Analysis of gene flow in Zea mays in consideration of the environment in main cultivation regions of Austria

L. Girsch, N. Angerer, J. Hartmann, H. Strelec and H. P. Stueger

Untersuchungen zur Fremdbefruchtungsrate in Maiskulturen unter Berücksichtigung der Umwelten in den Hauptanbaugebieten Österreichs

To develop strategies for coexistence fitting to Austria's agrarian conditions, experimental analyses were conducted. Maize (*Zea mays*) as a relevant GMO-crop was subject of this examination. Twelve field experiments were conducted in seven districts in Austria in four climatic zones for one season to determine the "maximum" gene flow by foreign pollen of neighbouring maize fields. Field experiments were arranged as a non-artificial field trial which means a randomised arrangement of pollen acceptor field and neighbouring pollinator fields. To determine the "maximum" gene flow it was necessary to castrate the plants of each pollen acceptor field to avoid pollination within the acceptor field. Three different methods were conducted to estimate the best measurement of distance in relation to the external gene flow from the pollinator fields to the pollen acceptor field:

1. the shortest distance between the border of the main pollinator field to the border of the pollen acceptor field;
2. the shortest distance between the border of the main pollinator field to the centre of the pollen acceptor field;
3. the distance between the centre of the main pollinator field to the centre of the pollen acceptor field. The results showed that gene flow represented as a successful fertilization in the pollen acceptor field was dependent upon numerous factors but mainly upon the distance to the pollinator fields. The statistical analyses of the three different methods for distance measurement constitute a new effort for the technical implementation of coexistence strategies.

Zusammenfassung

Diese Studie stellt einen Beitrag zur Erarbeitung wissenschaftlich fundierter Koexistenz-Richtlinien für den Maisanbau, wie sie von der EU gefordert werden, dar. Die Versuchsanlage spiegelt reelle Umwelten in den Maisanbaugebieten Österreichs wider. Es erfolgte die Messung des maximalen externen Gentransfers nach totaler Kastration der Pollenempfängerflächen. Die Höhe des maximalen erfolgreichen externen Gentransfers lag im Durchschnitt zwischen 4,5 % und 97,5 %. Als geeignete Methode zur Bestimmung der Mindestentfernung stellte sich die Messung vom nächstliegenden Rand der Pollenquellenfläche zum Mittelpunkt der Pollenempfängerfläche heraus.

Schlüsselworte: Gentransfer, *Zea mays*, Isolationsdistanz, Koexistenz, GVO.

Summary

This report was executed in consideration of the implementation of scientific based directives for a coexistence management. A field design representative for a natural cultivation situation of maize in Austria was selected. The "maximum" external gene flow as a successful fertilization in a castrated pollen acceptor field was measured. The average of the successful fertilization related to the entire pollen acceptor field was between 4.5 % and 97.5 %. The highest correlation was observed when measuring the shortest distance between the field border of the main pollinator field and the centre of the pollen acceptor field and it seems to be the most suitable way to define distances between a GMO-field and a non-GMO-field in coexistence management.

Key words: Gene flow, *Zea mays*, isolation distance, coexistence, GMO.

1 Introduction

On 23 July 2003 the European Commission adopted Recommendation 2003/556/EC on guidelines for the development of national strategies and best practices to ensure the coexistence of genetically modified crops with conventional and organic farming, which are intended to help Member States develop national legislative or other strategies for coexistence. In item 1.1 of this Recommendation "coexistence" is defined as follows: "coexistence refers to the ability of farmers to make a practical choice between conventional, organic and GM-crop production, in compliance with the legal obligations for labelling and/or purity standards".

