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Review

The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization

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The ongoing discussion on the co-existence between genetically modified (GM) and non-GM crops becomes more important in the European Union (EU). With the recent inscription of 17 GM maize varieties in the common EU catalogue of varieties of agricultural plant species, the acreage of transgenic maize for market purposes is expected to increase in some European countries. In the EU, specific tolerance thresholds have been established for the adventitious and technically unavoidable presence of GM material in non-GM produce, and member states are elaborating legal frames to cope with co-existence. As maize is a cross-pollinated crop relying on wind for the dispersal of its pollen, technical management measures will be imposed to reduce cross-fertilization between transgenic and non-transgenic maize. Various biological, physical and analytical parameters have been identified to play a role in the study of cross-fertilization in maize. This variability may hamper the comparison between research results and may complicate the definition of appropriate isolation distances and/or pollen barriers in order to limit out-crossing. The present review addresses these parameters and proposes containment measures in order to not exceed the legal labeling thresholds in maize.

Keywords: *Zea mays* L. / maize / pollen flow / cross-fertilization / co-existence / thresholds / isolation distances / pollen barriers / GM-crop-free zones

INTRODUCTION

Genetically modified (GM) maize (*Zea mays* L.) varieties providing resistance to specific insects (*e.g.* European corn borer (*Ostrinia nubilalis*), Mediterranean corn borer (*Sesamia nonagrioides*)) and/or to the non-selective herbicides that contain the active ingredients glyphosate or glufosinate are grown commercially. In 2004, the worldwide acreage of commercial plantings of GM maize reached 19.3 million hectares. The six principal countries where transgenic maize was grown were the United States, Argentina, Canada, Brazil, China and Paraguay (James, 2004). So far, the commercial cultivation of GM maize in the European Union (EU) has been restricted to Spain. From 1998 on, Bt176 varieties and from 2003 on, MON810 varieties have been cultivated and represented 4–7% of the total Spanish maize plantings, corresponding to about 20 000–32 000 ha (Alcalde, 2003; Brookes and Barfoot, 2003). In 2004, about 58 200 ha of these varieties were cultivated in Spain corresponding to 12% of the planted maize area (James, 2004; Ortega Molina, 2004). The cultivation of the Bt176 and MON810 GM varieties for commercial purposes in Spain was possible due to their EU approval for cultivation pursuant to Directive 90/220/EEC (part C) and their inscription in the Spanish catalogue of varieties of agricultural plant species (Tab. 1). With the inscription of 17 GM maize varieties derived from the event MON810 in the common EU catalogue of varieties of agricultural plant species on 8 September 2004, it is to be expected that these GM maize cultivars will be grown commercially in European countries where the European corn borer and Mediterranean corn borer are pests

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Table 1. Overview of commercially authorized and positively assessed GM maize events in the EU per commercial application (under Directives: 90/220/EEC, 2001/18/EC and Regulations: 258/97, 1829/2003) (BBS: http://www.biosafety.be; EC: http://europa.eu.int/comm/food/food/biotechnology/index_en.htm; EFSA: http://www.efsa.eu.int) (August 2005).

Commercial applications	Transgenic events	Traits	Notifiers
Growing	Bt176*	IR, HR	Ciba-Geigy Novartis
	T25*	HR	AgrEvo
	MON810**	IR	Monsanto
	1507†	IR, HR	Pioneer Hi-Bred, Mycogen Seeds
	Bt11†	IR, HR	Syngenta Seeds
Food (all products)	Bt176	IR, HR	Ciba-Geigy Novartis
	Bt11	IR, HR	Syngenta
	NK603	HR	Monsanto
	GA21†	HR	Monsanto
	MON863†	IR	Monsanto
	1507†	IR, HR	Pioneer Hi-Bred, Mycogen Seeds
	MON863 × MON810††	IR	Monsanto
	MON863 × NK603††	IR, HR	Monsanto
	MON863 × MON810 × NK603††	IR, HR	Monsanto
Food (only maize derivatives and products made with maize derivatives)	MON810	IR	Monsanto
	T25	HR	AgrEvo
	MON809	IR	Pioneer
Feed, industrial processing, import	Bt176	IR, HR	Ciba-Geigy Novartis
	T25	HR	AgrEvo
	Bt11	IR, HR	Novartis Seeds
	MON810	IR	Monsanto
	NK603	HR	Monsanto
	MON863	IR	Monsanto
	1507†	IR, HR	Pioneer Hi-Bred, Mycogen Seeds
	Bt11†	IR, HR	Syngenta Seeds
	MON863 × MON810††	IR	Monsanto
	MON863 × NK603††	IR, HR	Monsanto
	MON863 × MON810 × NK603††	IR, HR	Monsanto
Feed	GA21†	HR	Monsanto
	T25 × MON810††	IR, HR	Pioneer

* Genetically modified varieties derived from a transgenic event included in (a) national catalogue(s) (Bt176 and MON810 varieties in Spain and France, T25 variety in the Netherlands).
** Genetically modified varieties derived from a transgenic event included in the common EU catalogue of varieties of agricultural plant species (17 MON810 varieties).
† Unapproved events that received a positive opinion for marketing and for which a detection method is publicly available and for which a threshold of 0.5% may be applied.
†† GM hybrids containing two or three constructs obtained by crossing of GM inbred parental lines/events (also called stacked events).
Abbreviations: HR = herbicide resistance / IR = insect resistance.

(Bénétrix and Bloc, 2003; Demont and Tollens, 2004; Farinós et al., 2004). Preliminary data for 2005 suggest that MON810 GM varieties are grown in Czech Republic, France, Germany, Portugal and Spain (Tencalla, personal communication).

The Regulation (EC) N° 1829/2003 on GM food and feed that entered into force in April 2004 provides a legal basis for the national and/or regional implementation of co-existence frames in the EU (see article 43). As defined in the guidelines on co-existence (2003/556/EC), co-existence refers to the ability of farmers to make a practical choice between conventional, organic and GM crop production. All farming systems should be possible in the EU and the presence of one system should not exclude other systems in the neighborhood. The ability to maintain different agricultural production systems is a prerequisite for providing a high degree of consumer choice. This latter objective may be reached by segregation and identity preservation systems, and through traceability and labeling provisions. In reality, co-existence is a complex issue, since most of the crops are not grown under confined conditions, and the supply chains are rarely segregated. As a consequence, adventitious mixing of GM material with non-GM produce can occur in all the steps of production and supply chains. The potential sources of adventitious mixing between GM and non-GM material are the use of impure seed, the natural pollen flow between neighboring fields, the occurrence of volunteer plants originating from seeds and/or vegetative plant parts from previous crops, the human activities during sowing, harvesting, handling, transporting, storing, importing and processing, and to a lesser extent the presence of certain sexually compatible wild relatives and feral plants (Devos et al., 2004; Eastham and Sweet, 2002; Sanvido et al., 2005; Schiemann, 2003). As there are no wild relatives of maize in the EU, and as maize seeds or seedlings do not survive winter cold in most EU countries, the major potential biological source of mixing is pollen flow. Maize is a cross-pollinated crop relying on wind for the dispersal of its pollen.

