature review by Eastham K & Sweet J (2002) GMOs: the significance of gene flow through pollen transfer, European Environment Agency

b) Pollen viability

As discussed above, although pollen may be dispersed, it must be viable, land on the stigma of the receptor plant and compete with other viable pollen to be able to cross-pollinate (introgress):

- Jones and Brooks (1950) measured the percentage of outcrossing between large blocks of emitter and receptor crops over a three year period. The average level of cross-pollination in rows immediately adjacent to the crop were found to be 25.4% falling to 1.6% at 200 metres and 0.2% at 500 metres;
- Salamov (1940¹²) found cross-pollination levels of 3.3% at 10 metres from the pollen source, 0.5% at 200 metres, 0.8% at 600 metres and 0.2% at 800 metres;
- > Jugenheimer (1976) found levels of cross-pollination of 4.5% at 3 metres;
- ▶ Burris (2001) found cross-pollination of 1.11% at 200 metres;
- Baltazar & Schoper (2002) identified no out-crossing beyond 200 metres in very dry and calm conditions;
- In Bateman (1947), cross-pollination levels fell from 40% at 2.5 metres to approximately 1% at 20 metres;
- Messean (1999) measured 1% cross-pollination at a separation distance of 25-40 metres;
- Simpson (1999) found 1% cross-pollination at 18 metres from the pollen source;
- Loubet and Foueillassar (2003) showed that the fertilisation capacity (% of pollen grains able to fertilise) decreases with distance from the source: 4%-12 % at 100 metres and 2%-7 % at 250 metres. This work also identified that the lightest pollen grains are the least viable, yet travel the longest distances and pollen placed in a air flow (humidity 70 %) dies within 2 hours at a temperature of 20 °C or within 1 hour at a temperature of 30°C.

c) Disruption of pollen dispersal and viability

Studies have shown the following:

- Jones and Brooks (1952) experimented with barriers to cross-pollination and found a single row of trees and under bush reduced out-crossing by 50% immediately behind the barrier. The reduction was even greater when an intervening crop was used (it provides competing pollen) and when open ground or low growing barrier crops exist to isolate maize crops, it appears that the first few rows intercept a high proportion of the pollen, so that cross-pollination levels are highest in these rows and then decrease exponentially with distance;
- Outcrossing rates tend to be higher at field edges than within a maize field of comparable size. Therefore, the use of mechanical barriers (like hedges, a line of trees) is only effective if established around a recipient field (Meir-Bethke & Schiemann 2003)
- A study by Foueillassar & Fabie (2002) evaluated the level of cross-pollination between two maize crops (waxy and conventional dent maize) under conditions suitable for maximising the chances of cross-pollination occurring. Thus, the flowering times were the same in both crops (waxy and dent), there was minimal isolation distance between crops, a single source of competing pollen from the dent maize and a long border existed between the fields to increase the likelihood of cross-pollination. The research was also undertaken in a variety of field sizes (0.7 ha to 13 ha) so that a reasonable spread of farming conditions were simulated. The data showed that the level of cross-pollination between the crops across entire adjacent fields was under 0.9%. The highest level of cross-pollination in border rows was

¹² Levels of outcrossing in the immediate vicinity are lower than Jones and Brooks (1950) due to the placement of the Salamov's traps on the windward side (Treu & Emberlin, 2000)

6.2% (where the isolation distance was zero, the wind direction was from the dent to the waxy maize and the area of dent maize was several times the waxy maize along a relative long border length). The lowest level of cross-pollination in a border row was 0.43% (where the dent maize flowered 2 days earlier than the waxy maize and the crop areas where similar).

Overall, the key findings from studies into maize pollen dispersal and viability are summarised in Table 1. The reader should note that the levels of cross pollination at specified distances, cited in the table, overstate the likely levels of cross pollination of maize containing a single GM trait such as the Bt traits, Mon 810 or Bt 176. In the conventional maize studies, 100% of emitted pollen is of relevance to cross pollination levels, whilst in the case of GM (Bt) maize, only 50% of emitted pollen is of relevance (only 50% of emitted pollen contains the Bt trait). Thus, for example, when 99% of cross pollination in conventional crops is reported to occur within 18-20 metres of an emitter field border, for a GM maize containing a single trait such as Mon 810, the level of cross pollination carrying the GM trait is likely to occur at a reduced ratio. Specific research relating to cross pollination levels for GM maize crops in the EU (that take this factor into consideration) are summarised in section 3.2 below.

Issue	Most common findings
Pollen dispersal	98% of pollen is deposited within 25 metres of the emitter field, almost 100% within 100 metres
Cross pollination	99% of the cross-pollination that occurs outside the emitter field takes place within 18-20 metres of the emitter field borders
Influence of weather	Weather can influence pollen dispersal and cross-pollination: some studies show slightly higher levels of pollen dispersal and outcrossing at the 20-25 metre distance (eg, receptor crop downwind of emitter crop)
Influence of barriers	Physical barriers (eg, trees, hedges) can affect pollen dispersal and cross- pollination. Impact varies according to location of barrier to receptor crop. Barriers located immediately before a receptor crop tend to reduce cross pollination levels.
	If the barrier comprises rows of maize between emitter (eg, a GM crop) and receptor (eg, non GM) maize crops, this acts as a buffer, reducing levels of cross-pollination. One buffer row is roughly equal to 10 metres of separation.

