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Definition and feasibility of isolation distances for transgenic maize cultivation

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Abstract A major concern related to the adoption of genetically modified (GM) crops in agricultural systems is the possibility of unwanted GM inputs into non-GM crop production systems. Given the increasing commercial cultivation of GM crops in the European Union (EU), there is an urgent need to define measures to prevent mixing of GM with non-GM products during crop production. Cross-fertilization is one of the various mechanisms that could lead to GM-inputs into non-GM crop systems. Isolation distances between GM and non-GM fields are widely accepted to be an effective measure to reduce these inputs. However, the question of adequate isolation distances between GM and non-GM maize is still subject of controversy both amongst scientists and regulators. As several European countries have proposed largely differing isolation distances for maize ranging from 25 to 800 m, there is a need for scientific criteria when using cross-fertilization data of maize to define isolation distances between GM and non-GM maize. We have reviewed existing cross-fertilization studies in maize, established relevant criteria for the evaluation of these studies and applied these criteria to define science-based isolation distances. To keep GM-inputs in the final product well below the 0.9% threshold defined by the EU, isolation distances of 20 m for silage and 50 m for grain maize, respectively, are proposed. An evaluation using statistical data on maize acreage and an aerial photographs assessment of a typical agricultural landscape by means of Geographic Information Systems (GIS) showed that spatial resources would allow applying the defined isolation distances for the cultivation of GM maize in the majority of the cases under actual Swiss agricultural conditions. The here developed approach, using defined criteria to consider the agricultural context of maize cultivation, may be of assistance for the analysis of cross-fertilization data in other countries.

Keywords Genetically modified crops · Pollen-mediated gene flow · Coexistence · Cross-fertilization · *Bt*-maize · GIS

Introduction

There are concerns that the adoption of genetically modified (GM) crops in agriculture could lead to unwanted GM-inputs in non-GM crop production systems. This concern has become of particular interest when the European Union (EU) entered the first GM *Bt*-maize varieties into the Common EU Catalogue of Varieties in September 2004 (European Commission 2004), making it likely that the

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commercial cultivation of Bt-maize will further expand in several European countries (GMO compass 2007). Given the specific regulations in the EU on labelling, threshold values and traceability of GM organisms (European Union 2003a, b), an urgent need to define the conditions and measures required to ensure coexistence of GM and non-GM crops on a scientific, legal, and administrative basis has become apparent. The term "coexistence" is hereby defined as the ability of farmers to make a practical choice between conventional, organic and GM-crop production given that GM-free production is warranted (European Commission 2003). This, however, requires specific measures to prevent mixing of GM with non-GM products during crop production. Taking into account that adventitious presence of GM material cannot be entirely avoided, the EU has defined a 0.9% threshold as the maximum percentage of GM material that may be contained in food and feed without the need to be specifically labelled as containing GM material (European Union 2003a). Similar to the EU, Swiss legislation stipulates that protection of GM-free production and consumers' freedom of choice must be guaranteed if GM crops were commercially cultivated (GTG SR 814.91). Swiss legislation has also adopted the 0.9% threshold for food and feed defined by the EU (LGV SR 817.02).

As a general principle, the farmer introducing GM crops should bear the responsibility of implementing the farm management measures necessary to limit mixing of GM and non-GM crops (European Commission 2003; GTG SR 814.91). Apart from specific agronomic measures that are necessary to ensure the coexistence of GM and non-GM crop production systems, there are a number of economic and social aspects that also need to be clarified. These aspects include, for example, the costs of implementing and coordinating coexistence measures among GM and non-GM farmers, incentives for non-GM farmers to support specific measures, as well as liability issues related to accidental admixtures exceeding the legal threshold. Although these economic and social aspects are of fundamental importance when discussing the success of any coexistence-strategy, they exceeded the scope of the present study and were thus not addressed. In the present study, we focussed on the agronomic aspect of coexistence and particularly on the necessary isolation distances measures to reduce cross-fertilization between GM and non-GM maize.

Various mechanisms have been identified, which may lead to a mixing of GM and non-GM products in the agricultural production chain (Bock et al. 2002; Tolstrup et al. 2003; Sanvido et al. 2005). These mechanisms include introductions via seed impurities, volunteers from GM pre-cultures, cross-fertilization by pollen from GM crops, as well as seed dispersal through mixing in machinery during sowing, harvesting and transport. Taking into account the experiences from existing systems to maintain the purity of specific agricultural commodities (identity preservation) (Sundstrom et al. 2002), several technical and organizational measures have been proposed, which can help farmers to reduce these GMinputs (Bock et al. 2002; Tolstrup et al. 2003; Sanvido et al. 2005). Inputs due to seed impurities can be minimized by using certified seeds. Volunteers can be controlled by using crop rotation as well as by ensuring optimal soil preparation techniques after harvest or before sowing (Pekrun et al. 1998; Gruber et al. 2004). The extent of cross-fertilization between fields of GM and non-GM crops can effectively be reduced by using isolation distances and/or buffer zones as pollen barrier (Ingram 2000; Eastham and Sweet 2002). The risk of mixing in machinery can be reduced by adequate cleaning practices of machines after use on GM crop fields or by using machinery separately for GM or non-GM crops. A clear segregation of harvested material and the documentation of procedures during storage, processing and transport from field to delivery of harvest can further minimize the risk of mixing.

Among the various mechanisms that could lead to GM-inputs into non-GM crop production systems, cross-fertilization is certainly one of the most widely discussed issues. Given that maize is a cross-pollinating species and its pollen is transported by wind, the commercial cultivation of *Bt*-maize in several European countries has arisen specific concerns related to unwanted GM-inputs from GM maize fields into non-GM maize products through cross-fertilization. The importance of cross-fertilization in contributing to GM inputs is, however, still subject of controversy both amongst scientists and regulators. This is particularly apparent for the question of adequate isolation distances between GM and non-GM maize fields in order to keep GM-inputs below

the 0.9% threshold. Several European countries have proposed largely differing isolation distances for maize ranging from 25 m in the Netherlands (van Dijk 2004) up to 800 m in Bulgaria (GMO safety 2006). Most of these recommendations were based on scientific studies assessing cross-fertilization rates in maize, but a general interpretation of the different results is often difficult because experimental conditions usually differ between studies and various factors are known to influence cross-fertilizations rates (Ingram 2000; ACRE 2002; Devos et al. 2005). These factors include pollen viability and longevity, male fertility or sterility, synchrony in flowering between anthesis of the pollen donor and silking of the recipient field, wind direction and velocity, weather conditions, size, shape and orientation of both pollen source and recipient field, as well as distance, topography and vegetation between pollen source and recipient field.

There is a need for scientific criteria when analysing cross-fertilization data of maize to define isolation distances between GM and non-GM maize fields. The scope of the here presented study was therefore restricted to this particular aspect. The objectives of this study were (1) to review existing studies, which have assessed cross-fertilization in maize, (2) to establish relevant criteria for evaluating these studies, and (3) to apply these criteria for the definition of isolation distances. Evaluation criteria should consider biological and physical parameters, as well as the agricultural conditions relevant for maize cultivation. Since biological and physical parameters influencing cross-fertilization in maize have been largely reviewed by others (Raynor et al. 1972; Aylor 2004; Devos et al. 2005), they will not be specifically addressed here. The present study is focusing on agronomic criteria, while biological and physical criteria will only be considered where necessary. In addition, two different approaches were used to assess whether the proposed isolation distances could be implemented in Switzerland when growing GM maize under actual Swiss agricultural conditions. The first approach used statistical data on the acreage of maize cultivation in Switzerland, while the second approach consisted of an assessment of aerial photographs covering an agricultural landscape in the eastern part of Switzerland by means of a Geographic Information Systems (GIS).