The EU accepts an adventitious or technically unavoidable presence of authorized GM material in non-GM food and feed up to a 0.9% level. For EU unapproved events, a zero tolerance is applied, unless they have received a favorable scientific risk assessment for marketing and a detection method is publicly available. In the latter case, a threshold of 0.5% may be applied (Tab. 1). Up to July 2005, no threshold for GM adventitious content has been defined for seeds: the newest proposal for maize is 0.3%. Organic growers prefer a zero tolerance, but as our world is no longer completely free of transgenic pollen, a level between the limit of quantification of a DNA analysis (0.1%) and 0.9% might be defined in due time. Above the mentioned thresholds the product needs to be labeled as being consisting of, containing or produced from a genetically modified organism. The efforts needed to meet the legal labeling thresholds will affect farming and the supply chain management. Recently, research on pollen flow has been boosted in order to find out by which means adventitious presence by cross-fertilization can be kept below the threshold levels. Simultaneously, different EU member states are developing legal frameworks to cope with the agronomic consequences of the inevitable cross-fertilization.

The present review focuses on the state of knowledge on pollen flow and cross-fertilization between maize plants and fields. Different parameters affecting these processes are discussed, and measures to reduce cross-fertilization are proposed.

APPROACHES FOR STUDYING POLLEN DISPERSAL

It is important to mention that research approaches, analytical methods and experimental designs differ over studies, which may hamper the comparison between research results and complicate the definition of appropriate measures to limit cross-fertilization.

The approaches to study pollen dispersal in maize are (1) measurements of pollen concentrations at various distances and heights from a pollen source (Aylor et al., 2003; Bassetti and Westgate, 1994; Bateman, 1947; Brunet et al., 2003; Cervantes Martínez et al., 2001; Das, 1983; Jarosz et al., 2003; 2005; Kawashima et al., 2005; Lang et al., 2004; Pleasants et al., 2001; Raynor et al., 1972; Sears and Stanley-Horn, 2000; Westgate et al., 2003; Zangerl et al., 2001), (2) measurements of levels of cross-fertilization at various distances from a source through phenotypic traits and protein analysis (Bannert and Stamp, personal communication; Bateman, 1947; Burris, 2001; Byrne and Fromherz, 2003; Cervantes Martínez et al., 2001; Chilcutt and Tabashnik, 2004; Fabié, 2004; Foueillassar and Fabié, 2003; Garcia et al., 1998; Jones and Brooks, 1950; 1952; Luna et al., 2001; Ma et al., 2004; Messeguer, personal communication; Narayanaswamy et al., 1997; Paterniani and Stort, 1974; Salamov, 1940; Stevens et al., 2004) or through molecular markers (Bénétrix, 2004; Bénétrix and Bloc, 2003; Henry et al., 2003; Jemison and Vayda, 2002; Meier-Bethke and Schiemann, 2003; Melé, 2004; Messeguer et al., 2003; Ortega Molina, 2004; Stevens et al., 2004; Weber et al., 2005), and (3) calculations through modeling (Angevin et al., 2001; Arritt et al., 2003; Aylor et al., 2003; Belcher et al., 2005; Bock et al., 2002; Du et al., 2001; Jarosz et al., 2004; Klein et al., 2003; Loos et al., 2003; Novotny and Perdang, 2002; Tolstrup et al., 2003; Yamamura, 2004; Wolt et al., 2004).

(1) Approaches based on pollen counts do not always take into account pollen mortality, pollen competition, failure of pollen to land on silks, receptiveness of the silks and/or the developmental failure of the ovary, which may result in an overestimation of the maximum distance traveled by viable pollen and of the fertilization potential of pollen (Aylor et al., 2003; Fonseca and Westgate, 2005).

(2) Studies using maize as receptor plants indicate cross-fertilization truly. If one uses homozygously expressed phenotypic markers, cross-fertilization is detected based on xenia or on the presence of phenotypic off-types in the progeny. Xenia is the immediate effect of pollen on the developing maize kernel (Poehlman and Sleper, 1995). Hence cross-fertilization is quantified as a percentage of off-types in the kernels or in the progeny. In the GM era, cross-fertilization is spotted in hybrid progeny by the detection of transgenic DNA and/or proteins or by applying an appropriate selective pressure (*e.g.* herbicide application in the case of herbicide resistance). According to the EC recommendation on technical guidance for sampling and detection (2004/787/EC), DNA-based analyses are generally accepted to quantify inadvertent co-mingling in order to cope with the legal thresholds for adventitious mixing. Using DNA analysis, results are expressed as the number of target/transgenic DNA sequences per sequence specific to the target taxon, calculated in terms of haploid genomes. Results expressed as a percentage of genomes differ depending on the genetic constitution of the analyzed tissue (zygotic or maternal), the relative shares of these tissues in the sample, the ploidy levels of the tissue (triploid endosperm *vs.* diploid maternal tissue), the moment of sampling (early or late stage of seed/kernel development), the copy number of transgenic DNA, and on the DNA extractability, which may differ between plant tissues (for a detailed discussion of the problems see Taverniers, 2005). As a consequence, the results of a DNA analysis are not smoothly convertible to results obtained by phenotypic markers. Moreover, owing to the current production of GM hybrid varieties, the transgene generally is present only in the seed parent or in the pollinator. As a result, GM hybrid varieties are hemizygous for the transgenic trait. Hence only half of the pollen produced carries the transgene. Compared to a pollen donor that is homozygous for the screened trait only half of the cross-fertilization is measured.

Apart from the quantification approach, the results of field trials studying cross-fertilization rates may also differ according to the design implemented. In different studies, individual plants or small recipient plots have been planted at various distances from a source in order to measure how far viable maize pollen can successfully fertilize a maize ovule. However, such a design does not reflect the real agricultural situation and is not suited to quantify the cross-fertilization levels of recipient fields of commercial size. Due to the small and diluted pollen cloud hanging over individual plants or small recipient plots, incoming pollen is less hindered. Consequently, individual plants or small recipient plots are much more prone to cross-fertilization compared to large fields with a dense pollen cloud acting as a physical barrier and competitor for incoming pollen. As the probability of cross-fertilization diminishes with increasing distances, sampling must be done at different positions within the receptor field of representative size in order to calculate the average percentage of cross-fertilization over the whole field. The recommendation of isolation distances and/or pollen barriers, based on discrete cross-fertilization levels, may therefore be too conservative and thus larger than the ones actually needed.

(3) The application of different mathematical models to study pollen flow in maize is growing. Models are based on experimental data: the more experimental data become available, the better the validation of the models will be. We refer to the different models to study their underlying assumptions.

PARAMETERS AFFECTING CROSS-FERTILIZATION IN MAIZE

Apart from the previously discussed difficulties to compare research results, some consistent facts and patterns are observed over trials that have been conducted under different geographical and climatic conditions (Eastham and Sweet, 2002; Emberlin et al., 1999; Ingram, 2000; Sanvido et al., 2005; Treu and Emberlin, 2000).

Isolation distance between pollen source and recipient field

Compared to pollen of other wind-pollinated species, maize pollen grains are relatively large (an average diameter of 90 μm) and heavy (0.25 μg) (Aylor et al., 2003; Di-Giovanni et al., 1995; Raynor et al., 1972). Due to its pollen characteristics, maize pollen has a high settling speed and a quick deposition (Aylor et al., 2003; Di-Giovanni et al., 1995). Jarosz et al. (2005) observed pollen deposition rates ranging from 10 to 100 grains/m^{2}s at 10 m downwind distance from the source. At downwind distances of 800 and 1 000 m, the pollen deposition rates decreased to 0.001–0.0002 grains/m^2s. Pollen concentrations and consequently successful fertilizations decline rapidly with the distance from the source following a leptokurtic pattern with a long tail. *Ca.* 95–99% of the released pollen is deposited within about 30 m from the source. At distances further than 30–50 m, the levels of pollen dispersion are very low but there is no clear cut-off distance beyond which these levels reach zero (Aylor et al., 2003; Jarosz et al., 2003; 2005; Paterniani and Stort, 1974; Pleasants et al., 2001; Sears and Stanley-Horn, 2000).