In the last two decades new techniques of genetic modification were developed to create genetically modified (GM) plants. Especially for maize, numerous transgenic hybrids have been developed and are commercially cultivated. Maize is a monoecious plant with male (staminate inflorescence) and female (pistillate inflorescence) flowers formed in separate parts of the same plant, leading to a high degree of cross-pollination between plants (MA et al., 2004). Due to these facts maize is the main subject in the debate over the coexistence of GM and non-GM crop. With continued release of new transgenic maize hybrids gene flow (cross-fertilization) between maize genotypes gained increasingly in importance. Not only ecological effects on farmland biodiversity must be the subject of scientific analyses, also the economic effects in seed production as well as in the production of corn and silage should be considered. Growers of conventional or organic crops fear economic losses if GM fragments are present in their fields or harvested products. In this context the European Union issued article 26a of Directive 2001/18/EC which calls on Member States to take appropriate national measures on coexistence in order to avoid the unintended presence of GMOs in other products. EU Regulation (EC) No 1829/2003 on genetically modified food and feed has introduced a 0.9 % labelling threshold for adventitious presence of approved GM material in non-GM products. In the European Union a threshold of -0- for adventitious presence of not finally or not approved GM in food and feed exists, but an explicit threshold for seed does not exist at the moment. In Austria the Saatgut-Gentechnik-Verordnung defines a threshold of -0- (in 3000 seeds) in the first analysis and a tolerance value of 0.1 % in the check-up. On 23 July 2003 the Commission adopted Recommendation 2003/556/EC, which is intended to help Member States develop national legislative or other strategies for coexistence. Numerous research projects have been and continue to be conducted at national level as well as on Community level to develop coexistence measures.

The objectives of the present study were to determine the distance for gene flow control in maize between a pollinator and a pollen acceptor field and to advance the method for distance measurement in Austrian regions with different environmental and topographical features: large parts of Austria are mountainous, main areas for crop production are concentrated on lowland areas of Eastern Austria, and on smaller flat parts in Western and Northern Austria; cultivation areas are small and fragmented. Austria's climate is mainly dominated by the Alps and by a continental impact.

2 Material and methods

2.1 Site description

Twelve field experiments were conducted in seven districts in Austria for one growing season (2005). The seven districts were located in four different climatic zones with specific geographic and agronomic characteristics (Table 1).

2.2 Field experiment

For this field experiment a non-artificial field design was chosen. Each field trial represented a randomised selected field within an actual maize cultivation area in Austria. This implicated twelve completely different environments regarding to field size, the constellation of the pollinator fields and the pollen acceptor field, the topographic and agronomic situation etc. Each experimental field was surrounded by a different number of neighbouring pollinator fields. The distance between the border of the pollen acceptor field and the closest borders of the pollinator fields was variable and reached from 1m (adjacent) to 335 m. To evaluate the maximum gene flow represented as a successful fertilization it was necessary to prevent pollination within the experimental field. This was achieved by complete detasseling treatment of plants before flowering. This procedure was performed mechanically (e.g. by Castrix tassel cutter). Because of bad weather conditions with lots of rain the process of detasseling was difficult and in some cases it was not possible to detassel the whole selected area of the experimental field: just a small part within the field trial was completely detasseled; the remaining, undetas-

Table 1: Number and geographic location of the experimental fields in the different climatic zones of Austria and the suitable climate data with the average share of maize in the appropriate district related to the agricultural area

Tabelle 1: Anzahl und geographische Lage der Feldversuche in unterschiedlichen Klimazonen Österreichs mit den zugehörigen Klimadaten sowie dem Maisanteil an der gesamten landwirtschaftlichen Fläche im jeweiligen Bezirk

Climatic zone	District	Number of experimental sites	Sea level (m)	Climate data of the climatic zones		Average share of maize in the district related to the agricultural area*) (%)
				Annual precipitation (mm)	Average temperature (°C)	
North alpine zone	Linz-Land	2	270–300	1000–2000	8.2°	17.3
	Wels	1	332			24.6
	Steyr-Land	1	446			10.4
Pannonian Zone	Bruck/Leitha	1	157	< 600	9.6°	8.8
	Neusiedl/See	4	182			13.8
Illyricum	Hartberg	1	412	700–900	8.8°	15.7
South alpine zone	Voelkermarkt	2	460	800–2000	8.0°	28.8