Approach and methods

Selection of studies for the definition of isolation distances

Cross-fertilization rates in maize have principally been studied for two motivations: (1) the maintenance of a specific purity in maize seed production and (2) concerns related to the adoption of GM maize varieties leading to unwanted inputs into non-GM maize crop production systems. Existing cross-fertilization studies were evaluated according to six defined criteria in order to select studies, which were relevant for the definition of isolation distances between GM and non-GM maize fields. The first four criteria aimed at excluding studies that have been based on conditions not relevant for GM maize cultivation under current agricultural practice. Subsequently, one criterion was applied to identify studies that had been performed under conditions representing the agricultural context of modern maize cultivation and another criterion was used to allow for a better comparison of cross-fertilization rates among the considered studies. Studies not providing specific cross-fertilization rates as a function of a given distance from the pollen source were not considered (Garcia et al. 1998; Luna et al. 2001).

Studies assessing dynamics and mechanisms of maize pollen dispersal

When considering measures to reduce cross-fertilization, it is important to clearly distinguish crossfertilization from pollen dispersal, simply because pollen dispersal does not necessarily result in fertilization. Successful cross-fertilization is depending on a series of biological and physical factors once pollen has reached the receptor crop (Ingram 2000; Eastham and Sweet 2002; Devos et al. 2005). Although studies investigating the flowering dynamics and mechanisms of maize pollen dispersal (Raynor et al. 1972; Di Giovanni et al. 1995; Aylor et al. 2003; Klein et al. 2003; Lizaso et al. 2003; Aylor 2004; Fonseca et al. 2004; Yamamura 2004; Fonseca and Westgate 2005) are important in understanding these components, pollen dispersal rates are not equivalent to crossfertilization rates. These studies were therefore not considered for the here presented analysis.

Studies conducted under seed production conditions

In order to maintain the purity of maize varieties in seed production, cross-fertilization rates have been investigated in several early studies performed around the 1950s' (Salamov 1940; Bateman 1947; Jones and Brooks 1950, 1952), as well as in some more recent studies (Das 1983; Narayanaswamy et al. 1997; Burris 2001). The results of these studies, and the general experiences gained in seed production, were used to define recommendations for isolation distances in maize seed production, which subsequently entered various national legislations. Experimental data obtained from studies growing maize for hybrid seed production (Das 1983; Narayanaswamy et al. 1997; Burris 2001) was not taken into account, because common agricultural production of maize differs largely from hybrid seed production. Maize fields growing grain for use as food and feed contain 100% fertile parent plants, while fields for the production of hybrid seed, in contrast, contain rows of pollen-producing (male) plants alternating with rows of sterile or detasseled (female) plants acting as pollen receptors. Usually only about 20% of the plants in these fields produce pollen resulting in a low amount of competing pollen within the field. Female flowers are therefore much more receptive for fertilization of pollen from the male parent but also from neighbouring fields (Brookes et al. 2004).

Studies with experimental limitations

The studies by Bateman (1947) and by Salamov (1940) were excluded due to experimental limitations. While the study performed by Bateman (1947) was considered unsuitable due to its experimental setup using single maize plants as pollen receptors, the cross-fertilization results reported by Salamov (1940) were partially affected by seed impurities in the white kernel variety resulting in yellow kernel-producing plants growing within the white maize receptor field. Although Salamov (1940) reports a reduction of the cross-fertilization rate within the first 50 m, the number of yellow kernels (so-called xenia grains) in cobs of the white kernel variety located further away did not continuously decrease with distance (e.g., 0.02% at 400 m but 0.79% at 600 m). The author states that "finding xenia grains away from the yellow maize could not be taken as a marker for the effective flight of the pollen from the yellow grain maize plot, because xenia was found even in the (purest) white maize (seed), as is visible from the field testing data'' (Salamov 1940).

Studies performed with open-pollinated maize varieties

Open-pollinated maize varieties (i.e., varieties that were produced through open pollination of parental plant populations) were commonly used before the widespread introduction of modern maize hybrid varieties (Poehlman and Sleper 1995). A study conducted with open-pollinated varieties by Jones and Brooks (1950) was compared to studies conducted with modern hybrid varieties. Based on the results of this comparison, the study was not considered for the definition of isolation distances because cross-fertilization rates of open-pollinated varieties are hardly comparable to the currently grown hybrid varieties (for the detailed reasoning see *Results* section).

Studies performed under atypical agricultural conditions

Cross-fertilization rates obtained in a number of studies have probably been partly influenced by the size of experimental donor and receptor fields, as this determines the amount of competing pollen (Ingram 2000; Devos et al. 2005). A high donor to receptor ratio (large donor field, small receptor field) leads to a higher amount of pollen from the donor field resulting in high cross-fertilization rates in the receptor field due to low competition against incoming pollen (Jemison and Vayda 2001). On the contrary, a low donor to receptor ratio generally leads to lower crossfertilization rates due to a relatively large pollen cloud in the receptor field with competing incoming pollen (Bénétrix et al. 2003; Messeguer et al. 2003). Experimental studies performed with donor fields being more than fifteen times larger than the receptor fields (or vice versa) were excluded (Jemison and Vayda 2001; Bénétrix et al. 2003; Messeguer et al. 2003) because their experimental conditions are likely to represent atypical agricultural conditions for GM maize cultivation (Table 1c).

Table 1 Studies asses	sing cross-fer	tilization in m	aize in the context of coexistence of genetically modified	ed (GM) and non-GM crops		
Reference	Experim. design ^a	Seasons locations	Methods/marker used Reference cross-fertilization rate ^b	Pollen source field size/ variety	Pollen receptor field size/ variety	Country ^c
a) Studies considered	for direct con	nparison of cro	ss-fertilization rates and for definition of isolation distan	lces		
Bannert et al. 2003		1 season 1 location	Phenotypic marker (% yellow kernels per total kernels)	Approx. 1.5 ha Hybrid variety with	1.4 ha Hybrid variety with	СН
				yellow kernels	white kernels	
Bannert 2006		1 season	Phenotypic marker (% yellow kernels	1.1 ha and 1.8 ha	1.1 ha and 1.8 ha	СН
no syncmony or flowering		2 locations	per total kernels)	Hybrid variety with yellow kernels	Hybrid variety with white kernels	
Bannert 2006		1 season		0.13 ha	1.07 ha	
Synchrony of		1 location		Hybrid	Hybrid variety with	
nowering				variety with yellow kernels	white kernels	
Brookes et al. 2004		1 season	PCR (% transgenic DNA per grain sample)	1.28–6.1 ha	n.a.	Е
		14 locations		<i>Bt</i> -maize (Bt 176 and MON 810)		
Byrne and Fromherz		1 season	Exp. A. Phenotypic marker (% blue	n.a. (size)	n.a. (size)	USA
2003		2 locations	kernels per total kernels)	Exp. A: Hybrid variety with blue kernels		
			Exp. B. Germination test (% herbicide	Exp. B:	Conventional hybrid	
			tolerant seedlings per sowed seeds)	Herbicide tolerant maize (Roundur Ready)	variety lacking the respective trait	
Della Porta et al. 2006	I	1 Season	Phenotypic marker (% yellow kernels	0.62 ha	7.7 ha	Ι
Experiment 1		2 Locations	per total kernels)	Hybrid variety with yellow kernels	Hybrid variety with white kernels	
Della Porta et al. 2006		1 Season		1.4 ha	2×1.05 ha	
Experiment 2		2 Locations		Hybrid variety with yellow kernels	Hybrid variety with white kernels	
Ma et al. 2004		3 seasons	Phenotypic marker (% yellow kernels per	0.07 ha	0.68 ha and 1 ha	CAN
	I	3 locations	total kernels)	Hybrid variety with yellow kernels	Hybrid varieties with white kernels	
Matsuo et al. 2004		2 seasons	Phenotypic marker (% yellow kernels per	4.5 ha	4.5 ha	JP
		1 location	total kernels)	Hybrid variety with yellow kernels	Hybrid variety with white kernels	