Size of pollen source and recipient field

Recent field tests demonstrated that for a pollen donor of a given size, the levels of cross-fertilization decrease as the size of the recipient field increases (Melé, 2004; Messeguer et al., 2003; Ortega Molina, 2004). Melé (2004) showed that with a pollen donor of 0.25 ha, the levels of cross-fertilization expressed in percentage of genomes decreased from 1.77 to 0.83% in the grains when the size of the recipient field was increased from 0.25 to 1 ha. The larger the recipient field, the larger its own pollen mass will be. This pollen cloud, hanging over the recipient field, is a physical barrier and competitor for incoming pollen. The results of the research of Weber et al. (2005) with fodder maize in Germany during 2004 confirmed these results (donor fields of transgenic maize were surrounded by the isogenic non-transgenic variety). Moreover, they concluded that the overall cross-fertilization measured as a percentage of genomes in the chopped material did not exceed 0.9% if adjacent donor and recipient fields were of the same size.

Shape and orientation of pollen source and recipient field

Calculations investigating the effect of the alignment of the recipient fields towards the pollen donor showed that the amount of cross-fertilization can easily double with elongated recipient fields compared to rectangular ones of the same surface (Ingram, 2000; Meier-Bethke and Schiemann, 2003; Novotny and Perdang, 2002). With the long side of an elongated recipient field of 5 ha facing the source, cross-fertilization expressed in percentage of grains was calculated to be 10.7% compared to 3.4% for the short side (Novotny and Perdang, 2002). In other words: the deeper the recipient field, the less the cross-fertilization level of the total product.

Wind direction and velocity

Air currents at pollen dehiscence can lift pollen up high in the atmosphere and distribute it over significant distances within its viability period. Aerial pollen concentrations at a height of 60 m above maize fields varied between 0.2 to 8.1 grains/m^3 (Aylor et al., 2003). At heights between 800 and 2 000 m, the pollen concentrations decreased from 1.1 to 0.2 grains/m^3. Pollen viability decreased with height. However, at high altitudes, the lower air temperature favored pollen longevity. About 5 to 10% of the sampled pollen was found to be viable at 2 000 m (Brunet et al., 2003). The horizontal pollen flux (grains/m^2s) observed at 6.5 m height by Jarosz et al. (2005) was similar at both 3 and 10 m from the source. Depending on the wind velocity and air currents, pollen will settle by gravity nearby or far away from the source. Small pollen amounts have already been observed at 800 and 1 000 m from the source with pollen deposition rates ranging between 0.0002–0.001 grains/m^2s (Jarosz et al., 2005). Calculations and multi-year trials carried out on different sites indicated that pollen flows much farther downwind than upwind. Ma et al. (2004) reported that on average (in all the sites and years they tested), levels of cross-fertilization (expressed in percentage of grains) were lower than 1% at a distance of 28 m downwind: the same cross-fertilization level was detected at a distance of 10 m upwind.

Rain

Pollen released in the airflow can be absorbed into water droplets and/or can land on wet silks where it bursts and dies. Moreover, the release of pollen is delayed when it is raining due to the absence of dehiscence of the anthers. Jones and Brooks (1950) ran an experiment to study pollen dispersal during three consecutive years. They attributed the low levels of cross-fertilization in one of the three years to the rainy weather during the pollination season but did not quantify the affect of the rain. In one of the trials of Jarosz et al. (2005), reduced pollen release and concentrations were observed, probably resulting from the washing out effect of the pivot irrigation. So far, no published data are available allowing the quantification of the impact of rain on maize pollen dehiscence and flow. In general, maize pollen is released mainly during dry (and drying) conditions from the tassels with the major portion of daily release usually occurring during midmorning to midday (Aylor et al., 2003; Jarosz et al., 2003; 2005; Paterniani and Stort, 1974).

Local environment

Apart from gravity and absorption in rain droplets, pollen flow is directed or filtered by topography and structures as vegetation and buildings (Treu and Emberlin, 2000). The latter affect wind speed profiles, which may result in an additional depletion of airborne pollen (Di-Giovanni and Kevan, 1991; Du et al., 2001; Jones and Brooks, 1952; Raynor et al., 1974). Simulations of Du et al. (2001) revealed that a maize field in itself has a significant effect on wind speed and direction due to its height and the high density of its canopy. Wind speed near the leeward edge was very weak and the wind direction even reversed allowing a huge amount of pollen deposition near the leeward edge of the maize field (Du et al., 2001).

Pollen viability/longevity and water status of pollen

Pollen viability/longevity, which is defined as the ability of pollen to germinate, is an important requisite to complete fertilization. If the viability and vigor of the pollen grain is poor, its capacity to compete with fresher pollen produced in the vicinity of the receptor plant will be poor (Aylor et al., 2003). Maize pollen is susceptible to desiccation, and water loss in pollen grains reduces its ability to germinate on the stigma (Barnabas, 1985; Buitink et al., 1996; Fonseca and Westgate, 2005; Jones and Newell, 1948; Schoper et al., 1986; 1987; Vanryckeghem, 2000). At 30% pollen moisture content maize pollen becomes completely non-viable (Fonseca and Westgate, 2005). Pollen death after release from the anthers is mainly due to dehydration, which is primarily controlled by the vapor pressure deficit of the air (Aylor, 2003; 2004; Barnabas, 1985; Fonseca and Westgate, 2005; Jones and Newell, 1948; Luna et al., 2001), and may be genotype-dependent (Fonseca and Westgate, 2005; Herrero and Johnson, 1980; Schoper et al., 1987). Pollen viability was found to be relatively insensitive to solar radiation (Aylor, 2004). The period of time after dehiscence, during which the pollen grain retains its potential for fertilization is considered to be less than 24 h under French field conditions (Angevin et al., 2001). Aylor (2004) reported that depending on the atmospheric conditions, an exposure of 60 to 240 min to outdoor conditions (Iowa, US) would reduce pollen germination by 50%. Under Mexican outdoor conditions (Nayarit, Mexico), a relative loss in pollen viability of 80% in 1 h and 100% in 2 h was found in a multi-year trial. In the year with the driest atmosphere at anthesis, 100% of the pollen grains became non-viable within 1 h (Luna et al., 2001). Higher midday Belgian temperatures of 28 °C and lower relative humidity (65.6%) occurring on 30–31 July 1999, compared to 21 °C and 69.8% on 26–27 July, reduced pollen viability and vigor, and lowered seed setting on average by a fourth (Vanryckeghem, 2000). The water content of maize pollen does not only play an important role in pollen viability, but also in its flight dynamics (Aylor, 2002; 2003; Aylor et al., 2003). At pollen dehiscence, the water content of pollen is up to *ca.* 60% of its mass. During drying, the shape of maize pollen changes from a prolate spheroid to a crinkled, prismatic solid, and its density increases by *ca.* 16%, and its settling speed decreases by *ca.* 34%. These physical changes are expected to have an impact on potential transport distances. In general, the lightest pollen will travel the longest distances, but it will be the least viable (Aylor, 2002).