 Table 1: Maize pollen dispersal, viability and cross-pollination in <u>conventional</u> maize crops: summary of research findings

d) The specific case of hybrid maize seed production

To understand the impact of adventitious presence in maize caused by cross-pollination, it is important for readers to differentiate between <u>seed</u> and <u>grain</u> production. The primary difference between the two types of production relates to the amount of pollen present carrying the trait of interest.

Hybrid seeds are intended for planting and are produced by crossing two parental maize lines carrying selected traits. Fields for the production of hybrid seed usually contain rows of pollen-producing (male) plants alternating with rows of sterile or detasseled (female) plants acting as pollen receptors. Depending on the planting pattern¹³, as much as 80% of the plants in a field (the detasseled female plants) do not produce pollen. As a consequence, they are highly receptive to both the pollen from the male parent but also to 'adventitious pollen'

¹³ The most common planting pattern in maize seed fields is one row of pollen parent to four rows of seed parent (other patterns include a 1:2:1:4 (1 row pollen parent, 2 rows seed parent, 1 row pollen parent and 4 rows seed parent) and a 2:6 pattern). It is also common practice to destroy the pollen parent after pollination is complete by cutting down to prevent grain formation and possible seed contamination at harvest

carried in from neighbouring fields by the wind. Also, because of inbred depression, the male parent plants usually produce less pollen then other maize and very often this (pollen production) lacks timing synchrony with female plant maturity. In order to ensure a high degree of purity of the hybrid seed (usually 99.5%), strict growing conditions are respected. These include, for example large separation distances from neighbouring fields (eg, 200 metres).

In contrast, maize grain is grown for direct use (food, feed or industrial purposes) and fields contain 100% fertile parent plants. The amount of pollen present and its competitiveness are much higher than in seed production fields, so that the influence of adventitious pollen from neighbouring fields is smaller. Therefore, maintaining a degree of purity in a grain maize field in situations where this is desired (eg, a non GM maize crop located near a GM maize crop) requires less strict measures than in the case of seed production¹⁴.

3.2 Specific studies examining cross-pollination between GM and non GM maize crops in Europe

This section summarises the findings of GM-specific research into cross pollination of maize.

3.2.1 Melé et al (2004)

This study commissioned by the regional government of Catalunya (Spain), was conducted by IRTA in collaboration with Syngenta Seeds, at Lleida, Catalunya. A 50 x 50 metre plot of GM insect resistant (Bt) maize (of the variety Compa CB (Bt 176) from Syngenta Seeds) was planted in the middle of a field and surrounded by plantings of the non GM maize variety 'Brasco'. The total area of the trial was 7.5 hectares. It was located in a flat region where the normal growing conditions are dry and accompanied by high temperatures. Data collected from the local weather station found that there were two prevailing winds during flowering time; from the west in the morning and then from the south after midday.

Samples from the non GM maize fields (three cobs per sample) were taken at the end of September. Six samples were taken at distances of 1, 2, 5 and 10 metres from each side of the central GM crop square. The rest of the non GM field was divided into squares of 30 x 30 metres and from each one of these squares a further sample was taken. In total, 255 samples were taken and analysed with the RT-PCR technique (for Zein and Bt-176 genes) to establish the level of GM adventitious presence (measured as the ratio of transgenic DNA to the total DNA).

The research found that:

- The level of GM adventitious presence from gene flow found in the non GM maize crop decreased rapidly with distance from the GM emitter crop;
- the level of detected GM adventitious presence in the non GM crop located downwind of the GM emitter crop was less than 0.9% at a distance of 10 metres (from the GM emitter crop). The level of detected GM adventitious presence in the non GM crop located upwind of the GM emitter crop was less than 0.9% at a distance of 2 metres (from the GM emitter crop).

These results were then used to estimate the likely levels of GM adventitious presence in non GM maize fields of different sizes and distances downwind from a GM emitter crop. The

¹⁴ See for example, Burris (2003) Adventitious pollen intrusion into hybrid maize seed production fields, American Seed Trade Association

level of GM adventitious presence likely to be found in non GM maize crops (1 hectare in size) planted adjacent to a GM plot is an average of 0.83% (measured for the total harvest in the 1 hectare plot¹⁵). The level of GM adventitious presence likely to be found in non GM maize crops (0.25 hectares) planted adjacent to a GM plot is an average of 1.77% (for the total harvest of the 0.25 hectare plot). This would fall to 0.77% when a 6 metre separation distance is maintained between the GM and non GM crops.