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Reference	Experim. design ^a	Seasons locations	Methods/marker used Reference cross-fertilization rate ^b	Pollen source field size/ variety	Pollen receptor field size/ variety	Country ^c
 Studies considered gmo-safety.eu 2002; Meier-Bethke and Schiemann 2003 	for validation	n of the suggest 2 seasons 1 location	ed isolation distances PCR (% transgenic DNA per grain) Germination (% herbicide tolerant seedlings per sowed seeds)	1 ha Herbicide tolerant maize (Liberty Link T25)	5.5 ha Conventional hybrid variety	D
Weber et al. 2007		1 season 28 locations	PCR (% transgenic DNA per total DNA whole plant)	1–20 ha Br-maize (MON 810)	Approx. 4–12 ha Conventional hybrid variety	D
Foueillassar and Fabié 2003		2 seasons 12 locations	Phenotypic marker (% cross-fertilization per field)	0.6–12 ha Waxy maize	0.7–13 ha Conventional hybrid variety	ц
Henry et al. 2003		3 seasons 55 locations	PCR (% transgenic DNA per total DNA)	Approx. 5 ha Herbicide tolerant maize (Liberty Link T25)	Approx. 5 ha Conventional hybrid variety	UK
Messéan 1999		1 season 1 location	Phenotypic marker (% blue kernels per total kernels)	n.a. (size) Hybrid variety with blue kernels	n.a. (size) Hybrid variety with yellow kernels	ц
POECB 2004		1 season 1 location	PCR (% transgenic DNA per grain sample)	2.27 ha Br-maize (MON 810)	6.7 ha Isogenic hybrid variety	ц
c) Studies excluded be Messeguer et al. 2003; gmo-safety.eu 2004	scause of aty	pical agricultura 1 season 1 location	ıl conditions PCR (% transgenic DNA per total DNA)	0.25 ha Bt-maize (Bt 176) var.	7.5 ha Conventional hybrid	ш
temison and Vayda 2001		2 seasons 1 location	Germination test (% herbicide tolerant seedlings per sowed seeds)	0.35 ha Herbicide tolerant maize (Roundup Ready)	282 m ² (0.02 ha) Conventional hybrid variety	USA

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Table 1 continued

Table 1 continued					
Reference	Experim. design ^a	Seasons locations	Methods/marker used Reference cross-fertilization rate ^b	Pollen source field size/ variety	Pollen receptor field size/ Country ^c variety
Bénétrix and Bloc 2003; Bénétrix et al. 2003		1 season 3 locations	PCR (% transgenic DNA per grain sample)	0.4–0.8 ha <i>Bt</i> -maize (MON 810)	Approx. 16 ha F Isogenic hybrid variety
n.a. = no data available	0				
Not considered:					
 Studies not providing 	specific cro	ss-fertilization	rates as a function of a given distance from the pollen sc	ource (Garcia et al. 1998; Lui	na et al. 2001)
 Studies performed ur 	ider seed pro	duction conditi	ons, which are not transferable to the regular cultivation	n practice (Das 1983; Narayan	aswamy et al. 1997; Burris 2001)
 Studies with experim 	ental limitati	ions (Salamov	1940; Bateman 1947) and studies conducted with open-p	ollinating maize varieties (Jo	nes and Brooks 1950)
^a Experimental desigr	1 (dark = pol)	len source, ligh	t = pollen receptor)		
^b The method/marker the data is given in itali	used is princ cs, the respe	ipally determini ctive studies ma	ing to what the cross-fertilization rate is referring to (exce) ake no specific indications – the reference is therefore assu	ept where the cross-fertilization unned based on the method use	1 rate refers to the whole field). Where ed (PCR = Polymerase chain reaction)

CH = Switzerland, D = Germany, E = Spain, F = France, I = Italy, JP = Japan, UK = United Kingdom, USA = United States of America

Studies yielding distances to meet specific thresholds

Cross-fertilization rates are usually determined by taking samples at different distances from the pollen source. Cross-fertilization rates are thus expressed as a function of distance to the pollen source. Unfortunately, only few published studies include directly comparable single value results, while most studies summarize their results and primarily indicate distances where average cross-fertilization rates remain below a specific threshold (often 0.9%). The studies giving detailed cross-fertilization rates as percentage of cross-fertilization per distance (Table 1a) (Bannert et al. 2003; Byrne and Fromherz 2003; Brookes et al. 2004; Ma et al. 2004; Matsuo et al. 2004; Bannert 2006; Della Porta et al. 2006) are hardly directly comparable to studies indicating minimum distances to meet specific thresholds (Messéan 1999; Henry et al. 2003; Meier-Bethke and Schiemann 2003; POECB 2004; Weber et al. 2007). The latter were therefore not used for the analysis performed to define the required isolation distances. Nevertheless, because these studies have been performed under conditions relevant for GM maize cultivation, they were subsequently used to validate the results of the analysis (Table 1b). Similarly, the study by Fouillassar and Fabié (2003) could not be used for direct comparison, because cross-fertilization rates have been given for the entire field.

Definition of isolation distances between GM and non-GM maize

Consideration of the agricultural context

Given the uncertainty that other sources than crossfertilization such as seed impurities, mixing in machinery, or post-harvest procedures could lead to GM-inputs into the agricultural production chain, we believed that defining isolation distances between GM and non-GM maize to meet the legal threshold of 0.9% was not accurate. We concluded that GM-inputs from cross-fertilization should remain substantially below 0.9% in order to allow for a safety margin up to the labelling threshold in the final product. Assuming the worst-case of having impurities of 0.5% GM in the seed (corresponding to the proposed legal threshold in the EU), inputs from other sources

including cross-fertilization, mixing in machinery and post-harvest procedures should not exceed 0.4%. We further sensed that it was inappropriate to define an isolation distance to remain below the targeted 0.4% purely based on point wise measured crossfertilization rates (given as percentage per distance), because using this approach, one did not consider that harvest always represent a mixture of the harvested area. We believed that this fact had to be considered because this mixing process will substantially reduce the potential GM-content in the harvest. This reduction is due to the fact that cross-fertilization rates are usually higher at the field margin and decrease rapidly within the receptor field due to increasing competition from the therein produced pollen (Devos et al. 2005). However, the final GM-content in the harvest is depending on various factors such as field size and harvesting procedure, and modelling of this reduction is currently not possible. An alternative approach had thus to be chosen. This approach consisted in defining an arbitrary level of maximal 0.5% cross-fertilization at the margin of a non-GM maize field. Considering the above mentioned reduction of GM-contents induced by mixing during harvest, the chosen approach should ensure that the GM-content in the harvested product should remain considerably below 0.4% allowing for the required safety margin up to the legal threshold of 0.9%. Cross-fertilization rates of the studies considered (Table 1a) were then plotted (157 data points in total) and classified according to their distance to the pollen source into four categories (0-10, 10-25, 25-50 and above 50 m). Mean and standard deviation of the cross-fertilization rates were determined for each category, as well as the number of data points exceeding the set level of 0.5%.

Consideration of the different uses of harvested maize

The different uses of maize were considered relevant for the definition of isolation distances. In Europe, maize is primarily used as animal feed and as raw material for industrial products. Maize for animal feed is mostly harvested as entire plant (green and silage maize) and as grain maize. Two different isolation distances were defined for grain and for silage maize considering that cross-fertilization is only affecting maize kernels, and that vegetative plant parts are unaffected. The use of the entire plant for green or silage maize thus results in a reduction of the transgenic fraction. The reducing factor depends on the proportion of the kernels compared to the entire plant and this proportion varies among different varieties and stages of maturity during harvest. In Swiss maize varieties, for example, kernels account for approximately 35-45% of the dry matter of the entire plant (Mathias Menzi, Agroscope ART, personal communication). Assuming an average proportion of 40% grain in the whole dry plant tissue, any GM input is reduced by a factor of 2.5 when harvesting the entire plant as compared to grain maize harvest. The isolation distance valid for grain maize can thus be reduced by this factor when applied to silage maize.

Estimation of the capacity for spatial coexistence in Switzerland

Landscape structures are important when assessing the possibilities for a spatial coexistence of GM and non-GM agricultural systems. The cultivation of arable crops in Switzerland is restricted to the climatically favourable lower parts and is characterized by small fields of an average size of less than two hectares as well as by the application of crop rotation. Agricultural landscapes are thus relatively fragmented and typically consist of a mix of several crops and grassland. Because the cultivation of maize is of major importance in Switzerland and covers on average more than 20% of the total arable land, two different approaches were used to assess whether the proposed isolation distances could be implemented in Switzerland when growing GM maize. The first approach used statistical data on the acreage of maize cultivation in Switzerland obtained from the farm structure survey of the Swiss Federal Statistical Office, while the second approach was based on an aerial pictures assessment using Geographic Information Systems (GIS).