Male fertility or sterility

Compared to commercial maize fields where plants are fertile, the seed parents in hybrid seed production fields are emasculated. Female plants make up to 80% of the total plant number in a seed production field. Moreover, the inbred pollinator lines produce less pollen than hybrids due to their less developed tassel. Individual tassels of inbred lines grown in France produced 0.8 to 2×10^6 pollen grains, whilst hybrids produced 6 to 8×10^6 pollen grains (Angevin et al., 2001; Jarosz et al., 2005). Fodder maize hybrids, grown in Belgium at densities of 100 000 plants/ha, produced 1–2 g pollen per plant during anthesis, and inbred lines approximately 0.7 g per plant (Vanryckeghem, 2000), which roughly corresponds with the data mentioned above. Hence the probability of cross-fertilization is increased in seed production fields.

Synchrony in flowering times

The closer the synchrony between anthesis of the pollen donor and silking of the recipient, the higher the probability of cross-fertilization (Angevin et al., 2001; Bassetti and Westgate, 1994; Bock et al., 2002; Uribelarrea et al., 2002; Westgate et al., 2003). Compared to simultaneous sowing, a difference in sowing dates of on average one week reduced the cross-fertilization in the first row of the recipient fields by 50% in Spain. Cross-fertilization was reduced by 75% when sowing differences were on average three weeks (Brookes et al., 2004; Ortega Molina, 2004). Although the authors did not mention whether the earlier sowing was done with pollen donor or recipient, we presume that they installed the recipient before the pollen donor in order to minimize out-crossing.

ON-FARM MANAGEMENT IN ORDER TO REDUCE CROSS-FERTILIZATION

For decades, seed marketing legislation has specified worldwide statutory segregation measures between seed crops and any other mainstream crops of the same species to maximize varietal purity. In order to maintain a purity of 99.8%, expressed in number of plants visible in the next generation, a minimal isolation distance of 200 m between the hybrid seed production plot and neighboring maize pollen sources is prescribed in the EU. The isolation distance can be reduced to 100 m in the presence of a natural barrier between the seed production field and other maize fields. In addition, the seed production plots have to be bordered by at least two rows of the male parent. It is important to note that the seed certification standards are based on practical field experience, and that they take into account different field situations and year-to-year variation in prevailing weather conditions (Bock et al., 2002; Ingram, 2000; Schiemann, 2003). Apart from seed production, experience with co-existence is also available from the cultivation of different maize types that are grown for different uses. Fodder and grain maize co-exist with sweet and/or waxy maize in the EU. Production requirements vary between member states, but several points are common. Impurities of 4% are tolerated in waxy maize, and contracts between growers and processors define the growing conditions and the segregation measures to be respected (*e.g.* isolation distances, cleaning of machinery, restricting harvest to a specific period) (Bock et al., 2002; Schiemann, 2003).

On-farm measures to minimize cross-fertilization may rely on spatial isolation (*e.g.* distances between GM and non-GM maize fields, pollen barriers), temporal isolation (*e.g.* arrangements between farmers on planting period and/or coordination of crop rotations) and on GM-crop-free zones. In the following section on isolation distances and pollen barriers, research data on cross-fertilization are grouped per tolerance threshold (0.9% for food and feed, 0.5% for positively assessed GM events, 0.3% for seed and 0.1% for organic produce), since the thresholds determine the containment needed, and per use of maize, since the significance of adventitious presence and cross-fertilization varies with the use of maize. In grain maize, the potential mixing is restricted to the grain fraction of the plant: here the cross-fertilization level is expressed per grain lot. In corn cob mix and fodder maize, the cross-fertilization (expressed as a percentage of genomes) is expected to be diluted, since vegetative parts of the maize plant are maternal tissue. In non-processed fresh sweet maize, it might be necessary to monitor per individual ear.

The data on cross-fertilization are summarized in Table 2 according to the quantification method used.

Isolation distances

Given the leptokurtic distribution of cross-fertilizations over distance from the pollen source, separating fields with GM and non-GM maize by a zone of open ground or a zone with low growing crops will reduce the extent of cross-fertilization.

0.9% threshold in grains

Different cropping scenarios were tested with the "Matrix based Approach to Pollen Dispersal" (MAPOD) model. Tolstrup et al. (2003) presumed a situation with a moderate (10%) and extensive (50%) acreage of transgenic maize having a seed impurity of 0.5%. The model prescribed an isolation distance of 200 m between GM and non-GM maize in order to achieve the 0.9–1% threshold expressed in percentage of grains (Bock et al., 2002; Tolstrup et al., 2003). Based on published data, on calculations, and on seed production standards, the "Supply Chain Initiative on Modified Agricultural Crops" (SCIMAC) and Ingram (2000) recommended an isolation distance of 200 m. *In situ*, the extent of cross-fertilization expressed in percentage of grains was measured in small recipient plots planted at various distances from the source. In recipient plots of 9.3 m^2 planted at a distance of 300 and 200 m from a source of 3.1 ha, Jones and Brooks (1950) observed out-crossing levels of respectively 0.5 and 1.2%, whilst Narayanaswamy et al. (1997) measured a level of cross-fertilization of 0.50% in a plot (150 m^2) at 200 m from the source (900 m^2). Cross-fertilization levels of 1.04, 0.11 and 0.03% of grains were observed in the first, middle and last subplots (23.5 m^2) of a recipient plot of 0.03 ha at 30 m from the source (3 454 m^2) in 1999 (Jemison and Vayda, 2002). Counts on waxy maize kernels in recipient plots of variable size (0.6–12 ha) planted at different distances (0–25 m) from conventional maize (0.7–13 ha) in France revealed that the overall levels of cross-fertilization, expressed in percentage of grains, were ≤ 0.72% in all recipients in 2002 (Fabié, 2004; Foueillassar and Fabié, 2003). To reach the 0.9% threshold in the first 5 m of all the recipients, they recommended an isolation distance of 10 to 25 m. Bénétrix (2004) and Melé (2004) observed an overall level of cross-fertilization, expressed in percentage of genomes, of 0.4 and 0.83% respectively for the recipient fields planted

Table 2. Overview of studies on cross-fertilization in maize per quantification approach (July 2005).