The key conclusions that can be drawn from the research are:

- When non GM maize is planted in fields of over 1 hectare in size near to GM maize crop, normal harvesting practices (where adventitious presence levels of GM material in non GM maize are tested at the field level, post-harvest) should be sufficient to ensure that levels of GM adventitious presence in non GM crops are below the 0.9% EU labelling threshold;
- When non GM maize is planted in fields smaller than 1 hectare in size near to GM maize crop, the operation of a 6 metre separation distance between the crops (GM and non GM) and implementation of normal harvesting practices (where adventitious presence levels of GM material in non GM maize are tested at the field level, post-harvest) should be sufficient to ensure that levels of GM adventitious presence in non GM crops are below the 0.9% EU labelling threshold.

3.2.2 APROSE (2003/4)

This unpublished study was commissioned by Monsanto, Nickersons South and Pioneer Hi-Bred, and presented to the Spanish Biovigilence Committee in February 2004. Grain samples were taken at 14 commercial field sites in the Spanish provinces of Huesca, Lleida, Zaragossa and Navarra, to examine cross-pollination between GM (Bt) maize and conventional (neighbouring) maize. The samples were analysed for presence of the Bt gene (coming from both the Mon 810 and CG176 sources) using the real time PCR test¹⁶. Details of the results are shown in Table 2. The research explored cross-pollination levels between fields of differing sizes and when GM and conventional crops had different planting times. Key findings were:

- There was a clear decrease in cross-pollination levels with distance from the GM crop field;
- Crops with the same planting date: an average of 16.93% of grains in samples showed some level of cross-pollination in the nearest (adjacent) row of conventional maize (average separation distance of 0.95 metres), the corresponding proportion of cobs in samples showing some level of cross-pollination were 2.73% at the fourth row (3 metres average separation), 1.18% at row eight (6 metres average separation) and 1.02% at row sixteen (12 metres average separation);
- Crops with up to 10 days difference (an average of 7 days difference) between the planting date: an average of 7% of grains tested in the nearest (adjacent) row of

¹⁵ When crops like maize are harvested, production from each row in a field typically becomes mixed inside the combine and hence if a few border rows in the field had higher levels of adventitious presence than other rows in the field, the average level across the field will be lower than the levels found in the border rows. This therefore highlights an important point of difference between levels of adventitious presence recorded at the sample level (eg, taken in different rows in a field prior to harvest) in some research trials and levels of adventitious presence found under normal crop production conditions, where tests would be typically undertaken at the field level, in the post harvest crop

¹⁶ The reader should note that in this study and the Bénétrix & Bloc study (section 3.2.3), the tests for GM adventitious presence in the non GM maize relate to % adventitious presence per grain sample. This contrasts with the Melé et al study (section 3.2.1) which reported levels of adventitious presence in terms of % DNA

conventional maize (average separation distance of 5 metres) registered some level of GM adventitious presence. The proportion of grains sampled in other rows further away from a GM source registering some level of GM adventitious presence were 0.97% at the fourth row (average of 7.25 metres separation), 0.63% at row eight (average of 10.25 metres separation) and 0.6% row sixteen (an average of 16.25 metres separation);

Crops with over 10 days difference (an average of 22 days difference) between the planting date: an average of 3.56% of grains sampled in the nearest (adjacent) row of conventional maize (separation distance of 1.7 metres) registered some level of GM adventitious presence. The proportion of grains sampled in other rows registering GM adventitous levels were 0.84% at the fourth row (an average of 3.95 metres separation), 0.56% at row eight (an average of 6.95 metres separation) and 0.26% at row sixteen (an average of 12.95 metres separation).

Overall, the research confirmed that cross-pollination levels decline with distance from the pollen source and that differences in planting times between emitter (GM) crop and recipient (non GM) crop can also have a limited impact on cross-pollination levels.

As a result of this research, and the IRTA research (see 3.2.1 above), the GM Bt technologyproviding companies agreed a number of recommendations for GM crop stewardship (see section 3.3). In relation to measures for minimising the scope for adventitious presence of GM maize being found in nearby non GM maize crops through cross-pollination, the GM crop stewardship recommendations included if a neighbouring non GM maize field is closer than 25 metres (to the GM maize) and smaller than 1 hectare, four buffer rows of non GM maize should be planted between the two crops in the border of the GM field adjacent to the neighbours non GM maize crop. This crop should be harvested and labelled as GM.

	Same planting date	Up to 10 days difference in planting dates (average 7 days)	More than 10 days planting difference (average 22 days)
Average area of GM (Bt) crop (hectares)	1.28	1.5	6.1
Average distance to 1 st non GM row (metres)	0.95	5	1.7
Average % of grains samples with GM adv presence in 1st NGM row (separation distance: see above)	16.93	7	3.56
Average % of grains sampled with GM adv presence in 4th NGM row (separation distance = distance to 1^{st} row +2.25 metres)	2.73	0.97	0.84
Average % of grains sampled with GM adv presence in 8th NGM row (separation distance = distance to 1^{st} row + 5.25 metres)	1.18	0.63	0.56
Average % of grains sampled with GM adv presence in 16th NGM row (separation distance = distance to first row + 11.25 metres)	1.02	0.6	0.26
Source: APROSE 2003/4			

 Table 2: Results from the APROSE GM and conventional maize cross-pollination study

 2003

3.2.3 Bénétrix and Bloc (France) 2002 & 2003

This research was undertaken to contribute to the development of co-existence guidelines/recommendations. It took place in three locations across France, where GM (Bt) maize was simultaneously planted directly next to non-GM maize and the level of cross-pollination measured over a distance of up to 240 metres.