*) Reference: Agrarmarkt Austria 2004

seled part was considered as a neighbouring maize field. These experimental fields represented not a typical cultivation situation for the appropriate climatic zone but were representative for some other maize cultivation conditions in Austria. The FAO-Index of the varieties planted in the region where the experimental sites were located was between 270 and 300, except for the Pannonian region where the FAO-Index was between 320 and 400. To check the synchronisation of flowering dates of the neighbouring pollinators and silk elongation in the experimental fields the beginning of flowering/silking dates were collected by the farmers and project staff. According to the Austrian seed certification rules (ANONYMUS, 2007) the beginning of flowering date was defined as more than 5 % of the plants in the neighbouring pollinator fields shedding pollen or more than 5 % of the plants in pollen acceptor field showing silks.

2.3 Measurements

Daily temperature, relative humidity and rainfall data as well as wind velocity and wind direction during pollen shed and silking time were acquired from stations of the Central Institute for Meteorology and Geodynamics (ZAMG) located close to the experimental sites. A sampling plan was developed that allowed to take samples on defined spots within the entire field trial. Evaluation of gene flow was accomplished by counting the kernels per ear representing a successful fertilization. On each sample point the kernels of three ears were counted and the average calculated.

Gene flow as percent fertilization in relation to the mean total kernel number of a reference ear of the appropriate variety was calculated. To illustrate the fertilization pattern in the experimental fields a colour scale simulation model based on the data points found on the field was applied. The common method measuring the distance between the pollen acceptor field and the pollinator field is to determine the shortest distance between the field borders. To implicate field size and field constellation two additional methods for distance measurement were assessed: 1. shortest distance between the centre of the pollen acceptor field and the border of the main pollinator field; 2. distance between the centre of the pollen acceptor field and the main pollinator field. Distances were measured by GPS or by the Geographic Information System (GIS). Based on the data of fertilization with distance to the pollen source a regression analysis with following exponential model was calculated:

$$Y = \exp(a+b*X)$$

where Y is the fertilization (%) and X the distance (m) between the measuring points in the pollen acceptor field and the pollinator field. Linear regression analysis was performed using SAS 9.1.3 with the log-distance as independent variable. The determination coefficient has been taken as criteria for the goodness-of-fit.

3 Results

3.1 Weather pattern and synchronization of flowering

During pollen shed of the neighbouring pollinators and the silking period of the pollen acceptor field westerly wind was generally observed except in the district Hartberg and Voelkermarkt, where south and south-west wind prevailed. Pollen shed of the main neighbouring pollinators was extensively synchronized with the silking periods of the pollen acceptor fields. Days of flowering periods varied between the experimental sites and ranged from 12 to 20 days depending on weather conditions, date of sowing and variety.

3.2 Gene flow

The rate of gene flow represented as a successful fertilization varied in all experimental sites. The minimum rate of fertilization was 4.5 % with a distance to the border of the main pollinator field of 75 m. The maximum fertilization rate was 97.5 % observed in an experimental field which represented a detasseled part within a non-detasseled maize field (Table 2).

Fertilization rate decreased significantly with increasing distance. Within the experimental field the fertilization rate was significant higher in the surface zone than in the field core. An example of a fertilization pattern is shown in Figure 1.

3.3 Distance to the pollen source

The rate of fertilization with regard to different types of distance measurement to the main pollinator field is illustrated in Figure 2–4. Based on the data points an exponential curve was set into each scatter plot. Results of the regression analysis based on the exponential model are shown in Table 3.

