Conference
Coexistence of GM and non-GM crops

Scientific data, practical applications and perspectives for the next decade

June 9 and 10, 2005

Agroscope FAL Reckenholz
Reckenholzstrasse 191, CH-8046 Zurich, Switzerland

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Agroscope FAL Reckenholz, Swiss Federal Research Station for Agriculture and Agroecology, Reckenholzstrasse 191, CH-8046 Zurich

Program

June 9, 2005

09:00 **Registration, welcome coffee**

09:45 **Welcome address**
Peter Stamp, ETH Zürich, CH

Session 1: Introduction
Chair: Peter Stamp

10:00 **Living with GM crops – fact or fantasy**
Franz Bigler, Agroscope FAL Reckenholz, CH

10:30 **The regulation of GMOs in Switzerland and in the EU**
Olivier Félix, Swiss Federal Office for Agriculture, CH

11:00 **Implementation of GMO regulation by breeders and the seed industry**
Simon Barber, EuropaBio, B

11:30 **Coexistence - other legal aspects to consider**
Julian Kinderlerer, University of Sheffield, UK

12:00 **Discussion**

12:15 **Lunch**

Session 2: Existing experiences with coexistence issues
Chair: Michael Winzeler, Agroscope FAL Reckenholz, CH

13:45 **Cross-fertilization in maize - experiences with the cultivation of GM-maize in Spain**
Joaquima Messeguer, IRTA, E

14:15 **Coexistence and maize – experiences and results from the German "Erprobungsanbau" 2004**
W. Eberhard Weber, University Halle, D

14:45 **Cross-fertilization in maize – results from a Swiss study**
Michael Bannert, ETH Zürich, CH

15:15 **Discussion**

15:30 **Coffee break**

16:00 **Outcrossing of rapeseed**
Karin Förster, University Halle, D

16:30 **Managing spatial and temporal gene flow in oilseed rape**
Jeremy Sweet, UK

17:00 **Seed persistence of oilseed rape and population dynamics of its volunteers**
Carola Pekrun, University Göttingen, D

17:30 **Discussion**

17:45 **End of day 1**

June 10, 2005

Session 3: Molecular strategies for pollen containment: contributions of plant sciences to coexistence

Chair: Beat Keller, University Zürich, CH

09:00 **Preventing outcrossing now by cultivation of male sterile cms-maize**
Karl-Heinz Camp, DSP Delley, CH

09:30 **Is cytoplasmic male sterile maize really sterile? Review and outlook**
Peter Stamp, ETH Zürich, CH

10:00 **Cleistogamy in oilseed rape: a tool to minimise pollen flow**
Jacqueline Pierre, INRA, F

10:30 **Discussion**

10:45 **Coffee Break**

11:15 **Potential molecular strategies for pollen containment in the near future: pollen incompatibility in maize**
Mark Frei, Maize Technologies International GmbH, A

11:45 **Potential applications of apomixis in plant breeding and transgene containment**
Ueli Grossniklaus, University Zürich

12:15 **Discussion**

12:30 **Lunch**

Session 4: Coexistence: Background and concepts

Chair: Franz Bigler, Agroscope FAL Reckenholz, CH

14:00 **The concept of the Danish working group on coexistence**
Birte Boelt, Danish Institute of Agricultural Sciences, DK

14:30 **Consensus on coexistence in the Netherlands – the Dutch way**
José Rompelberg, Limefarm, NL

15:00 **A concept for coexistence of GM- and non-GM crops in Switzerland**
Olivier Sanvido, Agroscope FAL Reckenholz, CH

15:30 **Discussion**

15:45 **General discussion**

16:15 **Farewell**

Abstracts Presentations

Living with genetically modified crops – fact or fantasy?

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In 2004, genetically modified (GM) crops have been grown commercially on 81 million hectares which is about 2% of the total acreage of agricultural crops cultivated worldwide. The four most important GM plants were soybean, cotton, maize and oilseed rape, accounting together for almost 100% of the total GM crop acreage. More than half of GM crops were grown in the USA, but Argentina, Canada, Brazil and China are other important GM crop growing countries. It is generally assumed that the acreage with GM crops will further increase in the future, and it is expected that countries like China, India, Brazil and South Africa will contribute substantially to this increase in the next years. As an example, herbicide tolerant soybean and insect resistant cotton have recently been approved for commercial cultivation in Brazil. Herbicide tolerance, insect resistance and virus resistance are still the only three traits introduced into GM plants which are commercially grown. Spain is the only western European country so far, where insect resistant GM maize, containing the toxin of Bacillus thuringiensis (Bt), was commercially grown in 2004 on approximately 60'000 hectares. A number of Bt maize varieties were approved in 2004 for commercial cultivation in the European Union (EU) and it is to be expected that other European countries will grow such GM varieties in the near future. Major motivations and expectations of adopting GM crops by farmers are: a) increased economic values of GM crops by reducing potential losses due to insect pests, viruses, diseases and weeds, b) lower pesticide costs (e.g. insecticides in cotton), c) simplicity of pest and weed control methods, d) more flexibility of weed control by post-emergence applications and e) lower farmers' health risk by reducing insecticide use.