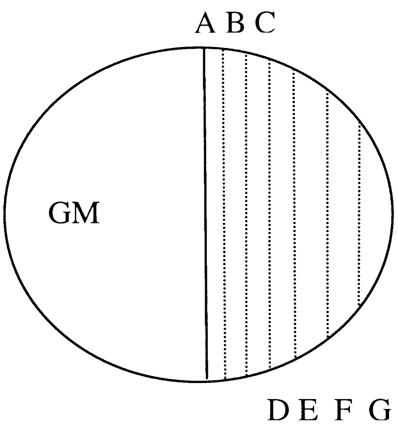
Experimental designs	Cross-fertilizations	References
Molecular quantification of **plants**: adventitious cross-fertilization expressed as a percentage of genomes		
<u>Germany / 2004</u> - 18 sites: source (0.3–23 ha) surrounded in all directions by a recipient area with a width of at least 60 m - per field 12 samples were taken from the harvester in 4 cardinal directions at 3 distances (0–10, 20–30 and 50–60 m strips) / in larger fields (≥ 60 m strips) additional samples were taken	- in 0–10 m strip: 1.15% / 20–30 m strip: 0.24% / 50–60 m strip: 0.15% - in 0–60 m strip: 0.43% / 0–80 m: 0.36% / 0–90 m: 0.33% / 0–100 m strip: 0.31%	Weber et al., 2005
Molecular quantification of **grains**: adventitious cross-fertilization expressed as a percentage of genomes		
<u>France / 2003</u> - 1 site: source (2.3 ha) surrounded by recipient (9 ha) or source (2.3 ha) adjacent to recipient (2.3 ha) in downwind direction - samples (6 × 5 ears) were taken in each subplot (variable size) of recipient	- at distances < 10 m in downwind direction: 1–2% / > 10 m: < 0.9% - in entire adjacent recipient: 0.4%	Bénétrix, 2004
<u>UK / 2000-2002</u> - 1 site: source (3.3 ha) separated by 142 m bare ground from recipient (3.3 ha) - 55 sites: source (3.3 ha) adjacent to recipient (3.3 ha) - samples (3–5 ears) were collected at distances of 2, 5, 10, 20 or 25, 50 and 150 m along 3 transects at *ca.* ¼, ½ and ¾ of recipient (6 transects were sampled in 2000) - at 2 sites samples were taken over larger distances (*e.g.* 650 m)	- at 2 m: 0.026 and 0.1% but none thereafter except at one sample point at 50 m: 0.06% - at 50 m; 55 sites tested; 34 sites: > 0.1%, 23 sites: > 0.3% - at 150 m; 44 sites tested; 12 sites: ≥ 0.1%, 7 sites: ≥ 0.3% - at 200 m; 3 sites tested; 0.14 and 0.42% - at 650 m; 2 sites tested; 0.14% - fitted cross-fertilization rates (regression equation); at 24.4 m: 0.9% / 80 m: 0.3% / 257.7 m: 0.1% / 650 m: 0.04% - total recipients; 26 sites ≥ 1% / after removal of 1st 80 m of recipient: 2 sites ≥ 1%	Henry et al., 2003
<u>Spain / 2003</u> - 1 site: a circle of *ca.* 46 ha with source (23.3 ha) adjacent to recipient (23.3 ha) - samples were taken at different distances 	- at 1 m: 6.86% / 2.8 m: 5.28% / 5.6 m: 3.22% / 8.4 m: 1.43% / 12.6 m: 0.68% / 16.1 m: 0.55% / 40.6 m: 0.45% / 90.3 m: 0.2% / 140.7 m: 0.07% / 190.4 m: 0.07% / 241.5 m: 0.04% / 290.5 m: 0.02% / 340.2 m: 0.05% - <u>in area A</u>; 1st 7 m (0.27 ha): 4.57% / after removal of 1st 4 r: 2.67% / after removal of 1st 8 r: 1.84% - <u>in area A+B</u>; 1st 14 m (0.53 ha): 2.73% / after removal of 1st 4 r: 1.79% / after removal of 1st 8 r: 1.20% - <u>in area A+B+C</u>; 1st 28 m (1.06 ha): 1.64% / after removal of 1st 4 r: 1.11% / after removal of 1st 8 r: 0.78% - <u>in area A+B+C+D</u>; 1st 70 m (2.6 ha): 0.94% / after removal of 1st 4 r: 0.68% / after removal of 1st 8 r: 0.55% - <u>in area A+B+C+D+E</u>; 1st 140 m (5 ha): 0.54% / after removal of 1st 4 r: 0.42% / after removal of 1st 8 r: 0.35% - <u>in area A+B+C+D+E+F</u>; 1st 210 m (8 ha): 0.36% / after removal of 1st 4 r: 0.28% / after removal of 1st 8 r: 0.24% - <u>in total area</u>; total recipient (23.3 ha): 0.26% / after removal of 1st 4 r: 0.20% / after removal of 1st 8 r: 0.17%	INIA/ITAP/CSIC/ Monsanto/Pioneer Hi-bred/ Nickersons Sur, 2004 in Ortega Molina, 2004

Table 2. Continued.

Spain / 2003 - 1 site: source (0.19 ha) surrounded by recipient (2.1 ha) - samples were taken at different distances	at 2 m: 16.4% / 4 m: 4.01% / 6 m: 1.18% / 9 m: 0.58% / 11 m: 0.38% / 13 m: 0.3% / 17 m: 0.24% / 22 m: 0.17% / 27 m: 0.09% / 40 m: 0%	INIA/ITAP/CSIC/ Monsanto/Pioneer Hi-bred/ Nickersons Sur, 2004 in Ortega Molina, 2004
Spain / 2003 - 1 site: source (0.25 ha) surrounded by recipient (7.5 ha) - samples (6 × 3 ears) were taken at distances of 1, 2, 5 and 10 m in 4 cardinal directions - behind 10 m recipient was divided in subplots (30 × 30 m) / 1 sample was taken from each subplot	- at 10 m downwind: < 0.9% / 2 m upwind: < 0.9% - throughout recipient: < 0.2% / hot spot at 40 m: 0.97% - in recipient area lying in line with donor in downwind direction; 1 ha: 0.83% / 0.25 ha: 1.77% / 0.25 ha starting 6 m from the donor: 0.77%	Melé, 2004; Messeguer et al., 2003
Germany / 2004 - 8 sites: source (1.8–18.3 ha) surrounded in all directions by a recipient area with a width of at least 60 m - per field 12 samples were taken from the harvester in 4 cardinal directions at 3 distances (0–10, 20–30 and 50–60 m strips)	- in 0–10 m strip: 0.98% / 20–30 m strip: 0.33% / 50–60 m strip: 0.11% - in 0–60 m strip: 0.44%	Weber et al., 2005
Molecular detection of grains, xenia in grains, germination of F_2-kernels on selective medium and/or herbicide spraying of F_2-seedlings: adventitious cross-fertilization expressed as a percentage of **grains**		
UK - 1 site: source (1 m^2), recipients: 2 single rows with a length of 23.8 and 25.6 m starting next to source and oriented in opposite direction - all plants were sampled	at 0.6 m: 70% / 1 m: 54–67% / 2.5 m: 32–46% / 3–9 m: 0–41% / 10–15 m: 0–7% / 16–20 m: 0–3% / 21–23 m: 0–2% / 24–26 m: < 1%	Bateman, 1947
France / 2002 - 3 sites: source (0.4 ha) next to recipient - samples (ears) were taken over a distance up to 240 m	- at distances of 10–12 m downwind: < 1% / 5–7 m upwind: < 1% / 25 m downwind: < 1% with very strong wind - entire recipients: < 0.9% / only > 0.9% in border rows	Bénétrix and Bloc, 2003; Brookes et al., 2004
US / 2002–2003 - 3 sites (1 in 2002, 2 in 2003): source (0.8 ha) surrounded by recipients (12.1–56.7 ha) with samples (10 ears) taken at distances between 0.8–305 m along 4–5 transects - 1 site (2003): recipient adjacent to source with samples (10 ears) taken at distances of 3 to 296 m along 2 transects	- for surrounding designs; at 0.8 m: 20%, 35% and 46% / 45.7 m: < 0.9% / farthest distance of detected cross-fertilization: 182.8 m - for adjacent design; at 3.1 m: 48% / 37 m: 0.75% / farthest distance of detected cross-fertilization: 82.3 m	Byrne and Fromherz, 2003
France / 2001–2002 - 27 sites (15 in 2001, 12 in 2002): plots in current agricultural situations: source plots (0.7–13 ha) sown to fodder maize in vicinity (0–25 m) of recipients (0.6–12 ha) sown to waxy maize - 90 ears (18 × 5 ears) were taken in 1st 6 rows / 150 ears dispatched in recipient field and grouped by distance (5–50, 50–100 and 100–150 m)	data for 2002 - plot separated by 0 m; at 1st 5 m: 6.20% / total: 0.41% - 8 plots separated by 2–8 m; at 1st 5 m: 2.34 / total: 0.16% - 2 plots separated by 10 m; at 1st 5 m: 1.03% / total: 0.54% - plot separated by 25 m; at 1st 5 m: 0.67% / total: 0.11% - total recipients: ≤ 0.72% / after removal of 1st 5 m: < 0.07% - hits indicated by xenia were divided by 2 to deliver these data	Fabié, 2004; Foueillassar and Fabié, 2003
Mexico / 1995–1997 - 5 sites (4 in 1996, 1 in 1997): isolated crossing blocks	at distances > 185 m: 0%	Garcia et al., 1998

Table 2. Continued.