Comparing the three ways of distance measurement the distance measured between the centre of the pollen acceptor field and the border of the main pollinator field shows the highest R^2 with 80.1 %. The lowest R^2 resulted from the distance measurement from the border of the pollen acceptor field to the closest border of the main pollinator

Table 2: Fertilization rate (%) of each experimental field in the different climatic zones with the three different distances measured from the pollen acceptor field to the pollinator field. Fertilization is further shown as the number of counted kernels per ear versus a reference ear

Tabelle 2: Befruchtungsrate (%) der einzelnen Versuchsfelder in den entsprechenden Klimazonen mit den drei unterschiedlichen Distanzen, gemessen von der Pollenempfängerfläche zur Hauptpollenquelle. Weiters Darstellung der Befruchtung in Form der gezählten Körner pro Kolben im Vergleich zum Referenzkolben

Climatic Zone	Experimental field (pollen acceptor field) (n)	Fertilization per ear (number of kernels)			Reference ear (number of kernels)	Mean fertilization of the entire pollen acceptor field (%)	Distance** between pollen acceptor field and main pollinator		
		Mean (n)	min (n)	max (n)			Border: Border (m)	Centre: Border (m)	Centre: Centre (m)
North alpine zone	Field 008	339	56	540	451	75.2	1	20	100
	Field 016*	376	200	496	402	93.5	1	150	160
	Field 007*	466	384	544	478	97.5	1	5	20
	Field 006	324	36	576	497	65.2	1	30	60
Pannonian zone	Field 012	19	0	165	420	4.5	75	460	640
	Field 014	116	12	280	408	28.4	45	60	75
	Field 015	66	6	500	420	15.7	5	230	500
	Field 017	160	11	576	418	38.3	10	215	230
	Field 013	46	3	465	420	11.0	5	150	260
Illyricum	Field 002	250	25	520	450	55.6	15	45	80
South alpine zone	Field 009	333	176	528	470	70.9	1	60	135
	Field 010	306	130	560	460	66.5	1	40	110

*) Experimental field which is a small detasseled part within a non-detasseled maize field

**) Estimated distance

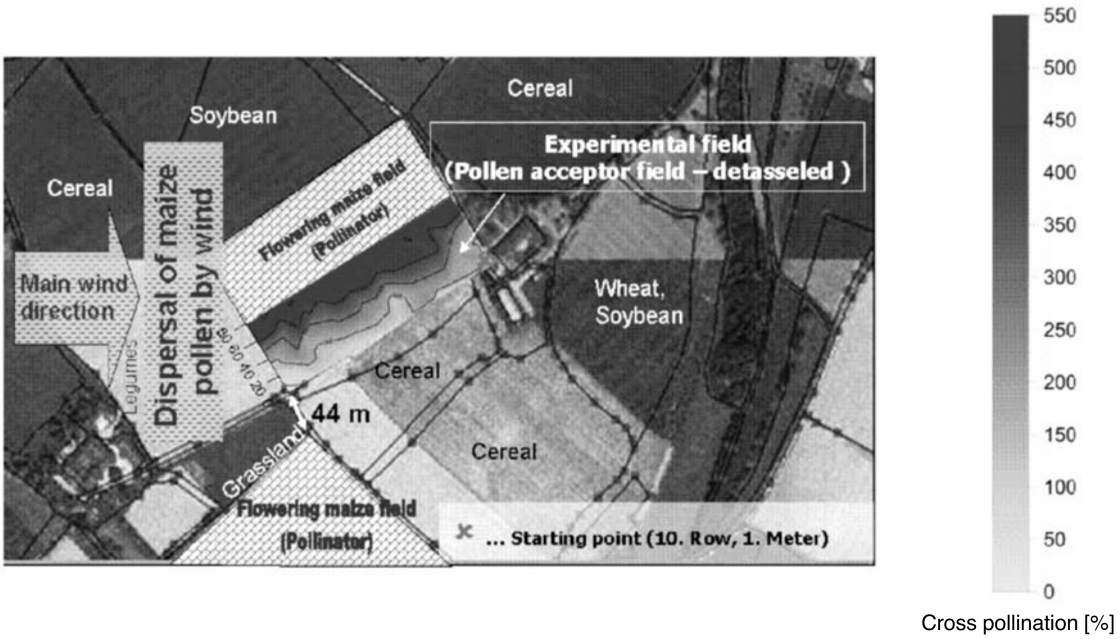

Figure 1: Example of an experimental field: acceptor field is surrounded by two pollinators. The main pollinator is adjacent to the experimental field. From row 80 to approximately row 60 400 kernels per ear were counted on average. The number of kernels per ear is decreasing toward south-east direction. The pollen impact of the pollinator in southern direction is incapable of measurement. Colour scale describes the cross pollination as number of counted kernels per ear (%)