One constraint in adopting GM crops in Europe is the obligation of the EU member states to establish national regulations and strategies that should enable coexistence of all agricultural production systems, be it with or without GM crops. The European Commission explicitly states that neither of these production systems should be excluded, and farmers should have free choice of production systems. The consumer's freedom of choice between food produced with different agricultural methods should be facilitated to the greatest possible extent. The consequence of this is a strict separation of non-GM and GM crops and their products on-farm, during processing of the harvest and during retail. The European Commission considers that measures for coexistence should be developed and implemented by the Member States. As national regulations and strategies for coexistence have been established in a few Member States only and as there is no practical experience gained so far, coexistence measures are highly debated both in politics and in the public.

Costs for GM crops may accrue to farmers if stringent coexistence measures will be established by governments. This is especially the case if economic damage to non-GM crop farmers has to be fully refunded by individual GM crop farmers. Additional costs may result for GM crop farmers due to increased labour, i.e. for more elaborate documentation, agreements with neighbours, segregation of harvests at transport and storage on the farm. In addition, separate processing of goods by food industry and the management of additional food chains by retailers may contribute to higher costs, which may result in increased food prices for consumers. National liability regulations may have further impacts on the decision of GM crop farmers to undertake the risk, and consequently, this will strongly influence adoption of GM crops in Europe.

Coexistence measures will be largely influenced by intrinsic properties of the cultivated plants with regard to their mechanisms of out-crossing on non-GM crops (e.g. self- or cross-pollination, pollen transport by wind or insects), their potential to build volunteers, to overwinter under specific climates and the possibilities of applying new techniques for genetic containment of gene flow. A list of crop plants is provided showing an overview of relevant properties from which efforts for coexistence measures at the farm level can be deduced.

Swiss and EU Regulations Concerning GMOs

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The law of 21 March 2003 on gene technology in the non-human field is the basis of existing Swiss legislation on the subject[1]. This law came into force on 1 January 2004 and aims to protect “humans, animals and the environment against abuses of gene technology” as well as to “serve the welfare of humans, animals and the environment in the application of gene technology”.

In line with the terms of directive 2001/18/EC issued by the European Parliament and Council on 12 March 2001 concerning the voluntary dispersal of genetically modified organisms in the environment, the Gene Technology Act stipulates that official authorisation must be obtained to test GMOs in the field as well as to supply GMOs intended for use in the field. The law sets out stringent conditions for the granting of such authorisation, notably with regard to the protection of humans, animals and the environment. Moreover, it forbids the provision of GMOs intended for use in the field which contain genes that cause resistance to the antibiotics used in human or veterinary medicine. From 31 December 2008 on this ban will also apply to the provision of GMOs for experimental purposes. It is also forbidden to supply genetically modified vertebrates for purposes other than science, therapy or medical or veterinary diagnosis.

According to the terms of the Gene Technology Act, authorisation or notification is also necessary with regard to activities involving GMOs in contained systems. This stipulation conforms to the terms of directive 90/219/EEC issued by the Council on 23 April 1990 relating to the use of genetically modified organisms in contained systems.

The law sets out the notion of respect for the integrity of living organisms in the case of modification of their genotype; this integrity has not been respected if, in particular, “species-specific traits, functions or habits are substantially impaired or if [such impairment] is not justified by preponderant legitimate interests”.

The law also sets out specific regulations regarding civil responsibility in relation to GMOs. It stipulates in particular that if a GMO causes damage to the products of farmers or foresters or to consumers of their products, the holder of the corresponding authorisation to use GMOs will be held solely responsible for that damage. People who use GMOs are required to do so according to instructions.

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[1] not legally binding in the English translation: http://www.umwelt-schweiz.ch/imperia/md/content/stobobio/biotech/17.pdf

The Gene Technology Act contains important principles with regard to coexistence. For example, "persons who handle genetically modified organisms must ensure that those organisms, their metabolites and the resulting waste do not impair production that does not involve genetically modified organisms or consumers' freedom of choice". Moreover, it is the responsibility of any person who supplies genetically modified organisms to provide the recipient with the necessary instructions for their use to ensure that the terms of the law are respected, notably in relation to products that have not been genetically modified and consumers' freedom of choice. The law also stipulates that genetically modified products must be processed separately in order to avoid any undesired mixing with organisms that have not been genetically modified; GMOs must be labelled as such when they are supplied.

The terms of the Gene Technology Act are in line with the relevant regulations laid down by the European Union; Swiss legislation on the matter also includes certain aspects such as questions of civil responsibility, however, that are not included in the EU regulations that refer specifically to GMOs.