All of the conditions for the research were designed to assess the worst-case scenario (varieties planted at the same time and with the same flowering time, no use of buffer crops or separation distances and the non GM crop planted downwind of the GM crop), so as to contribute towards the identification of robust co-existence measures. Essentially, the research focused on evaluating the impact of the direction and strength of the wind on the distance of pollen movement and level of introgression. Its key findings were:

At the sample level (ie, testing undertaken from samples taken from the in-field crops):

- ▶ 98% of pollen travelled no further than 10 metres;
- Where the prevailing wind at time of flowering was blowing from the GM crop towards a non GM crop, the level of GM adventitious presence found in the non GM crop was less than 1% beyond a distance of 10-12 metres;
- Where the prevailing wind at time of flowering was blowing from the non GM crop towards a GM crop, the level of GM adventitious presence found in the non GM crop was less than 1% beyond a distance of 5-7 metres;
- In strong wind conditions (blowing from the GM towards the non GM crop), the level of adventitious presence was over 1% up to a distance of 25 metres.

At the field level (ie, testing undertaken from post-harvest crops at the field level (normal harvest practice)):

- levels of GM adventitious presence in excess of the 0.9% EU labelling threshold were only found in border rows of the non GM maize closest to the GM emitter crop;
- the implementation of good farming practice (including on-farm segregation of crops) and normal harvesting practices is usually sufficient to ensure that GM adventitious presence levels in non GM maize are below the 0.9% EU labelling threshold. This applies even under 'worst case' conditions (eg, no separation distances, non GM crops being downwind of the GM crop, no use of buffer crops).

3.2.4 Henry et al (UK) 2003

This research explored gene flow from GM to non GM forage maize over the three year period of 2000-2002. Plots of GM herbicide tolerant maize were planted adjacent to non GM plots. Fifty five sites were included, from which cobs at 1,152 sample points were taken and tested (each sample consisted of 3-5 cobs, or in excess of 1,000 grains). The key findings of the research were:

- There is a rapid decrease in the rate of cross-pollination with increasing distance from the GM crop within the first 20 metres from the donor crop and beyond this distance the rate of decrease slows;
- Evidence of very limited cross-pollination was found up to 200 metres away from a GM (donor) crop in two out three sites sampled. The highest level of foreign GM gene detection at this distance (at one site) was 0.42%. In addition, the presence of the foreign gene was detected at a level of 0.14% at one site in the nearest row of a non GM crop facing the GM donor crop at a distance of 650 metres;

- At 50 metres into the non GM crop, the foreign GM gene was detected in 62% of samples taken¹⁷, of which 42% (of the total) had levels greater than 0.3%. Samples taken at a 150 metre separation distance showed evidence of cross-pollination in 43% of cases, with 27% (of the total) having a level of adventitious presence of greater than 0.1% and less than 0.3%, and 16% (of the total) with an adventitious presence level of greater than 0.3%;
- Wooded areas and hedges around fields influence gene flow by creating turbulence or reducing wind speed as pollen reaches the barrier. This can lead to some pollen being deposited in the immediate vicinity of the barrier and hence raising the level of crosspollination in this area above the level that would otherwise have been expected if no barrier had been present. Thus landscape can affect cross-pollination levels and create 'hotspots' of cross-pollination at distances of 100-150 metres when, for example pockets of airborne pollen get blown up into the air by turbulence around a barrier and deposited at a greater distance away from the GM source;
- Very low levels of cross-pollination occurred where there was set-aside land between a GM and non GM crop (ie, the adventitious presence level was only 0.1% at two metres into the non GM crop on the side nearest the GM donor crop). This may be the result of the first few rows of the non GM crops intercepting a higher proportion of the incoming (GM) pollen when it has travelled across open ground and not been subject to disturbance by barriers such as hedges;
- Relating the adventitious presence levels found in the samples taken in each non GM crop to the levels that would be expected at the field level (ie, grossing up the sample findings to the non GM field level), the study estimated that an isolation distance of 24.4 metres would be required to meet the 0.9% labelling threshold, an isolation distance of 80 metres would be sufficient to deliver adventitious levels below 0.3% and 258 metres would be needed to keep levels below 0.1%.

It is important to note that the analysis above refers to the ratio of cross-pollination at a fixed distance from an emitter crop, with samples taken for measuring adventitious presence levels from crop rows before harvest. As such, the adventitious presence levels identified do not take into account the dilution effect of normal harvesting practices (see above). Under normal harvesting practices, the upper thresholds for adventitious presence identified in the UK-based research are highly unlikely to be reached in harvested maize crops.