Statistical data from the Swiss farm structure survey

The first approach was based on statistical data provided by the farm structure survey of the Swiss Federal Statistical Office yielding data of the maize acreage and the total arable land of 2206 communes (the smallest administrative district in Switzerland) cultivating maize (BFS 2003). In order to evaluate whether the available arable land in these communes was large enough to allow for isolation of an assumed GM maize cultivation area, five assumptions were made: (1) 10% of the total maize area of every commune is cultivated with GM maize and needs to be isolated from the remaining non-GM maize area, (2) the arable land represents a continuous entity in each commune, (3) all maize fields are evenly distributed within the arable land of each commune, (4) all maize fields are of equal size and rectangular shape, either 1 ha $(50 \times 200 \text{ m})$ or 2 ha $(100 \times 200 \text{ m})$, and (5) each GM maize field is surrounded by an isolation belt of the respective isolation distance. In order to determine whether the available arable land in a specific commune allowed for spatial isolation of 10% GM maize, the total isolation area needed per commune (i.e., the sum of all isolation belts) was compared to the available arable land minus the total maize area. Spatial coexistence in a commune was defined being possible if the arable land was larger than the area needed for isolation of GM maize (isolation area \leq total arable land-total maize area).

Geographic Information Systems (GIS)-analysis of a selected agricultural region

The second approach was based on an assessment of aerial photographs covering a 164 km² area in the eastern canton Zurich (Schüpbach et al. 2003). The selected region is an agricultural landscape in the eastern part of the Swiss plateau where arable land is often distributed within a mix of urban areas, forests, lakes and rivers. Infrared-aerial photographs taken in August 2000 were used for a supervised semiautomatic land cover classification by means of a Geographic Information System (GIS). The approach allowed localizing maize areas over the whole region with a resolution of 25×25 m. A grid square $(25 \times 25 \text{ m})$ was classified containing "maize" when at least 50% of the grid area was covered with maize. For quality assurance, the classification was further revised by a visual screen on aerial photographs. An error rate of 5% (i.e., classifying a grid square mistakenly as containing maize although this was not the case and vice versa) was determined by verifying the results of the classification in a field survey on location. The software FRAGSTATS (McGarigal et al. 2002) was used to analyse the resulting map and to calculate the shortest distance between each maize area and its nearest neighbouring maize area (Euclidian Nearest-Neighbour Distance). Due to the resolution of the applied grid and because the distances between two grids were calculated from the centre of the grid squares, the minimum measurable distance between two maize areas was set to be 50 m (=25 m between two grids and two times 12.5 m from the centre to the border of each grid). Considering the highly diverse landscape structure of the selected region, the distances between maize areas obtained with the GIS-analysis were compared considering a previously performed landscape typology of the region (Szerencsits et al. 2004). Statistical analysis for each type of landscape included calculation of means and medians of resulting distances between maize areas, as well as calculation of the percentage of areas, which were located within 100 and 200 m to another maize area.

Results and discussion

Comparison of cross-fertilization in open-pollinated and hybrid maize varieties

A comparison between studies conducted with modern hybrid varieties (Table 1) and a study conducted with open-pollinated varieties (Jones and Brooks 1950) clearly showed that cross-fertilization rates among open-pollinated varieties were distinctively higher than those reported for hybrid varieties (Fig. 1). Unfortunately, the results reported by Jones and Brooks (1950) are often used to estimate isolation distances in the context of coexistence of GM and non-GM maize (Ingram 2000; Feil and Schmid 2002; Wolt et al. 2004). We believe that such a comparison is critical because this early study aimed at providing recommendations on isolation distances needed for maize seed production of open-pollinated varieties. Open pollinated varieties were probably more receptive for cross-fertilization from other maize fields as compared to current modern hybrid varieties. This could be due to the biology of maize flowering. In order to avoid self-fertilization, maize is a protandric species, i.e., pollen is released from the tassels before female flowers (silks) on the same plant are receptive. The

silks of an individual plant are therefore usually fertilized by pollen from another maize plant in the same field. In open-pollinated varieties the variability of individual traits was higher because seeds of these varieties were produced through open pollination of a heterogeneous parental plant population (Poehlman and Sleper 1995). In a maize field containing an open pollinated variety, individual plants were less homogenous for particular traits such as flowering time of female flowers, which is specifically relevant for fertilization. Maize fields with open-pollinated varieties thus probably had a larger time frame in which female flowers were receptive, especially after own (in-field) pollen shed was completed. This in turn increased the probability that these maize plants would be fertilized by pollen originating from neighbouring fields.

In modern hybrid varieties, in contrast, the likelihood that cross-fertilization occurs from neighbouring fields is smaller, because a major breeding target for modern hybrid varieties is to minimize protandry and to synchronize the development of male and female flowering in order to shorten the anthesis-silking interval to attain higher yield stability (Duvick 2005). This leads to a relatively small variability in modern hybrid varieties for individual traits such as pollen shed and receptivity of female flowers, which in turn increases pollination by plants of the same field. The synchrony between male and female flowering is thus higher in modern hybrid varieties, reducing the time period in which female silks are still receptive after male pollen shed is terminated. This reduces the probability of crossfertilization by pollen from neighbouring fields. This difference between open-pollinated and modern hybrid varieties allows to explain the results obtained by Jones and Brooks (1950), who to our opinion do not provide the necessary information for defining recommendations on isolation distances between GM and non-GM maize. It is, however, important to distinguish asynchrony in flowering between male and female flowers in the same field, which is due to protandry of maize, and asynchrony in planting dates and flowering between different maize fields of different farmers. The latter generally leads to a decrease in cross-fertilization rates between fields (Brookes et al. 2004), although an increase may be possible if pollen shed is finished and female silks are still receptive.



Fig. 1 Distinction of early cross-fertilization studies conducted with open-pollinating maize varieties (Jones and Brooks 1950) and more recent studies performed with modern hybrid varieties (see Table 1). Cross-fertilization rates of openpollinated varieties are distinctively higher than those of modern hybrid varieties

Definition of isolation distances between GM and non-GM maize

Results of studies considered for direct comparison of cross-fertilization rates (Table 1a) (Bannert et al. 2003; Byrne and Fromherz 2003; Brookes et al. 2004; Ma et al. 2004; Matsuo et al. 2004; Bannert 2006; Della Porta et al. 2006) showed that cross-fertilization rates rapidly decreased with increasing distance (Fig. 2). While some studies showed cross-fertilization rates up to 46% at the immediate border between the two fields (Byrne and Fromherz 2003), crossfertilization rates dropped in most studies below 1% within a distance of 10 m and then decreased to a very low level, but did not reach zero. A relatively high variability was found within the first 10 m with a mean cross-fertilization rate of 5.72% (SD $\pm 9.67\%$) and 45 out of 48 data points exceeding the 0.5% threshold (Table 2). In contrast, mean cross-fertilization rates in the three categories above 10 m showed a rapid decrease of the cross-fertilization rate with 0.35% (SD ± 0.30%) at 10-25 m, 0.23% $(SD \pm 0.24\%)$ at 25–50 m, and 0.19% $(SD \pm 0.13\%)$ above 50 m. Likewise, data points exceeding the arbitrary 0.5% level dropped from ten (out of 41) in the category 10-25 m down to two (out of 35) in the 25-50 m category. In spite the variability among the data due to varying experimental conditions, the analysis showed that mean cross-fertilization rates of the considered studies

generally remained below 0.5% above a distance of 50 m from the pollen source (Table 2). One data point (out of 33) was exceeding the 0.5% level above 50 m, indicating a cross-fertilization rate of 0.55% at 57 m (Della Porta et al. 2006). This one data point was estimated not contradicting the general trend observed, considering the large amount of data points lying below the defined level of 0.5%. The analysis suggests that an isolation distance of 50 m for grain maize is sufficient to meet the arbitrary level of 0.5%at the field border. Considering the rapid decrease within the first 10 m and given the reduction of the transgenic fraction in silage maize, the isolation distance of 50 m for grain maize can be reduced by a factor of 2.5 resulting in an isolation distance of 20 m for silage maize (for the detailed reasoning see Approach and Methods section).