Spain / 2003 - 14 sites: *in situ* situations where GM maize was planted close to conventional maize (plots of variable size, variable distances between sources and recipients) - samples (100 ears) were taken at the 1st, 4th, 8th and 16th row	- fields with same sowing dates; 1st r: 16.93% / 4th r: 2.73% / 8th r: 1.18% / 16th r: 1.02% - fields with < 2 weeks difference in sowing dates; 1st r: 7% / 4th r: 0.97% / 8th r: 0.63% / 16th r: 0.6% - fields with > 2 weeks difference in sowing dates; 1st r: 3.56% / 4th r: 0.84% / 8th r: 0.56% / 16th r: 0.26%	INIA/ITAP/CSIC/ Monsanto/Pioneer Hi-bred/ Nickersons Sur, 2004 in Brookes et al., 2004
US / 1999–2000 - 1 site (1999): 2 recipient plots (0.03 ha) at 30 and 350 m distance from source (0.35 ha) - 1 site (2000): 2 recipient plots (0.03 ha) at 30 and 100 m distance from source (0.35 ha) - recipient plots were divided in 12 subplots (23.5 m^2) - samples (50 ears) were harvested from each subplot	- plots at 30 m; 0 m: 1.04–1.65% / 5 m: 0.11–0.86% / 10 m: 0.03–1.14% - plot at 100 m; 0 m: 0.65% / 5 m: 1.04% / 10 m: 1.38% (this plot was sown with impure seed) - plot at 350 m; 0 m: 0%	Jemison and Vayda, 2002
US / 1947–1949 - 1 site (1 each year): 7 recipient blocks (9.3 m^2) at 0, 25, 75, 125, 200, 300, 400 and 500 m downwind from source (3.1 ha) - in each recipient block ears of 10 plants per row were sampled	- at 0 m: 28.6% / 25 m: 14.2% / 75 m: 5.8% / 125 m: 2.3% / 200 m: 1.2% / 300 m: 0.5% / 400 m: 0.2% / 500 m: 0.2% - at 0 m; 1st 5 r: 51.7%, 20–25 r: 9.5% / 25 m; 1st 5 r: 32.9%, 20–25 r: 5.6% / 75 m; 1st 5 r: 16%, 20–25 r: 3% / 125 m; 1st 5 r: 7.3%, 20–25 r: 1.6% / 200 m; 1st 5 r: 4.4%, 20–25 r: 0.7% / 300 m, 1st 5 r: 1.8%, 20–25 r: 0.2% / 400 m; 1st 5 r: 0.5%, 20–25 r: 0.2% / 500 m; 1st 5 r: 0.5%, 20–25 r: 0.1%	Jones and Brooks, 1950
Mexico / 1997–2000 - 3 sites (1 in 1998, 2 in 1999): recipient blocks (12.8 m^2) located in 4 cardinal directions at 100, (150), 200, 300 and 400 m distance from source (0.4 ha) - sampling protocol not defined	- 3 cases of cross-pollination whereof 1 at 100, 150 and 200 m - 300 m: 0%	Luna et al., 2001
Canada / 2000–2002 - 3 sites (3 each year): source (729 m^2) surrounded by recipient (total area: 0.68–1 ha) - ear samples were taken at rows 1, 7, 13, 19, 25, 31 or 33, 37, 43 and 48 from every 10th plant	prevailing wind direction; at 0.8 m: 2.2–62.3% / 5.3 m: 0.3–13.1% / 9.9 m: 0–15.6% / 14.4 m: 0–4.5% / 19 m: 0–2.7% / 23.6 m: 0–3.1% / 28.1 m: 0–3.2% / 32.8 m: 0–2.1% / 36.5 m: 0–0.6%	Ma et al., 2004
India / 1993, 1995 - 2 sites (1 in 1993, 1 in 1995): recipient plots (150 m^2) with rows of female and male plants at 100, 200, 300, 400, 500 and 600 m from source (900 m^2) - ears were sampled in each row	at 100 m: 2.802–2.890% / 200 m: 0.483–0.500% / 300 m: 0.144–0.145% / 400 m: 0.052–0.055% / 500 m: 0.006% / 600 m: 0.001%	Narayanaswamy et al., 1997
Brazil / 1962, 1964 - 4 sites (3 in 1962, 1 in 1964): 1 source plant completely surrounded by recipient area (300 m^2, 475 m^2, 740 m^2 and 0.16 ha) - the ears from all recipient plants were harvested	at 1 m: 0–4.11% / 2 m: 0–0.42% / 3 m: 0.06–0.25% / 4 m: 0.02–0.15% / 5 m: 0.04–0.17% / 6 m: 0.03–0.13% / 7 m: 0–0.08% / 8–34 m: < 0.04%	Paterniani and Stort, 1974
USSR - 1 site: recipient (10 ha) downwind from source (2 ha) - at each distance samples (30 000 seeds) were taken from 50 plants	at 12 m: 3.3% / 50 m: 0.33% / 100 m: 0.36% / 150 m: 0.25% / 200 m: 0.54% / 400 m: 0.02% / 500 m: 0.08% / 600 m: 0.79% / 700 m: 0.18% / 800 m: 0.21%	Salamov, 1940 in Jones and Brooks, 1950

Abbreviation: r = row.

adjacently to or surrounding the pollen source. In a recipient sampling area of 5 and 23.3 ha adjacent to a source of 23.3 ha, Ortega Molina (2004) reported respectively 0.54 and 0.26% GM presence expressed in percentage of genomes.

0.5% threshold in grains and plants

Based on a literature study, Sanvido et al. (2005) concluded that an isolation distance of 50 m for sweet maize and 25 m for fodder maize would be sufficient to keep the cross-fertilization levels below the 0.5% at the border of the non-GM crop. Due to the dilution by mixing the material of an entire field, the authors assumed that the average cross-fertilization rate would be definitely less than 0.5%.

0.3% threshold in grains

The isolation distance needed between GM maize and non-GM maize seed production has been estimated to be 300 m by MAPOD (Bock et al., 2002; Tolstrup et al., 2003), and 200 m by SCIMAC and Ingram (2000). In Mexico, Garcia et al. (1998) found complete pollen control at a distance larger than 184 m in different small-scale seed production experiments.

0.1% threshold in grains

In the context of Mexican experimental field trials with commercially unapproved transgenic maize, Luna et al. (2001) recommended an isolation distance of 185–200 m. Only three cases of cross-fertilization were observed via xenia, whereof one at 100, 150 and 200 m. At 300 m distance from the source, no cross-fertilization from the source was detected (Luna et al., 2001). In a recipient plot (3.3 ha) in the UK that was separated by 142 m bare ground from the source (3.3 ha), the levels of cross-fertilization expressed in percentage of genomes did not exceed 0.1% in the harvested grains (Henry et al., 2003).