Abbildung 1: Beispiel einer Versuchsfläche: die Pollenempfängerfläche ist von zwei Pollenquellen umgeben. Die Hauptpollenquelle grenzt an die Versuchsfläche an. Von Reihe 80 bis etwa Reihe 60 wurden durchschnittlich 400 Körner pro Kolben gezählt. Die Anzahl der Körner pro Kolben nimmt mit zunehmender Südost-Richtung ab. Die im Süden befindliche Pollenquelle zeigt keinen messbaren Polleneintrag. Farbliche Skalierung der Fremdbefruchtungsrate als Anzahl gezählter Körner pro Kolben (%)

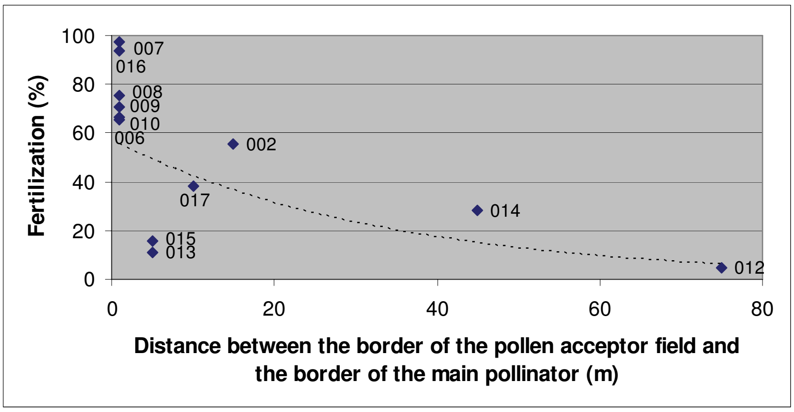

Figure 2: Mean rate of fertilization of each experimental field with the shortest distance measured between the border of the pollen acceptor field and the border of the main pollinator field

Abbildung 2: Durchschnittliche Befruchtung der einzelnen Versuchsflächen bezogen auf die kürzeste Distanz zwischen dem Feldrand der Pollenempfängerfläche und dem Feldrand der Hauptpollenquelle

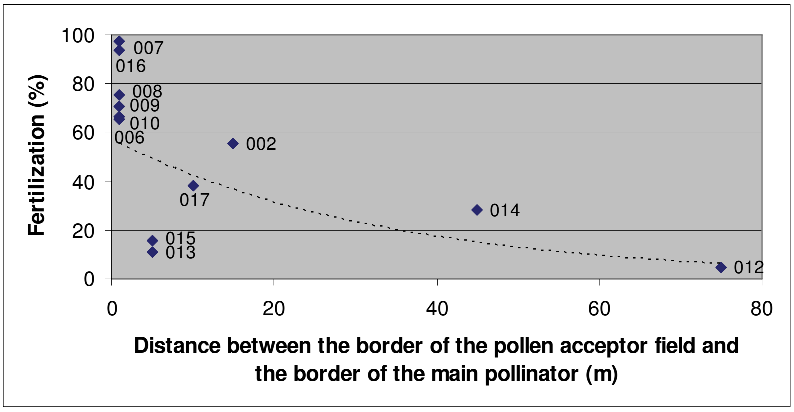

Figure 3: Mean rate of fertilization of each experimental field with the shortest distance measured between the centre of the pollen acceptor field and the border of the main pollinator field

Abbildung 3: Durchschnittliche Befruchtung der einzelnen Versuchsflächen bezogen auf die kürzeste Distanz zwischen dem Feldmittelpunkt der Pollenempfängerfläche und dem Feldrand der Hauptpollenquelle