The results of a number of recent large-scale studies performed in several European countries (Table 1b) support the here proposed isolation distances. A study conducted during the Farm Scale Evaluations in the United Kingdom including 55 locations over three seasons showed that average cross-fertilization rates remained below 0.9% at a distance of 25 m, and that distances of 80 m and 258 m, respectively, were sufficient to reach levels of 0.3% and 0.1% (Henry et al. 2003). Results of the German "Erprobungsanbau" revealed similar results for grain as well as for silage maize, indicating cross-fertilization rates of 0.98% for grain maize (1.12%)



Fig. 2 Comparison of cross-fertilization rates of studies considered for the definition of isolation distances between genetically modified (GM) and non-GM maize (see Table 1a). The box represents a magnification of the original graph with a limited scale of the respective axis

for silage maize) at 0–10 m, 0.34% (0.24%) at 20– 30 m and 0.11% (0.18%) at 50–60 m distance from the *Bt*-maize field (Weber et al. 2007). A number of other studies confirmed that distances ranging from 25 to 50 m were sufficient to keep cross-fertilization rates either below 0.9 or 1% (Messéan 1999; Foueillassar and Fabié 2003; Meier-Bethke and Schiemann 2003; POECB 2004). Given that all these studies have been performed under conditions relevant for GM maize cultivation, their results are useful for validating the above performed analysis and the proposed isolation distances.

Detection and quantification methods

Comparison of results from different studies is challenged by the different methods used to detect and quantify cross-fertilization rates in maize. Crossfertilization rates are always expressed as percentage of a certain entity, but the reference varies depending on the method used (Table 1). Detection of crossfertilization events is thereby based on identifying the presence of defined traits in the progeny such as the presence of yellow kernels in a white kernel maize variety, or the detection of specific transgenic DNA sequences. The presence of a defined trait in the progeny, however, depends on its genetic background, i.e., if the trait is present as a homo- or heterozygous allele in the respective parental plants. The phenotypic marker for yellow kernel colour, for example, is a dominant trait, which is expressed in every pollen grain if the maize variety used as pollen donor is homozygous. Every pollination event there-

 Table 2
 Statistical analysis of cross-fertilization studies (see

 Table 1a) considered for the definition of isolation distances

 between genetically modified (GM) and non-GM maize

Distance from	Cross-fert	ilization-rate (%)	Data points		
pollen source	Mean	±SD	Exceeding 0.5%	Total	
0–10 m	5.72	9.67	45	48	
10–25 m	0.35	0.30	10	41	
25–50 m	0.23	0.24	2	35	
Above 50 m	0.19	0.13	1 ^a	33	

^a Data point from Della Porta et al. 2006 (57 m/0.55%)

Cross-fertilization rates were classified into four categories according to their distance to the pollen source and mean and standard deviation was determined for each category fore leads to a transfer of the yellow kernel colour trait. In contrast, most transgenic traits in currently commercialized GM maize hybrid varieties (e.g., MON 810) are hemyzygous (i.e., there is only a single copy of the inserted transgenic DNA sequence instead of the customary two copies). This is because current GM maize hybrid varieties are usually produced by crosses of a specific selected non-GM line with a GM line (Brookes et al. 2004; Devos et al. 2005; Pla et al. 2006). As a result, only half of the pollen carries the transgenic trait and only every second pollination event is leading to a transfer of the GM trait. Quantification based on DNA analysis should thus theoretically result in only half of the GM amount when compared to methods using a homozygous phenotypic marker like the yellow kernel colour. In fact, a recent study indicated an excellent correlation between cross-fertilization rates obtained by phenotypic estimation and a PCR based quantification method when the percentage of cross-fertilization obtained through phenotypic marker was divided by a factor of 2 (Pla et al. 2006).

Experimental design

Cross-fertilization rates can be influenced by the experimental design of donor and receptor fields as this determines the amount of competing pollen within a given distance. The experimental design of donor and receptor fields can be divided into three different types, i.e., adjacent, separated and concentric fields (where the pollen source is surrounded by the pollen receptor) (Table 1). Isolation distances should ideally be defined based on experimental studies that were performed with separated fields given that in actual situations of coexistence, maize fields are often separated by other crops or structures. The majority of cross-fertilization studies has, however, been performed using either the adjacent or the concentric design. Given that such an experimental design does only partially represent a situation with separated maize fields, cross-fertilization rates obtained in studies with adjacent or concentric experimental designs have to be transferred with care to the coexistence context. This is mainly due to the fact, that a pollen barrier consisting of non-GM maize has proven to reduce cross-fertilization rates more effectively than an isolation of the same distance with open ground or low growing crops (Messeguer et al. 2006; Pla et al. 2006). Mean cross-fertilization rates obtained in studies with an adjacent or concentric experimental design typically drop below 0.5% at a distance ranging from 10 to 25 m (Table 2). In situations with separated fields, where the observed decrease may be less pronounced, isolation distances between donor and receptor fields have to be increased. Considering the rapid decrease of crossfertilization rates within 25 m obtained in studies with adjacent fields, the here proposed isolation distance of 50 m for grain maize represent a cautious approach adding a safety margin for separated fields. In fact, cross-fertilization rates in agricultural maize fields of comparable size and isolated by distances ranging from 52 to 4440 m always remained below 0.02% when calculated for the entire field (Bannert 2007). In addition, a number of recent studies demonstrated that for a pollen donor of a given size, cross-fertilization rates decreased with increasing recipient field sizes (Devos et al. 2005), indicating that a cautious approach may be particularly relevant for small-scale agricultural systems as they are typical for Switzerland.

A cautious approach was also chosen because a number of factors, which could influence the amount of adventitious GM inputs under different agricultural conditions, are either difficult to consider in an experimental setup or still have to be clarified. These include five different points: (1) the point of reference for applying the threshold "maximum of 0.9% GM of the food ingredients" as referred to in the European legislation (European Union 2003a) and how the threshold will be applied in practice. Although the term ingredient is defined as "... any substance, including additives used in the manufacture or preparation of a foodstuff and still present in the finished product, even if in altered form" (European Community 2000), it is still not fully understood to what the labelling threshold of 0.9% is referring to, i.e., how it can be translated to the molecular level (Weighardt 2006), (2) methodological challenges for the detection and quantification of adventitious GM amounts in agricultural products considering the hemizygosity of transgenic traits, and the dilution of transgenic fractions by vegetative plant parts (Ma et al. 2005; Weighardt 2006), (3) the different use of maize plant products and the influence of post-harvest processes. Currently, only few studies (POECB 2004) have investigated the influence of these processes (such as drying, storage and transport) on resulting GM amounts in the final product, (4) the influence of multiple GM sources (e.g., different Bt-maize events) on a landscape level. Apart from Messeguer et al. (2006), cross-fertilization has not been assessed considering the influence of various GM maize fields with different Bt-maize events within an agricultural landscape, (5) the consequences of low seed impurities up to the proposed legal threshold of 0.5% GM on the GM amounts in the final product.

The proposed isolation distances should be applied when no additional measures are used to minimize cross-fertilization. If additional measures are applied, it may be possible to use shorter isolation distances resulting in similar reductions of cross-fertilization rates. Separate harvest of the first few rows of a non-GM field facing a GM crop field, for example, is an effective measure to reduce the level of crossfertilization in the recipient plot, as it has been shown by a number of studies (Devos et al. 2005). In the case of insect-resistant Bt-maize, isolation can also be provided by planting the necessary non-GM maize refuges for insect resistance management at one or more borders of the GM field. In addition, shorter isolation distances may be applied if isolation is provided by natural barriers in the agricultural landscape such as trees or hedgerows.

Given that probably not all questions may be resolved using an experimental set up, approaches for modelling maize pollen dispersal and cross-fertilization between fields on a landscape level (Di Giovanni et al. 1995; Angevin et al. 2001; Aylor et al. 2003; Klein et al. 2003; Lizaso et al. 2003; Aylor 2004; Fonseca et al. 2004; Yamamura 2004; Fonseca and Westgate 2005) may help to gain additional information and assist decision-makers.