3.2.5 Summary of findings from cross pollination & co-existence studies between GM and non GM maize

A summary of the key findings from the four pieces of research referred to in sub-sections above is presented in Table 3.

Study	Circumstance	Relevant distance	Likelihood of
		(metres) to meet	adventitious
		0.9% labelling	presence levels
		threshold	being above

Table 3: Summary of findings: GM and non GM maize co-existence research

¹⁷ The reporting of GM detection levels per sample may give the impression that the incidence of adventitious presence is high relative to levels reported in the other research reviewed. This high level of adventitious presence largely reflects the reporting of adventitious presence levels on a per sample basis, with each sample comprising at least 1,000 grains, compared to the other research which reports adventitious presence levels in terms of % of grains. Although the way in which the data in Henry et al is reported does not allow direct comparisons on a % of grains basis to be shown, the key conclusions from the work are consistent with the findings of the research from Spain and France

			0.9%
IRTA	Non GM field over 1 ha in size (adventitious presence measured at field level according to normal harvesting practice)	No separation distance required	No
IRTA	Non GM field under 1 ha in size (adventitious presence measured at field level according to normal harvesting practice)	6 metres	No
APROSE	Crops with similar (up to 10 days difference) planting dates (GM & non GM), no buffer crops (adventitious presence measured on sample basis before harvest from the crop)	6.25 metres	Unlikely
APROSE	Crops with the same planting date (GM & non GM), no buffer crops, adventitious presence measured on sample basis before harvest from the crop	25 metres	Unlikely
Benetrix & Bloc	Non GM crop downwind of GM crop, no buffer crop (adventitious presence measured on sample basis before harvest from the crop)	10-12 metres	Unlikely
Benetrix & Bloc	Non GM crop downwind of GM crop, very strong wind conditions, no buffer crop, (adventitious presence measured on sample basis before harvest from the crop)	25 metres	Unlikely
Benetrix & Bloc	Application of good farming practices and normal harvesting practices (adventitious presence measured at field level according to normal harvesting practice), no buffer crops	No separation required	No
Henry et al	Adventitious presence measured on sample basis (before harvest from the crop), including border rows of non GM	24.4	No

3.3 Practical experience of co-existence and minimising levels of cross-pollination between GM and non GM maize crops

Dealing with adventitious presence is nothing new in the maize production sector. Farm level practices (eg, separation of crops by space and time, communicating with neighbours, use of good husbandry, planting, harvest and storage practices) to minimise levels of adventitious presence (and hence delivering good/successful co-existence) have been in operation, by farmers, for many years (eg, for waxy maize production).

Some practical examples in relation to GM and non GM maize crops are summarised below.

3.3.1North America

a) Co-existence aspects

Ensuring co-existence has involved actions being taken by both GM and non GM grower. All suppliers of GM seed to farmers in North America provide farmers with 'Technology Use Guides' or 'Crop Stewardship Guides'. These contain recommendations for use of the GM products (eg, herbicide use for weed control recommendations) and some advice on 'co-existence issues' that target maintaining the purity of non GM crops growing on GM crop planting farms, on nearby farms, in storage or when supplied to buyers. Issues covered include:

Pollen movement: ways of minimising the chances of cross-pollination through the siting of crops in relation to prevailing wind directions, use of buffer crops and barriers, timing of plantings, varieties planted (with different flowering times),

separation distances and removal (ie, separate harvesting and segregation) of outer strips of crop in a field (eg, some speciality corn crops require the removal of the outer 9 rows of a crop to ensure the removal of impurities from adjacent (non speciality) corn crops);

- Holding discussions with neighbours about planting intentions;
- Holding discussions with grain buyers to ensure that contractual requirements are identified (eg, a buyer servicing markets that require certified non GM maize, or a market where only some GM maize varieties are approved for importation and use). This prompts the implementation of appropriate on-farm measures to facilitate segregation and channelling of maize crops to different markets.

In addition, non GM growers, especially those in the organic sector, are provided with advice on similar measures from some of their advisors and certifying bodies.

- b) Other crop management issues
 - All farmers of herbicide tolerant crops (including non GM herbicide tolerant crops) are also provided with advice on managing volunteers in crops¹⁸. This advice covers aspects of an integrated weed management system, the majority of which is equally applicable to non GM varieties of these crops, and includes crop rotation, rotation of herbicides, rotation of herbicide tolerant traits, rotation of timing of herbicide applications, rotation of timing of tillage and use of certified seed;
 - ii) Farmers planting insect resistant (Bt) maize in the USA are also required to implement an insect resistance management plan (IRM) to contribute to minimising the possibilities of target pests (corn borers and corn earworms) developing resistance to the Bt trait. As such, this is not directly related to meeting economic and market 'co-existence' issues but can, through compliance with the IRMs, contribute to indirectly facilitating co-existence. The IRM programme includes guidelines on separation distances and insecticide usage:
 - At least 20% of total corn plantings must be to non Bt varieties, on the basis of a minimum of 8.1 hectares (20 acres) of non Bt per every 32.38 hectares (80 acres) of maize planted. If the Bt corn is also planted in regions where Bt cotton is present this non Bt refuge requirement rises to 50% of the corn crop (because cotton and maize have a common pest problem that is the target of the Bt trait);
 - A non Bt refuge must be planted within half a mile of each Bt corn field, and preferably within one quarter mile;
 - Refuges can be in the form of strips; lateral, within or around the Bt crop, or as blocks between Bt crops;
 - Non Bt corn refuges can only be treated with conventional insecticides if target pest pressure reaches economic thresholds;
 - ▶ Bt-based foliar insecticides are not allowed to be used on the refuge areas.