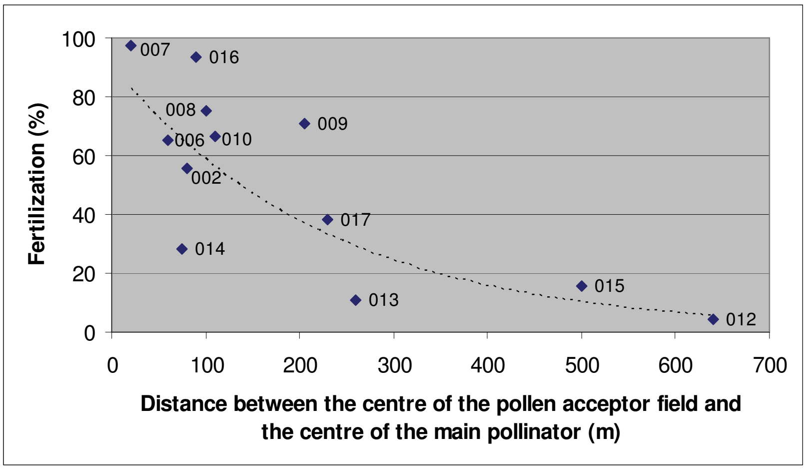

Figure 4: Mean rate of fertilization of each experimental field with the distance measured between the centre of the pollen acceptor field and the centre of the main pollinator field

Abbildung 4: Durchschnittliche Befruchtung der einzelnen Versuchsflächen bezogen auf die Distanz zwischen dem Feldmittelpunkt der Pollenempfängerfläche und dem Feldmittelpunkt der Hauptpollenquelle

Table 3: Results of the regression analysis for the fertilization rate with three different types of distance measurement between pollen acceptor field and the main pollinator field

Tabelle 3: Ergebnisse der Regressionsanalyse für die Befruchtungsrate bezogen auf die drei unterschiedlichen Varianten zur Messung der Distanz zwischen der Pollenempfängerfläche und der Hauptpollenquelle

Regressor	Parameter	Estimated value	Std. error	p-Value	R-squared
Border (Pollen acceptor) – Border (Pollinator)	Intercept	4.045	0.240	0.0000	50.8 %
	Slope	–0.0298	0.0093	0.0093	
Centre (Pollen acceptor) – Border (Pollinator)	Intercept	4.356	0.172	0.0000	80.1 %
	Slope	–0.0064	0.0010	0.0001	
Centre (Pollen acceptor) – Centre (Pollinator)	Intercept	4.507	0.218	0.0000	74.3 %
	Slope	–0.0044	0.0008	0.0003	

field. Rate of fertilization (%) showed a high significant association to all types of distance measurement (Table 3).

4 Discussion

The experimental field design used in this study represents an effective way to measure the maximum gene flow in view of the coexistence management in maize seed production as well as in corn production and silage. The field design expresses an authentic field constellation situation found in different Austrian maize cultivation areas. Detasseled plants in the experimental field create the possibility to measure the maximum gene flow as a successful fertilization because only maize pollen of neighbouring pollinators is able to fertilize the silks of the pollen acceptor field. Fertilization rate of the experimental fields varied between 4.5 % and 97.5 % depending on different environmental conditions (Table 2).

A similar field experiment was conducted by FOUEILLASSAR et al. (2005) in 2002/2003. Maize plants in different distances downwind from a maize field were detasseled. The resulting maximum fertilization capacity was at 50 m 52 %, at 100 m 27 %, at 250 m 7 % and at a distance of 400 m 4 %. The maximum gene flow evaluated in our study is higher resulting mainly from the differences of the experimental designs. The experimental design used by FOUEILLASSAR et al. (2005) was based on just one pollinator which was situated in one direction to the detasseled maize field, a cultivation situation that is not common especially in areas with intensive maize cultivation. With the following short presentations of results from current scientific works an overview of precautionary principals like isolation distance and buffer zones should be given.