Estimation of the capacity for spatial coexistence in Switzerland

Statistical data from the Swiss farm structure survey

The results of the approach based on data of the Swiss farm structure survey showed that, depending on the chosen field size and isolation distance, between 93.0 and 95.6% (Table 3) of Swiss communes disposed of sufficient arable land to allow for spatial isolation of

10% GM maize. The results further demonstrate that the feasibility for spatial isolation largely depends on the region and the prevalent agricultural system (Fig. 3a). While spatial isolation was possible within most communes situated in the main maize cultivating regions in the Swiss plateau (Fig. 3b), the area required for spatial isolation was not available in some communes in the pre-alpine regions. The area allowing for isolation declines if the proportion of maize within the arable land increases, and if the proportion of arable land decreases within the total agricultural land of a commune. Both factors are typical for the pre-alpine regions, where maize typically covers more than 40% of the available arable land and where arable land is often concentrated in the plane areas of the communes,

Two points have to be considered when interpreting the results of this approach:

whereas the slopes are mainly used as pastures or

meadows.

- (1)The chosen approach represents a "worst-case" scenario for the isolation area needed since it was based on the assumption that each maize field needs a complete isolation belt. Actually, the isolation area per field decreases if two GM fields are adjacent and their isolation belts partially overlap. The approach does further not consider the whole area available for isolation in an agricultural landscape, because statistical data of the used parameter "arable land" does not include permanent grassland, which could allow for additional isolation. Moreover, the arable land of a commune does probably not form a continuous entity, but is likely to be fragmented. Trees, hedgerows or other structures may be located between maize fields representing "natural" isolation barriers further reducing the isolation area required.
- (2) Calculations were made for an assumed cultivation of 10% GM maize. The spatial situation will become more critical with an increasing percentage of GM maize. However, a higher percentage of GM maize will also increase the likelihood of GM maize fields to border other GM maize fields resulting in a reduction of the required isolation area. On the other hand, a higher percentage of GM maize fields will further increase the ratio of donor to recipient field area, thus potentially increasing

Table 3 Number (and percentage) of Swiss communes in which the remaining arable land cultivated with other crops than maize is large enough to incorporate the area needed to isolate an assumed cultivation of 10% genetically modified maize from conventional maize

Field size	Isolation distance	
	20 m	50 m
1 ha	2097 (95.1%)	2052 (93.0%)
2 ha	2108 (95.6%)	2081 (94.3%)

cross-fertilization rates in non-GM fields due to a higher amount of competing GM maize pollen.

The results of the approach also showed that the area needed for isolation is depending on the field size of the GM fields since smaller fields need proportionally more isolation than larger fields (Table 3). Considering that the average maize field size in Switzerland is between 1 and 1.5 ha, the isolation area needed for each field is relatively high when compared to the effective field size. Larger



Fig. 3 (a) Results of the approach estimating the capacity for spatial coexistence in Switzerland using statistical data from the Swiss farm structure survey. In the communes depicted in red, arable land is not sufficiently large to apply an isolation distance of 50 m for an assumed cultivation of 10% GM maize and a field size of 1 ha. The perimeter of the agricultural area selected for the GIS-analysis (see Fig. 4) is indicated in the northeastern part of Switzerland. Communes where no maize is cultivated are shown in grey. (b) Percentage of maize cultivation in Switzerland relative to the total agricultural land per commune. Data basis: Generalized Commune Boundaries of Switzerland 2003, Farm Structure Survey 2003, Swiss Federal Statistical Office

maize fields would decrease the isolation area needed.

Geographic Information Systems-analysis of a selected agricultural region

The results of the GIS-analysis indicated that mean distances between maize areas in the communes of the selected agricultural region (Fig. 4) varied between 75 m (SE ± 3 m) and 149 m (SE ± 10 m) among the different landscape types, with a mean distance of 112 m over all landscape types considered (Table 4). Distances between maize areas were shorter in landscape types, which are more suited for arable farming (such as the valley bottom area), than in landscape types with less arable farming such as the upland area. The median distance among all considered landscapes was 90 m, i.e., half of the maize areas in the selected region were separated by more than 90 m from the next maize area. In the valley bottom area, the region with the highest maize density, the median distance was 56 m. The analysis further showed that 57% of all maize areas in the selected region had a neighbouring area within a distance of 100 m, while 88% had one within a distance of 200 m.

Because the GIS-analysis was based on an interpretation of aerial photographs, two points should be considered when interpreting the results of this approach:

(1) The calculated distances between two maize areas could be overestimated, because the distance between two grids was calculated from the centre of the grid squares. The effective distance could therefore be shorter than the minimum measurable distance of 50 m between two maize areas. The resolution of the applied grid $(25 \times 25 \text{ m})$ did, unfortunately, not allow to calculate the percentage of fields located within a range of 20-50 m. This is especially important considering that the median shows that in the valley bottom area nearly half of the maize areas have a neighbouring maize field close to or below the minimal measurable distance of 50 m, which equals the proposed isolation distance for grain maize. The situation is, however, less critical considering that approximately two thirds of the maize area cultivated in Switzerland is used as silage maize where an isolation distance of 20 m could be applied.

(2) The number of maize areas may be underestimated because two adjacent fields lying in the same grid were interpreted as one contiguous maize area. If these two fields would belong to two different farmers, the cultivation of GM maize would not be possible in that particular case, because minimum distances cannot be respected.

Although the approach using data of the Swiss farm structure survey showed that all communes lying within the agricultural region selected for the GISanalysis disposed of sufficient arable land to allow for spatial isolation of 10% GM maize (Fig. 3a), the more precise GIS-analysis demonstrated that depending on the landscape type both the density of maize cultivation as well as the distances between maize fields can strongly differ between landscape types within a smallscale (Table 4). Taking into account the limitations of the selected approach, the GIS-analysis also showed that the proposed isolation distances of 50 m for grain maize and 20 m for silage maize may be implemented in many cases, because over the whole selected region half of the maize areas were separated by more than 90 m from the next maize area. Nevertheless, only in a few cases, the cultivation of GM maize will be possible without coordination with neighbouring farms. Certain landscape types such as the valley bottom area revealed



Fig. 4 Localization of maize fields in an 164 km^2 area in the eastern canton of Zurich based on an assessment of aerial photographs using a land cover classification by means of a Geographic Information Systems (GIS) (Schüpbach et al. 2003). The map was used to calculate the shortest distance between maize fields. (Reproduced by permission of swisstopo BA071302)

Туре	of landscape	Mean distance ^a		Median distance ^a	No. maize areas	% of areas neighbouring another area within a range of	
		(m)	±SE	(m)	(<i>=n</i>)	100 m	200 m
1	Hill slopes	142	12	95	94	50	76
2	Alluvial cones of the lake	102	8	75	50	56	92
3	Valley bottom	75	3	56	137	80	100
4	Drumlin landscape	110	3	100	473	50	89
5	Moraine landscape	103	5	79	159	58	92
6	Upland area	149	10	103	126	46	78
Total	area Greifensee	112		90		57	88

Table 4Means (\pm SE) and medians of shortest distances between maize areas, as well as percentage of areas which were locatednearer than 100 and 200 m to another maize field in six different types of landscapes in the selected agricultural region

^a Based on calculation of "Euclidian Nearest Neighbour distance" (ENN)

a relatively high density of maize cultivation making agreements between farmers necessary for nearly half of the fields when implementing an isolation distance of 50 m. In order to facilitate farmer agreements, GM site registers, possibly coordinated by public administration, would help to facilitate compliance with the necessary coexistence measures. In fact, public GM site registers are mandated by the European legislation (European Community 2001) for monitoring possible effects of GM crops on the environment. They could also be used for other purposes provided that privacy is warranted. It is further conceivable that such registers could be based on existing data bases that are already supported by public administration (e.g., for the coordination of agricultural subsidies). The German Federal Office of Consumer Protection and Food Safety (BVL), for example, coordinates an internet based GM site register listing all sites where farmers intend to cultivate a GM crop variety. Every farmer is thereby obliged to notify the planned cultivation three months prior to seeding.