The higher isolation distances recommended to obtain cross-fertilization levels below 0.1%, probably results from the small percentage of pollen in the tail of the pollen distribution curve. The small pollen fraction may still be translated into relevant out-crossing amounts at considerable distances from a source. Erratic cross-fertilization events have been measured up to 650 m from the GM source and cross-fertilization hot spots (0.4% of genomes) were seen at distances of 100–150 m from the GM source (Henry et al., 2003). Also, older studies reported low levels of cross-fertilization at larger distances. Salamov (1940), cited in Jones and Brooks (1950), found 0.2% at 800 m and Jones and Brooks (1950) 0.2% at 500 m. Jones and Brooks used the white colored sweet maize "Honey June" as a receptor and a yellow dent fodder maize as a pollinator: any cross-fertilization resulted in a non-shriveled yellow kernel on the recipient plants. By this double control they could clearly distinguish cross-fertilization from a potential transposon activity known to be present in some white maize lines. Transposon activity may result in some colored grains, overestimating cross-fertilization. Any seed impurity of "Honey June" would have had the same result, but the authors did not give information on the seed purity. Convective air currents resulting from the warmed up air above a crop could explain the aerial concentrations of pollen above maize fields and the occurrence of cross-fertilization hot spots over longer distances than expected. Another explanation for the cross-pollination hot spots may be the occurrence of late developing plants (*e.g.* due to genetic impurity or to field heterogeneity), provided that there is a good flowering synchrony with a sympatric late flowering pollen donor. Due to the smaller pollen cloud hanging over the recipient, late developing plants are more prone to cross-fertilization. Bannert and Stamp (personal communication) observed that from the 5.5% classified late (small) ears, 64% had cross-fertilization rates of up to 80% contributing to 47% of the out-crossing of the whole field. From the 74% normal ears, only 8.5% had cross-fertilized kernels generally limited to a few cross-fertilized kernels per ear.

Pollen barriers

Plants sown around the source or recipient field can function as pollen barrier. If the outer parts of fields function as a barrier, the distance between the inner parts increases. Moreover, barriers introduce competing pollen (if the barrier is of the same species as the crop) and/or may serve as a physical barrier to air and consequently pollen flow. A physical barrier will deplete some pollen from the airflow by impaction and filtering, and will create a sheltered zone in the lee (Du et al., 2001; Emberlin et al., 1999; Raynor et al., 1974; Treu and Emberlin, 2000). Jones and Brooks (1952) compared the effectiveness of bare ground, a barrier of trees and a barrier of maize in reducing cross-fertilizations. The maize and tree barrier reduced cross-fertilizations more effectively than bare ground. The levels of cross-fertilization were reduced by 50% immediately behind the barrier. The reduction of cross-fertilization was considerably less with the tree barrier than with a maize barrier, presumably because the trees did not provide any competing pollen.

0.9% threshold in grains

Henry et al. (2003) mimicked worst-case commercial on-farm situations in the UK through the use of a split-field design. They quantified the presence of transgenic material in grain samples in terms of percentage of genomes, and inferred from the results that at a distance of 24.4 m the average level of cross-fertilization in the recipient fields remained under 0.9%. In Spanish studies

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with an adjacent pollen donor and recipient, out-crossing levels of 0.68 and 0.58% of genomes were observed at 12.6 and 9 m in the recipient fields, respectively (Ortega Molina, 2004). Meier-Bethke and Schiemann (2003) studied cross-fertilization in different situations: 1) pollen donor and recipients adjacent to each other, 2) recipient fields separated by the donor by gaps of different lengths, and 3) two recipient fields in line and separated by gaps from the donor and each other. The first rows of the fields after a gap showed about five times more cross-fertilizations than measured at the same distance in adjacent continuous fields (*e.g.* 1.5 *vs.* 0.3% of grains at 50 m from the source). The peak fell quickly, and within a few rows out-crossing levels were comparable. This phenomenon was also observed in the second recipient plot planted behind the first. The message is that when different fields are separated by gaps, the first rows of recipient fields are more cross-fertilized than in a continuous field at the same distance. Other studies confirmed the high interception of pollen by the first few maize rows when open ground or low growing barrier crops separate maize fields (Bannert and Stamp, personal communication; Burris, 2001; Henry et al., 2003; Jones and Brooks, 1950; 1952; Meier-Bethke and Schiemann, 2003; Messeguer, personal communication; Ortega Molina, 2004). The removal of the first few rows of a plot facing a GM crop prior to harvest might be worthwhile to reduce the total level of cross-fertilization in the recipient. Recent observations suggest that after a 10 to 20 m barrier of maize, the 0.9% GM presence expressed in percentage of genomes almost never was exceeded in the harvested grains (Bénétrix, 2004; Melé, 2004; Messeguer, personal communication; Ortega Molina, 2004; Weber et al., 2005). According to other studies where the source and recipient were planted next or close to each other, the removal of 4 to 8 rows was effective to end up with a cross-fertilization level of the entire field below 0.9% (Fabié, 2004; Foueillassar and Fabié, 2003; Ortega Molina, 2004). In a Spanish study where the source and recipient of equal size were sown next to each other as two halves of a circle (*ca.* 46 ha), the adventitious presence of GM material expressed in percentage of genomes was 4.57, 2.73, 1.64, 0.94, 0.54, 0.36 and 0.26% for the sampling strips (design see Tab. 2) of 0.27, 0.53, 1.06, 2.6, 5, 8 and 23.3 ha respectively (Ortega Molina, 2004). Removing the first four rows in all sampling strips decreased the presence of transgenic material to 2.67, 1.79, 1.11, 0.68, 0.42, 0.28 and 0.20% respectively. The lowest levels of adventitious presence being 1.84, 1.20, 0.78, 0.55, 0.35, 0.24 and 0.17% were observed after the removal of the first eight rows (Ortega Molina, 2004). In French maize fields of 0.6–12 ha, the removal of the first 5 m reduced the levels of cross-fertilization from 0–0.72% of grains to levels below 0.07% of grains in all recipient fields (Fabié, 2004; Foueillassar and Fabié, 2003).

0.9% threshold in plants

The only experiment where entire plants were harvested and chopped for analysis, inferred that in 6 of the 18 sites the levels of cross-fertilization expressed in percentage of genomes exceeded the 0.9% threshold in the first 10 m strip of the recipient fields adjacent to the pollen donor fields. The amounts of cross-fertilization were on average 0.24, 0.15, 0.43 and 0.31% in 20–30, 50–60, 0–60 and 0–100 m strips away from the edge with the pollen donor. Behind a strip of 20 m the 0.9% threshold was not exceeded (Weber et al., 2005).

0.3–0.1% thresholds in grains

In the UK, the average level of cross-fertilization expressed in percentage of genomes in grain samples remained under the 0.3 and 0.1% at a distance of 80 and 257.7 m in the recipient fields respectively (Henry et al., 2003). At 90.3 and 140.7 m in the recipient, the levels of cross-fertilization were respectively 0.2 and 0.07% of genomes in Spain (Ortega Molina, 2004).

Scheduling different crop production cycles

The within-year isolation in time of GM and non-GM maize in order to prevent cross-fertilization is a theoretical co-existence measure, as the synchronization of pollen dispersal and silking has been demonstrated to be crucial in determining the extent of out-crossing in maize (Bassetti and Westgate, 1994; Uribelarrea et al., 2002; Westgate et al., 2003). A difference in sowing dates may result in a difference in flowering time, hence limiting cross-fertilization. However, this approach may not be realistic all over the EU or only of limited use. Because of its frost susceptibility, maize can not be planted earlier in the season. Depending on climate conditions, postponing the sowing might be at the expense of yield. Early varieties tend to flower earlier than later varieties, but early varieties are less productive and the dry matter content of late varieties may be too low at harvest time. Moreover, Weber et al. (2005) could not avoid overlapping flowering periods by choosing different sowing dates or varieties differing in the development in the German trials carried out in 2004. In Mediterranean European countries, however, this approach may be workable without losses in yield (Brookes et al., 2004; Messeguer, personal communication; Ortega Molina, 2004). Again theoretically, farmers might adjust their crop rotations in order to schedule maize crops over different years. Such a strategy will demand very tight discipline and good agreements between neighbors. It will be hampered by market-driven production strategies, by the share of the maize crop in a specific region and by growing maize in monoculture as it is practiced frequently in a number of member states.