WEBER at al. (2005) conducted a field trial on large scale with maize at different sites in Germany based on the labelling threshold of 0.9 % for adventitious presence of GM material in non-GM products. Bt-Maize fields with sizes between 1.8 and 18.3 ha were surrounded by conventional maize. A quantitative molecular assay was used to detect GMO presence in maize seeds (maize crop) or maize plants (silage maize). The results of 2004 for maize crop showed that at four out of eight sites in a band of 0–10 m adventitious GM values higher than 0.9 % were measured; after 20 m the threshold of 0.9 % was not exceeded in any of the eight sites. The results are comparable with these from silage maize. The conclusion of this field trial was that a buffer zone of 20 m of the conventional maize field has to be harvested separately to achieve a GMO-presence lower than 0.9 % in the remaining maize field. If the conventional maize field has a depth of at least 60 m, the GMO-presence of the entire harvest will be lower than 0.9 %.

The study of the Institute for Prospective Technological Studies (MESSEAN et al., 2006) considers two model scenarios for the presence of GM crops in the landscape (10 % and 50 % share of GM crop) and different target thresholds for the level of adventitious GM presence (0.1 % and 0.9 % for crop production and 0.1 %, 0.3 % and 0.5 % for seed production). To assess the levels of adventitious GM presence and the effect of changes in farming practices, a combination of expert opinion and gene flow models were used. Based on the simulation model (MAPOD®, see MESSEAN et al. 2006) isolation distances for Maize crop were determined to keep adventitious GM presence below a desired threshold for different field sizes and wind orientations. The simulation model is adapted from landscape data of the French region Poitou-Charentes, a region where maize fields are usually clustered around water supply points. Coexistence between clusters for a threshold at 0.9 % may be feasible if shared harvesters are properly cleaned. Intra-cluster coexistence would also be technically possible for 85 to 90 % of the maize area, but to achieve 100 % compliance for a 0.9 % threshold additional measures would be necessary. Intra-cluster coexistence at a 0.1 % threshold would not be technically feasible.

LANGHOF et al. (2008) compared in a German field experiment in 2005 the ability of a sunflower crop versus a clover-grass crop to reduce outcrossing when planted as buffer between a yellow kernel pollen donor plot and a white kernel recipient maize plot. Results showed no general effect of different buffer-crops on gene flow.

The results of the presented studies are based on completely different experimental designs, evaluation methods (GM-DNA-presence or % kernels per ear) and differences in landscape and climate. Results of quantitative DNA-analyses can not transformed in results of qualitative analyses without any difficulty (DEVOS, 2008). The comparison of the results of different studies should demonstrate the variability of this topic.

The maximum gene flow does not characterize a real cultivation situation but represents a worst case scenario as it is possible in hybrid maize seed production. Based on the maximum gene flow a risk assessment can be developed which assures a high safety standard for future coexistence of GM and non-GM maize. The results of this study show that the level of gene flow as a successful fertilization was significant high, resulting from a couple of influencing factors. Wind direction, wind velocity and geographical barriers were not considered in this first approach even though wind conditions seem to be one of the most important factors affecting gene flow. Wind velocity and wind direction can not be influenced and are not controllable under field conditions. Further more the distances between the weather stations and the experimental sites varied between 7 and 24 km so it was not possible to observe the local wind currents which seem to play an important role in the dispersal of maize pollen. The farm-scale evaluation study conducted by HENRY et al. (2003) used a split-field design where a GM maize and a non GM-maize field were placed together on the same site in a wide range of locations and environments in England. One of the conclusions of HENRY et al. (2003) was that there are a number of factors that affect pollination rates in maize but wind speed and direction and surface turbulence can also affect pollination rates. These factors make it difficult to forecast the effect of one maize field on another.