Conclusions

The definition of isolation distances should be based on experimental studies that have assessed crossfertilization in maize under realistic agricultural conditions including similar field sizes of both pollen donor and receptor. In order to infer the necessary isolation distances between GM and non-GM maize fields, current experimental data has to be carefully evaluated, mostly because actual agricultural conditions have only been partially considered in some studies. When applying the here defined criteria, relevant cross-fertilization studies can be selected and recommendations for isolation distances between GM and non-GM maize fields can be made. Although several factors (experimental design, seasons, locations, methods used, size of pollen donor and receptor fields) were varying considerably among the studies considered, all studies showed a characteristic rapid decrease of cross-fertilization rates with increasing distance. A major challenge in the interpretation of cross-fertilization results lies within the adaptation of the results to the agricultural context of current maize cultivation. Both distribution of cross-fertilization within the field as well as the different uses of maize (green or silage maize) have to be considered when deducing science-based isolation distances. The results of the performed analysis showed that an isolation distance of 20 m for silage maize, and 50 m for grain maize, respectively, is sufficient to keep GM-inputs from cross-fertilization below an arbitrary level of 0.5% at the border of a non-GM maize field. The results of a number of studies performed under agricultural conditions in several European countries (Henry et al. 2003; POECB 2004; Bannert 2006; Della Porta et al. 2006; Weber et al. 2007) suggest that the here proposed isolation distances represent a rather conservative approach leaving an additional safety margin up to the current legal threshold of 0.9% in the final product. The two chosen approaches assessing whether the proposed isolation distances could be implemented in Switzerland when growing GM maize under actual Swiss agricultural conditions showed that the potential for spatial coexistence is strongly depending on the prevalent landscape structures. The results of both approaches demonstrated that in the main maize cultivating areas in Switzerland, the isolation of GM maize fields using the proposed isolation distances is possible in the majority of the cases. In regions with a high ratio of maize cultivation within the arable land, however, agreements between farmers will probably be necessary in half of the cases when implementing an isolation distance of 50 m. The results of the present study confirm the conclusions of a number of studies, which state that coexistence between GM and non-GM maize cultivation would be possible in European agriculture (van Dijk 2004; Devos et al. 2005; Messéan et al. 2006).

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References

- ACRE (2002) Background paper: gene flow from genetically modified crops. DEFRA report. Department for Environment, Food and Rural Affairs—Advisory Committee on Releases to the Environment, London
- Angevin F, Klein E, Choimet C, Meynard JM, de Rouw A, Sohbi Y (2001) Modélisation des effets des systèmes de culture et du climat sur les pollinisations croisées chez le maïs: le modèle MAPOD. Rapport du groupe 3 du programme de recherche "Pertinence économique et faisabilité d'une filière sans utilisations d'OGM". INRA— FNSEA, Paris
- Aylor DE (2004) Survival of maize (*Zea mays*) pollen exposed in the atmosphere. Agric For Meteorol 123:125–133
- Aylor DE, Schultes NP, Shields EJ (2003) An aerobiological framework for assessing cross-pollination in maize. Agric For Meteorol 119:111–129
- Bannert M (2006) Simulation of transgenic pollen dispersal by use of different grain colour maize. Thesis, Swiss Federal Institute of Technology, Zurich
- Bannert M, Stamp P (2007) Cross-pollination of maize at long distance. Eur J Agron 27:44–51
- Bannert M, Stössel F, Orsini E, Stamp P, Soldati A (2003) Fremdbefruchtung bei Mais: Eine realistische Simulation transgenen Pollenflugs. Bericht über die Versuchstätigkeit 2001–2003. Swiss Federal Institute of Technology, Zurich
- Bateman AJ (1947) Contamination of seed crops II. Wind pollination. Heredity 1:235–246

- Bénétrix F, Bloc D (2003) Maïs OGM et non OGM—possible coexistence. Perspect Agricoles 294:14–17
- Bénétrix F, Bloc D, Foueillassar X, Naïbo B (2003) A study to evaluate co-existence of GM and conventional maize on the same farm. In: Boelt B (ed) Proceedings of the GMCC-03—1st European Conference on Co-existence of Genetically Modified Crops with Conventional and Organic Crops, Helsingør, 13–14 November 2003, 204 pp
- BFS (2003) Farm structure survey. Swiss Federal Statistical Office, Neuchâtel
- Bock AK, Lheureux K, Libeau-Dulos M, Nilsagård H, Rodriguez-Cerezo E (2002) Scenarios for co-existence of genetically modified, conventional and organic crops in European agriculture. Technical Report Series of the Joint Research Center of the European Commission. Institute for prospective technological studies, Sevilla
- Brookes G, Barfoot P, Melé E, Messeguer J, Benetrix F, Bloc D et al (2004) Genetically modified maize: pollen movement and crop co-existence. PG Economics, Dorchester
- Burris JS (2001) Adventitious pollen intrusion into hybrid maize seed production fields. In: Proceedings of the 56th Annual Corn and Sorghum Research Conference, Washington DC
- Byrne PF, Fromherz S (2003) Can GM and non-GM crops coexist? Setting a precedent in Boulder County, Colorado, USA. Food Agric Env 1:258–261
- Das KGS (1983) Vicinity distance studies of hybrid seed production in maize (*Zea mays* L.) at Bangalore. Mysore J Agric Sci 20:340
- Della Porta G, Ederle D, Bucchini L, Prandi M, Pozzi C, Verderio A (2006) Gene flow between neighboring maize fields in the Po valley. CEDAB—Centro documentazione agrobiotecnologie, Milan
- Devos Y, Reheul D, de Schrijver A (2005) The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. Environ Biosafety Res 4:71–87
- Di Giovanni F, Kevan PG, Nasr ME (1995) The variability in settling velocities of some pollen and spores. Grana 34:39–44
- Duvick DN (2005) The contribution of breeding to yield advances in maize (*Zea mays* L.). Adv Agr 86:83–145
- Eastham K, Sweet J (2002) Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer. Environmental Issue Report No. 28. European Environment Agency, Copenhagen
- European Commission (2003) Commission Recommendation of 23 July 2003 on guidelines for the development of national strategies and best practices to ensure the coexistence of genetically modified crops with conventional and organic farming. Commission of the European Communities, Brussels
- European Commission (2004) Inscription of MON 810 GM maize varieties in the Common EU Catalogue of Varieties. Press release IP/04/1083. Available via www.europa.eu. int/rapid/pressReleasesAction.do?reference=IP/04/1083& format=HTML&aged=1&language=EN&guiLanguage=en. Cited 9 Aug 2004
- European Community (2000) Directive 2000/13/EC of the European Parliament and of the council of 20 March 2000

on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs. European Parliament and the Council of the European Union, Brussels

- European Community (2001) Directive 2001/18/EC of the European Parliament and of the council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. European Parliament and the Council of the European Union, Brussels
- European Union (2003a) Regulation (EC) No. 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. European Parliament and the Council of the European Union, Brussels
- European Union (2003b) Regulation (EC) No. 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. European Parliament and the Council of the European Union, Brussels
- Feil B, Schmid JE (2002) Dispersal of maize, wheat and rye pollen: a contribution to determining the necessary isolation distances for the cultivation of transgenic crops. Shaker Verlag, Aachen
- Fonseca AE, Lizaso JI, Westgate ME, Grass L, Dornbos DL (2004) Simulating potential kernel production in maize hybrid seed fields. Crop Sci 44:1696–1709
- Fonseca AE, Westgate ME (2005) Relationship between desiccation and viability of maize pollen. Field Crops Res 94:114–125
- Foueillassar X, Fabié A (2003) Waxy maize production, an experiment evaluating the co-existence of GM and conventional maize. Poster presented at the GMCC-03-1st European Conference on Co-existence of Genetically Modified Crops with Conventional and organic Crops, Helsingør, 13–14 November 2003
- Garcia M, Figueroa J, Gomez R, Townsend R, Schoper J (1998) Pollen control during transgenic hybrid maize development in Mexico. Crop Sci 38:1597–1602
- gmo-safety.eu (2002) Out-crossing from transgenic maize and quantifying outcrossing rates. Available via www. gmo-safety.eu/en/safety_science/106.docu.html. Cited 15 Aug 2002
- gmo-safety.eu (2004) Spanish study of maize and outcrossing—large fields remain below the threshold. Available via www.gmo-safety.eu/en/archive/2004/279.docu.html. Cited 12 Mar 2004
- GMO compass (2007). Available via www.gmo-compass.org/ eng/home/
- GMO safety (2006) Coexistence in the countries of the EU—a European patchwork. Available via www.gmo-safety.eu/ en/focus/coexistence/513.docu.html
- Gruber S, Pekrun C, Claupein W (2004) Population dynamics of volunteer oilseed rape (*Brassica napus* L.) affected by tillage. Eur J Agron 20:351–361
- GTG (SR 814.91) Bundesgesetz über die Gentechnik im Ausserhumanbereich (Gentechnikgesetz). Systematische Sammlung des Bundesrechts, Bern