3.3.2 Spain

Here, as in North America, farmers are advised by seed suppliers about possibilities of adventitious presence of GMOs from their crops being found in non GM crops and how best to minimise this occurring. Recommendations to growers build directly on the findings of the IRTA and APROSE research referred to in section 3.2 and specifically include provisions for 'worst case' scenarios. The recommendations include the following:

¹⁸ See for example CropLife Canada, Controlling herbicide tolerant volunteers in a succeeding crop: a best practice guide. www.croplife.ca

- ➢ GM farmers must inform their neighbours about their intention to plant GM varieties;
- Advice is provided on the siting of GM and non GM refuges to minimise possibilities of cross-pollination occurring (taking into consideration prevailing wind directions, flowering dates of different varieties and the planting of refuges in bands between the GM crops and neighbouring non GM crops);
- At least four rows of conventional maize planted between GM crops and non GM crops are recommended when the neighbouring (non GM crop) crop is closer than 25 metres and smaller than 1 hectare. This should be planted after planting of Bt varieties and should be harvested and labelled as GM;
- Planting and combining equipment should be thoroughly cleaned after working with Bt maize or in the nearest 2,000 metres square (to the GM maize) of non GM maize used as a buffer crop.

Also, as part of IRM requirements, the planting of refuges is advised to anyone planting over 5 hectares of Bt maize. These should be equal to at least 20% of the total maize crop and be planted close to the GM crop. Refuges can be strips; lateral, within or around the Bt crop, or as blocks between Bt crops.

Overall, the commercial experiences of growing GM maize alongside/near to non GM maize, both in North America and Spain shows that cross-pollination between different (maize) crops has been successfully managed, without causing economic or commercial problems.

In over 90% of cases where Bt maize has been grown in Spain, neighbouring fields have either been Bt maize or a conventional maize variety being sold for feed usage, where the buyer does not differentiate between GM and non GM sources of supply. In such circumstances, there has been no need to implement co-existence measures and farmers have often agreed between themselves to grow GM maize adjacent to non GM maize without formally applying any co-existence measures and simply labelling the maize sold off-farm as containing GM. Where GM maize has been grown near to non GM maize which has been sold to markets which require the crop to be certified as non GM (eg, to starch manufacturers), the application of the co-existence recommendations has successfully enabled the two crops to be planted near to each other, without compromising the purity requirements of buyers in the non GM sector.

A very small number of instances of adventitious presence of GM events have been found in non GM and organic maize crops (and resulted in possible rejection of deliveries by buyers or imposition of contractual price penalties) but this has usually been caused by deficiencies in application of good co-existence practices rather than any failure of the practices themselves¹⁹.

4 Conclusions

The possibility of GM adventitious presence occurring in a non GM crop because of crosspollination in maize crops is well researched. It draws on practical (commercial) experience of growing specialty maize crops (eg, waxy maize), GM crops, and specific research studies.

Maize pollination essentially relies on wind dispersal of pollen. As such, levels of crosspollination are generally closely related to distance of a receptor plant from a pollen donating plant, with the level of cross-pollination falling rapidly the further away the recipient plant is from the pollen source (as maize pollen is fairly heavy, the vast majority is deposited within a

¹⁹ For further information see a series of four papers on GM and non GM crop co-existence in North America, the EU, the UK and Spain by PG Economics. These can be found on www.pgeconomics.co.uk

short distance of any emitter plant). On average, almost all maize pollen travels no further than 100 metres and nearly all potential cross-pollination <u>between fields of non GM maize</u> occurs within 18-20 metres of an emitter crop. In respect of GM maize containing a single trait such as insect (Bt) resistance, the presence of the GM trait in only 50% of pollen means that almost all cross pollination (of pollen with the GM trait) will occur at a reduced distance from the GM emitter crop.