Despite of detasseling the experimental site the rate of fertilization was unexceptionally high. One of the reasons for this circumstance can be explained by the short distances to the pollinators. Most of the experimental fields were adjacent to one or more pollinator fields which caused a high pollen pressure in the experimental field. The fertilization pattern shows that the fertilization rate in the surface zone was much higher than in the field core. Based on this fact the fertilization rate in the first ten rows and first ten meters (based on 70 cm distance between the rows) was measured (data not shown). This circumstance, in view of the implementation of buffer zones, could be an interesting fact for fields which are bigger in size. In this study this approach was not further tracked because buffer zones play a subsidiary role in Austria's coexistence management.

The use of a non-artificial experimental design gave reason to associate the resultant variety in field size, constellation and field shape with the rate of fertilization. Emanating from the fact that the field sizes of the pollen acceptor field and the pollinator field play a role in view of the fertilization pattern and fertilization rate the existing method of defining the distance between both fields was advanced. The method to measure the distance from the centre of the pollen acceptor field to the closest border of the pollinator field is based on the consideration that a small pollen acceptor field shows a higher rate of cross-fertilization than a field which is bigger in size when the pollinator is situated in the same distance. The method measuring the distance from the centre of the pollen acceptor field to the centre of the main pollinator field is based on the additional aspect of the field size of the pollinator. A similar approach was tracked by ŠUŠTAR-VOZLIĀ (2008) in a forecast-model for Slovenia presented in an oral lecture 2008: minimum-distance (shortest distance between sample point and border of donor maize), centre-distance (shortest distance between sample point and centre of donor maize), visual angle = angle between the corner of the donor field and the sample point (0–180°) and meteorological parameters were subject matters of the forecast-model to explain outcrossing. Results showed that the geometry of the donor and receptor field played a crucial role in the process of outcrossing. More than 80 % of variation was explained with distance and position of the sampling point.

The statistical analysis of the present study show that the method measuring the distance from the centre of the pollen acceptor field to the closest border of the pollinator field has the highest R^2 of 80.1 %. In comparison the method measuring the shortest distance between the field borders shows a low R^2 of 50.8 %. In further analyses the correlation of the position of the pollinator field to the pollen acceptor field on the rate of fertilization should be determined. Outgoing from a common rectangular field it is to expect that a pollinator field situated along the length of the pollen acceptor field affects a higher rate of cross fertilization than a pollinator field situated along the broadside of the pollen acceptor field.

The results of this study demonstrate that the rate of gene flow depended on numerous factors. Coexistence management decisions are required before planting the fields. Insofar an applicable coexistence management ought to be concentrated on the factors which can be directly influenced. Precautionary principals on the field should contain primary the isolation distance including the method of measurement and the varietal purity regarding adventitious presence of GM in view of the labelling threshold of 0.9 %. In this context a validation of the conclusions based on seed impurity in the applied model of the study of the Institute for Prospective Technological Studies (MESSEAN et al., 2006) seems to be useful and justifiable. Even though the presented results are based on data of just one year under field conditions they should help to develop technical strategies for a coexistence management applicable for authentic cultivation situations in Austria. In the mean time an additional study was performed in 2007 with modified design and methods and a summary report is in development.

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Address of authors

Leopold Girsch, Österreichische Agentur für Gesundheit und Ernährungssicherheit, Bereich Landwirtschaft
Nives Angerer, Österreichische Agentur für Gesundheit und Ernährungssicherheit, Institut für Saatgut
Josef Hartmann, Österreichische Agentur für Gesundheit und Ernährungssicherheit, Institut für Saatgut
Harald Strelec, Department für Raum, Landschaft und Infrastruktur, Institut für Angewandte Statistik und EDV
Hans Peter Stueger, Österreichische Agentur für Gesundheit und Ernährungssicherheit, Institut für Biostatistik

Corresponding author

Nives Angerer, Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH – Austrian Agency for Health and Food Safety, Institut für Saatgut, Abteilung Zertifizierung, Spargelfeldstraße 191, 1220 Wien, Österreich
E-Mail: nives.angerer@ages.at
Tel.: +43(0)50555 34812, Fax: +43(0)50555 34808

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