- Henry C, Morgan D, Weeks R, Daniels RE, Boffey C (2003) Farm scale evaluations of GM crops: monitoring gene flow from GM crops to non-GM equivalent crops in the vicinity—Part I. Forage maize. DEFRA report. Central Science Laboratory Sand Hutton, Centre for Ecology and Hydrology, Dorchester
- Ingram J (2000) The separation distances required to ensure cross-pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape. Plant Var Seeds 13:181–199
- Jemison JM, Vayda ME (2001) Cross pollination from genetically engineered corn: wind transport and seed source. AgBioForum 4:87–92
- Jones MD and Brooks JS (1950) Effectiveness of distance and border rows preventing outcrossing in corn. Technical Bulletin No. T-38. Oklahoma Agricultural Experimental Station
- Jones MD and Brooks JS (1952) Effect of tree barriers on outcrossing in corn. Technical Bulletin No. T-45. Oklahoma Agricultural Experimental Station
- Klein EK, Lavigne C, Foueillassar X, Gouyon PH, Laredo C (2003) Corn pollen dispersal: Quasi-mechanistic models and field experiments. Ecol Monogr 73:131–150
- LGV (SR 817.02) Lebensmittel- und Gebrauchsgegenständeverordnung. Systematische Sammlung des Bundesrechts, Bern
- Lizaso JI, Westgate ME, Batchelor WD, Fonseca A (2003) Predicting potential kernel set in maize from simple flowering characteristics. Crop Sci 43:892–903
- Luna S, Figueroa J, Baltazar B, Gomez R, Townsend R, Schoper JB (2001) Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Sci 41:1551–1557
- Ma BL, Subedi K, Evenson L, Stewart G (2005) Evaluation of detection methods for genetically modified traits in genotypes resistant to European corn borer and herbicides. J Environ Sci Health B 40:633–644
- Ma BL, Subedi KD, Reid LM (2004) Extent of cross-fertilization in maize by pollen from neighboring transgenic hybrids. Crop Sci 44:1273–1282
- Matsuo K, Amano K, Shibaike H, Yoshimura Y, Kawashima S, Uesugi S et al (2004) Pollen dispersal and outcrossing in Zea mays populations: a simple identification of hybrids detected by xenia using conventional corn in simulation of transgene dispersion of GM corn. In: Proceedings of the 8th International Symposium on the Biosafety of Genetically Modified Organisms, Montpellier, 26–30 September 2004, pp 282
- Meier-Bethke S and Schiemann J (2003) Effect of varying distances and intervening maize fields on outcrossing rates of transgenic maize. In: Boelt B (ed) Proceedings of the GMCC-03—1st European Conference on Co-existence of Genetically Modified Crops with Conventional and Organic Crops, Helsingør, 13–14 November 2003, pp 77–78
- Messéan A (1999) Impact du développement des plantes transgéniques dans les systèmes de culture—rapport final. dossier no.96/15-B—Impact des plantes transgéniques. CETIOM—Centre Technique Interprofessionnel des Oléagineux Métropolitains
- Messéan A, Angevin F, Gomez-Barbero M, Menrad K, Rodriguez Cerezo E (2006) New case studies on the

coexistence of GM and non-GM crops in European Agriculture. Technical Report Series of the Joint Research Center of the European Commission. Institute for prospective technological studies, Sevilla

- Messeguer J, Ballester J, Peñas G, Olivar J, Alcalde E, Melé E (2003) Evaluation of gene flow in a commercial field of maize. In: Boelt B (ed) Proceedings of the GMCC-03—1st European Conference on Co-existence of Genetically Modified Crops with Conventional and Organic Crops, Helsingør, 13–14 November 2003, 220 pp
- Messeguer J, Penas G, Ballester J, Bas M, Serra J, Salvia J et al (2006) Pollen-mediated gene flow in maize in real situations of coexistence. Plant Biotechnol J 4:633–645
- Narayanaswamy S, Jagadish GV, Ujjinaiah US (1997) Determination of isolation distance for hybrid maize seed production. Curr Res 26:193–195
- Pekrun C, Hewitt JDJ, Lutman PJW (1998) Cultural control of volunteer oilseed rape (*Brassica napus*). J Agric Sci 130:155–163
- Pla M, La Paz JL, Penas G, Garcia N, Palaudelmas M, Esteve T et al (2006) Assessment of real-time PCR based methods for quantification of pollen-mediated gene flow from GM to conventional maize in a field study. Transgen Res 15:219–228
- POECB (2004) Operational programme for evaluation of biotechnology crops (POECB). ARVALIS Institut du végétal, Montardon, France
- Poehlman JM, Sleper DA (1995) Breeding field crops, 4th edn. Blackwell Publishing, Ames IA
- Raynor GS, Ogden EC, Hayes JV (1972) Dispersion and deposition of corn pollen from experimental sources. Agron J 64:420–427
- Salamov AB (1940) About isolation in corn (title translated from Russian; original: O prostranstwennoi isoljazii kukuruzy). Selekcija i semenovodstvo 3:25–27
- Sanvido O, Widmer F, Winzeler M, Streit B, Szerencsits E, Bigler F (2005) Konzept für die Koexistenz verschiedener landwirtschaftlicher Anbausysteme mit und ohne

Gentechnik in der Schweiz. Schriftenreihe der FAL Nr. 55. Agroscope FAL Reckenholz, Eidgenössische Forschungsanstalt für Agrarökologie und Landbau, Zürich

- Schüpbach B, Szerencsits E, Walter T (2003) Integration von Infrarot-Ortholuftbilddaten zur Modellierung einer nachhaltigen Landwirtschaft. In: Strobl J, Blaschke T, Griesebner G (eds) Angewandte Geographische Informationsverarbeitung, Vol. 15. Wichmann, Heidelberg, pp 481–490
- Sundstrom FJ, Williams J, van Deynze A, Bradford K (2002) Identity preservation of agricultural commodities. University of California—Division of Agriculture and Natural Resources, Davis
- Szerencsits E, Schüpbach B, Buholzer S, Walter T, Zgraggen K, Flury C (2004) Landschaftstypen und Biotopverbund. Agrarforschung 11:428–433
- Tolstrup K, Andersen S, Boelt B, Buus M, Gylling M, Holm P et al (2003) Report from the Danish working group on the co-existence of genetically modified crops with conventional and organic crops. Ministry of Food, Agriculture and Fisheries—Danish Institute of Agricultural Sciences, Copenhagen
- van Dijk J (2004) Co-existence in the primary sector. Report by the temporary committee chaired by J. van Dijk, committee members: Biologica, LTO Nederland, Plantum NL, Platform Aarde Boer Consument. The Hague
- Weber WE, Bringezu T, Broer I, Eder J, Holz F (2007) Coexistence between GM and non-GM maize crops tested in 2004 at the field scale level (Erprobungsanbau 2004). J Agron Crop Sci 193(2):79–92
- Weighardt F (2006) European GMO labeling thresholds impractical and unscientific. Nat Biotechnol 24:23–25
- Wolt JD, Shyy YY, Christensen PJ, Dorman KS, Misra M (2004) Quantitative exposure assessment for confinement of maize biogenic systems. Environ Biosafety Res 3:183– 196
- Yamamura K (2004) Dispersal distance of corn pollen under fluctuating diffusion coefficient. Pop Ecol 46:87–101