Not surprisingly, it is possible to find examples of research that identified rates of crosspollination (and hence levels of adventitious presence) at variance with these rates, because of the influence of a number of other factors. These include:

- Timing of planting (and flowering) of different maize crops: the greater the difference between planting times of crops of the same variety, the lower the levels of crosspollination;
- Varietal differences: recommendations for planting times and the time each variety takes to flower (and produce/be receptive to pollen) usually varies by variety. Consequently, varietal differences can contribute differences in the timing of flowering and hence to the chances of cross-pollination occurring (see above);
- Buffer crops: the planting of (non GM) buffer crops affects cross-pollination levels. This is because a non GM buffer crop (of maize) can act as a interceptor to a large proportion of GM pollen and can provide additional non GM pollen that 'crowds out' the GM pollen (further reducing the chances of the GM pollen introgressing with the non GM crop in which adventitious presence is to be minimised). One row of buffer crop is considered to be roughly equal to 10 metres equivalent of separation distance;
- Temperature and humidity levels: the drier and hotter conditions are at time of flowering the lower the levels of cross-pollination and vice versa;
- The strength and direction of wind: levels of cross-pollination are highest in receptor crops that are typically downwind of donor crops. Not surprisingly, the stronger the wind at time of pollen dispersal, the greater the likelihood of cross-pollination being recorded at greater distances;
- Barriers: objects such as hedges and woods, as well as topography can affect levels of cross-pollination by interrupting and diverting airborne pollen flow. These barriers can cause pollen to be diverted upwards (and hence could travel further than otherwise would be the case) and sometimes this can result in pollen being deposited in 'hot spots';
- Length of border/shape of fields: the longer the border between a GM and non GM crop, the greater the chances of cross-pollination occurring and vice versa;
- Volunteers. The presence of volunteer maize plants from an earlier crop may increase the level of adventitious presence in a crop. Whilst this possible source of adventitious presence is potentially highest in regions which do not have low enough average winter temperatures to kill volunteer plants, farm level experience (eg, in Spain) shows that this is a very minor source of adventitious presence.

These factors of influence are known to growers of specialty maize crops (eg, waxy maize) and to the organisations that typically supply seed to farmers and/or buy (specialty) maize from farmers. As a result, the application of a variety of measures (such as separation distances, the use of buffer crops, varying the time of planting or varieties used), and taking into consideration the dilution effect on adventitious presence levels of normal harvesting practices (see section 3.2^{20}), usually delivers required levels of purity. More recently, the

²⁰ The key point being that it is normal practice to test crops for adventitious presence of all unwanted material (eg, the presence of GM material in non GM crops that are required to be certified as non GM, weed material, dirt, seed off types etc) after harvest. As a result, levels of adventitious presence of any unwanted material tend to be lower in harvested crops than might be the case if testing was undertaken in the field before harvest

same principles and practices have been successfully applied in respect of GM maize crops where a non GM maize market has developed.

The key point to note about the competent application of measures to deliver the purity levels required in different markets (including the 0.9% EU labelling threshold) is that adventitious presence levels in excess of the required purity levels are rare. This is because the measures taken are based on years of experience and usually operate to 'worst case' scenarios. The evidence from the GM-specific co-existence research in Spain, France and the UK (section 3.2) identified that the application of good farming practices and normal harvesting practices alone (ie, without the formal application of co-existence measures) are likely to deliver the 99.1% purity threshold set by the 2004 EU labelling legislation (ie, the maximum GM adventitious presence level permitted is less than 0.9%). However, by additionally applying specific co-existence measures in isolation or in combination the probability of GM adventitious presence being found in a non GM maize crop (grown in close proximity) is reduced further to an extremely low level.

Spanish experience illustrates that the application of four buffer rows of non GM maize between a GM crop (on the GM growing farm) and a non GM crop (on an adjacent farm in plots of under 1 hectare) as a single measure has delivered effective co-existence. Alternatively a separation distance of 6 metres is likely to be equally effective. Application of greater separation distances (eg, 10-12 metres identified in the French co-existence research²¹) appears to offer additional provision for worst case scenarios and reducing further the probability of GM adventitious presence occurring to minute levels.

Overall, evidence from both commercial practice, and research shows that GM, conventional and organic growers²² of maize can co-exist and maintain the integrity of their crops without problems.

²¹ A separation distance of 25 metres referred to in some of the research work (eg, APROSE and Bénétrix & Bloc) represents an extreme 'worst case' scenario and would probably lead to GM adventitious presence levels being below the 0.9% labelling threshold even if adventitious presence levels were tested in individual rows of a non GM crop field closest to a GM crop field prior to harvest ²² In respect of organic growers this assumes application of the EU legal (labelling) threshold of 0.9%. It does not

²² In respect of organic growers this assumes application of the EU legal (labelling) threshold of 0.9%. It does not consider the threshold applied by some organic certifying bodies of zero detectible presence because it is not possible to meet such a threshold in any form of agricultural production system

Bibliography

APROSE (2004) Evaluation of cross pollination between commercial GM (Mon 810) maize and neighbouring conventional maize fields. Analytical survey of 14 commercial Bt fields in 2003 by Monsanto, Nickersons and Pioneer Hi-Bred International, presented to the Spanish Bio-Vigilance Commission, unpublished

Bateman, A. J. (1947) Contamination of seed crops — II. Wind pollination. Heredity 1: 235-246.