GM-crop-free zones

A report of the European Parliament adopted on 18 December 2003 (2003/2098(INI)) recognizes that member states have the right to prohibit completely the cultivation of transgenic crops in geographically restricted areas in order to safeguard co-existence. The rationale behind this report is that the voluntary or regionally restricted renunciation of cultivation of transgenic crops is probably the most effective and least costly measure to ensure co-existence. Important conditions to install GM-crop-free zones are that farmers jointly decide on a voluntary basis not to grow transgenic crops in a specific region, and that a bottom up approach is followed. Then the competent authority can declare a ban on the cultivation of transgenic crops for a limited period of time in that region. On the opposite, farmers wishing to grow GM crops can demand the creation of a GM crop production zone. Only economic considerations (*e.g.* protection of local traditional agriculture) will be taken into account in the decision for the creation of GM-crop-free zones. To date, different zones have been declared GM-crop-free in Austria, France, Germany, Greece, Hungary, Italy, Poland, Spain and the UK.

CONCLUSIONS

Various parameters, *e.g.* isolation distance between the pollen source and recipient, size, shape and orientation of the pollen source and recipient, wind characteristics, rain, local environment, pollen viability, water status of pollen, male fertility, flowering synchrony, commercial destination of maize, sampling protocol, approaches used to study pollen dispersal, quantification methods, and analyzed plant material, with varying levels of relative importance have been identified to play a role in the study of cross-fertilization in maize. Some of these are under little or no human control, whereas others can be managed properly. Considering this variability, generic co-existence measures may not be appropriate to limit the adventitious presence of GM material in non-GM product resulting from pollen-mediated gene flow. A certain degree of flexibility and adaptability to different situations should be accepted.

Existing data on pollen dispersal in maize demonstrated that the levels of cross-fertilization drop rapidly over the initial meters around the pollen source. Most of the released pollen is deposited within about 30 m of the source. At distances farther than 30–50 m from the source, pollen dispersal is very low but not zero. Due to convective air currents, erratic cross-fertilization hot spots have been observed up to 650 m from a known GM source (Henry et al., 2003). The occurrence of late-developing plants may also explain cross-pollination peaks in recipient fields (Bannert and Stamp, personal communication).

Based on the available information the following situations may be considered. If the 0.9% threshold is to be achieved in *maize grains* of an entire recipient field, three different cases could be distinguished based on the size (and thus the width) of the recipient plot. (1) Recent experiments mimicking worst-case commercial on-farm situations (*e.g.* pollen source next to or completely surrounded by recipient) indicated that the overall levels of cross-fertilization remain under the 0.9% threshold when the receptor plots are over 5 ha. Owing to the huge pollen cloud above such large recipient fields an isolation distance is not required. (2) For plots within the range of 5 to 1 ha, containment may be necessary. Three different strategies may be followed or combined. (2a) An isolation distance increasing with decreasing size of the recipient may be foreseen between the pollen source and recipient fields. However, defining the length of the appropriate isolation distance under commercial agronomic settings is not possible at the moment. Levels of cross-fertilization were only measured in small recipient plots planted at various distances from the source. A range of isolation distances lying between 10 to 50 m may be recommended based on cross-fertilization data and pollen counts done at various distances from the source. If an isolation distance is not possible, the recipient and/or donor plot can be bordered by a pollen barrier. (2b) In the receptor field, the outer maize rows may be considered as this pollen barrier. After a pollen barrier of maize of 10–20 m, almost none of the remaining maize contains more than 0.9% GM material. At harvest, the outer rows of a recipient field can be discarded or be classified as "GM maize" if the GM content exceeds the tolerance threshold. (2c) From a political/legal point of view, bordering the transgenic maize with a pollen barrier of non-GM maize might be preferred, since GM crop production is generally regarded as the "newcomer" in most European countries. Farmers growing GM crops will thus be required to take the appropriate on-farm measures limiting adventitious mixing. It is, however, unclear if a pollen barrier around the donor will reduce cross-fertilizations as effectively as a barrier of the same width around the recipient. (3) For recipient plots smaller than 1 ha and/or plots of low depth, an isolation distance of at least 50 m may be recommended especially in the main wind direction.

If the 0.9% threshold expressed in percentage of genomes is to be achieved in *fodder maize* the results of Weber et al. (2005) indicated that (1) a pollen barrier of 20 m is sufficient to maintain the rest of the field under the 0.9% threshold and that no isolation is required when (2a) the recipient field is deeper than 90 m or when (2b) adjacent donor and recipient plots are of the same size.

Because data are actually scarce in commercial situations with thresholds tighter than 0.9%, it is difficult to recommend reliable isolation distances. Under these conditions, pollen dispersal occurring over larger distances is expected to be of greater importance but needs further investigation. Also the effectiveness of pollen barriers in reducing cross-fertilization over long distances is questioned, because pollen that is coming down from warm upstream air layers probably will not be hindered much by barriers growing close to the ground (Meier-Bethke and Schiemann, 2003).

The proposed containments may be subject to future refinement. First, they are based on data obtained from studies relying on experimental designs with a single pollen source. If the cultivation of transgenic maize expands, different pollen sources with different GM events will have to be considered. Depending on the relative share of GM maize and its distribution pattern in the agricultural area, distances may need to be adjusted. When data become available from areas where GM and non-GM maize are growing together in different proportions, fine-tuning will be possible. In this situation, models may be validated to predict pollen flow at the landscape level, under different spatial distributions of maize cultivars and different cropping systems. Second, in practice, adventitious mixing may occur within a field owing to impure seed, while in nearly all experiments the seed was considered as genetically pure. Third, except for the experiments of Weber et al. (2005) data on fodder maize are lacking. Fourth, some of the cited experiments continue to run and will provide further data in the near future (*e.g.* Bannert and Stamp, personal communication; Bénétrix, 2004; Henry et al., 2003; Messeguer, personal communication; Weber et al., 2005). Attempts to fill most of the knowledge gaps and to collect data in a more uniformly standardized way are currently pursued within the framework of the "Sustainable Introduction of GMOs into European Agriculture" (SIGMEA) and the "GM and non-GM supply chains: their Co-Existence and traceability" (Co-Extra) projects. Both projects are funded and performed within the context of the Sixth EU Framework Programme (FP6) for Research and Technological Development and Demonstration (RTD). Detailed information on the SIGMEA and Co-Extra projects is available on http://sigmea.dyndns.org/ and http://www.coextra.org/, respectively.

There are important differences between seed production fields and the production of food and feed crops. Usually thresholds are tighter in seed production, particularly in the production of pre-basic and basic seed. As a seed crop has a high added value, it is acceptable and affordable for the seed producer to apply a strong protection management. The seed producer can decide to grow seed crops in special areas with low out-crossing risks, while farmers usually cannot move crops (*e.g.* in GM-crop-free zones). Under current agricultural practices, the use of isolation distances and/or pollen barriers seems to be the most important tool to minimize unwanted cross-fertilization. In regions with a very high share of maize in crop rotations, the introduction of additional strategies, such as agreements between farmers concerning crop sequences, sowing dates, and choice of varieties aiming at different maturity groups, may be necessary to improve co-existence. If nothing helps, GM-crop-free zones may be the ultimate solution.

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