Balthazar & Schoper (2002) Crop to crop gene flow: dispersals of transgenes in maize: proceedings of the 7th symposium on biosafety of GMOs, cited in Henry et al Bénétrix F & Bloc D (2003) Mais OGM et non OGM possible coexistence. Perspectives

Agricoles No 294 Burris J.S. (2003) Adventitious pollen intrusion into hybrid maize seed production fields. American Seed Trade Association

DEFRA (2003) Review of knowledge of the potential impact of GMOs on organic farming. <u>http://www2.defra.gov.uk/research/project_data/More.asp?I=OF0193&SCOPE=1&M=CFO&</u> <u>V=JIC&LvI=0&Cat1=&Cat2=&Cat3=&Cat4=&Cat5</u>= (accessed October 2004)

Eastman K & Sweet J (2002) Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer. A review and interpretation of published literature and recent/current, European Environment Agency, ISBN 92 9167 4117

Emberlin, J., Adams-Groom, B. & Tidmarsh, J. (1999) The dispersal of maize (Zea mays) pollen. A report commissioned by the Soil Association: National Pollen Research Unit, University College Worcester, UK.

http://www.soilassociation.org/web/sa/saweb.nsf/b0062cf005bc02c180256a6b003d987f/8025 6ad800554549802568660075e5b4!OpenDocument&Highlight=2,Treu (accessed October 2004)

Foueilassar X & Fabie A (2003) Waxy maize production, an experiment evaluating coexistence of GM and conventional maize, ARVALIS, France

Henry C et al (2003) Farm scale evaluations of GM crops: monitoring gene flow from GM crops to non GM equivalents in the vicinity: part one forage maize, DEFRA report EPG/1/5/138

Ingram J (2000) Review of the use of separation distances between GM and other crops, MAFF research project, RG0123

Jones, M. D. & Brooks, J. S. (1952) Effect of tree barriers on outcrossing in corn. Oklahoma Agricultural Experimental Station, Technical Bulletin No. T-45.

Jurgenheimer R (1976) in Corn improvement, seed production and uses, Wiley Interscience, cited in Henry et al (2003)

Klein, E. K., Laredo, C. & Lavigne, C. Estimation of pollen dispersal function from field experiments, cited in Eastham K & Sweet J (2002)

Loubet, B and Foueillassar, X. et al., (2003) INRA Thiverval-Grignon Etude mécaniste du transport et du dépôt de pollen de maïs dans un paysage hétérogène.Rapport de fin de projet Convention INSU N° 01 CV 081

Ma B et al (2004) Crop ecology, management & quality: extent of cross-fertilisation in maize by pollen from neighbouring transgenic hybrids, Crop Science 44, 1273-1284, Crop Science Society of America, USA

Melé E et al (2004) First results of co-existence study: European Biotechnology Science & Industry News No 4, vol 3

Messean, A. (1999) Impact du development des plantes transgeniques dans les systemes de culture: rapport final. Dossier No. 96/15- B: Impact des plantes transgeniques. http://www.acta.asso.fr/cr/cr9924.htm (accessed October 2004) Meir-Bethke & Schiemann J (2003) Effect of varying distances and intervening maize fields on outcrossing rates of transgenic maize, Proceedings of the 1st European conference on the co-existence of GM crops with conventional and organic crops, Denmark, November 2003 Paterniani, E. & Stort, A. C. (1974) Effective maize pollen dispersal in the field. Euphytica 23: 129–134.

Pleasants, J. M., Hellmich, R. L. & Lewis, L. C. (1999) Pollen deposition on milkweed leaves under natural conditions (presentation at the Monarch Butterfly Research Symposium, Chicago, 1999).

Salamov, A. B. (1940) About isolation in corn. Sel. I. Sem., 3. (Russian translation by Michael Afanasiev in 1949).

Sears, M. K. & Stanley-Horn, D. (2000) Impact of Bt corn pollen on monarch butterfly populations. In: Fairbairn, C., Scoles, G. & McHughen, A. (Eds.) Proceedings of the 6th International Symposium on The Biosafety of Genetically Modified Organisms. University Entension Press, Canada.

Simpson, E. C., Norris, C. E., Law, J. R., Thomas, J. E. & Sweet, J. B. (1999) Gene flow in genetically modified herbicide tolerant oilseed rape (Brassica napus) in the UK. In: Gene Flow and Agriculture: Relevance for Transgenic Crops. Lutman, P. (Ed.). BCPC Symposium Proceedings No. 72.

Treu, R. & Emberlin, J. (2000). Pollen dispersal in the crops Maize (Zea mays), Oil seed rape (Brassica napus ssp oleifera), Potatoes (Solanum tuberosum), Sugar beet (Beta vulgaris ssp vulgaris) and wheat (Triticum aestivum). Report for the Soil Association.

http://www.soilassociation.org/web/sa/saweb.nsf/b0062cf005bc02c180256a6b003d987f/8025 6ad800554549802568660075e5b4!OpenDocument&Highlight=2,Treu (accessed October 2004)