Most of the provisions for the application of the Gene Technology Act have already come into force. For example, the ordinance of 25 August 1999 on the dispersal of GMOs in the environment[2] sets out how and under what conditions this may be done. The ordinances of 1 March 1995 on foodstuffs[3] and of 26 May 1999 on animal fodder[4] contain regulations regarding authorisation and labelling of GMOs in these two sectors. These regulations take into account the terms of (EC) regulation no. 1829/2003 issued by the European Parliament and Council on 22 September 2003 concerning genetically modified foodstuffs and animal fodder.

A particularity of Swiss legislation is an article in the ordinance of 7 December 1998 on seeds relating to the chance presence of GMOs in seeds that have not been genetically modified.

As far as concerns coexistence, certain articles in the Gene Technology Act have not yet been set out in detail in ordinances for application of the law. One aspect in particular that remains to be dealt with is the way in which instructions for ensuring GMO-free production are to be passed on by the holder of the supply permit to the recipient. Regulations concerning the separate processing of genetically modified products also need to be drawn up in detail. This concerns in particular the cultivation of genetically modified crops and harvesting techniques on farms.

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[2] RS 814.911
[3] RS 817.02
[4] RS 916.307

Coexistence - legal aspects to consider

Julian Kinderlerer

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When transgenic foods were first considered for use in Europe there was little public reaction, and where there was, it was positive. A tomato paste produced from genetically modified tomatoes was on sale in two supermarkets in the UK during 1998 and sold extremely well. At the turn of the century, the public response to the technology had turned, with a clear rejection of its use. Although the public in many countries has been fearful of the introduction of the products of biotechnology, Parliaments have not been as reticent, and have recognised both benefits that may arise from its use and the risks that it theoretically poses. In 1993 the Parliamentary Assembly of the Council of Europe passed recommendation 1213 (13th May 1993) on developments in biotechnology, for which there were many wonderful prospects, but also for which there were many concerns. Unlike North America where foods produced using modern biotechnology have attracted governmental support and if nothing else, public apathy, many European Governments have imposed an effective moratorium on these products. Whereas a small number of genetically modified foods were approved for growing and/or for food use in the late 1990s, very few have been allowed this century, even though a new directive on the release and marketing of genetically modified organisms (2001/18) and regulations on genetically modified food and feed (1829/2003/EC) and on “Traceability and Labelling of Genetically Modified Organisms and the Traceability of Food and Feed Products produced from Genetically Modified Organisms” (1830/2003/EC) have now been introduced.

The concerns in Europe are based on many things, including the pace of the introduction, opposition to globalisation and fear of damage to both human health and of the environment due to the insertion of foreign genetic material into foods. Many (if not most) consumers in Europe are concerned that transgenic crops will ‘pollute’ or damage the environment and may affect their health. The ‘pollution’ or damage includes the impact of these new products on traditional or organic crops as well as the impact on the natural environment, weeds, birds etc. Although agriculture is recognised as having to be efficient, it is also part of the perceived natural environment, and consumers expect respect for the environment. Wholesale use of chemicals to increase yields, or modification of the agricultural practices is possibly not acceptable if it means that birds and wild flowers disappear. Coexistence now becomes an important issue, where the impact of growing transgenic crops in proximity to both traditional agriculture and organic agriculture has to be taken into account. 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 [1]

It is only those transgenic products that pass an extensive safety review which are permitted onto the market. The review includes risk assessment as set out in the directive and regulations and that incorporated into the Cartagena Protocol on Biosafety (which is primarily concerned with the protection of biological diversity). Directive 2001/18 is clear that if there is a risk of an adverse effect that cannot be managed it should not be authorised. Croplife Canada[2] have asserted that "[a]ll agricultural systems that are deemed safe should have an equal opportunity to contribute to the agri-food production system under free market conditions. Preference of one system over another must not be the result of artificial and impractical standards. Coexistence of different agricultural systems can play an important role in sustainable agri-food production system in Canada and globally. Coexistence is not a safety issue as all products currently available in the marketplace have been thoroughly tested for food and environmental safety." This has been accepted by the European Union where a discussion document states that no form of agriculture, be it conventional, organic, or agriculture using GMOs, should be excluded in the European Union. "In principle, farmers should be able to cultivate the types of agricultural crops they choose - be it GM crops, conventional or organic crops. None of these forms of agriculture should be excluded in the EU" In addition, the ability to maintain different agricultural production systems is a prerequisite for providing a high degree of consumer choice.[3] The document further states that "[t]he ability of the food industry to deliver a high degree of consumer choice goes hand in hand with the ability of the agricultural sector to maintain different production systems. This means that, if food has been certified as safe for human and animal consumption and that its impact on the environment is minimised as required under the Directive, "the pending issues still to be addressed in the context of co-existence concern the economic aspects associated with the admixture of GM and non-GM crops".[3] The Commission therefore recommended that the Member states implement management systems individually." It is seen as important that "[s]trategies and best practices for coexistence need to be developed and implemented at national or regional level, with the participation of farmers and other stakeholders and taking account of national and regional factors" Management measures for co-existence should build on and take into account already existing segregation practices/methods and available agricultural experience about handling of identity preserved crops and seed

[1] Commission Recommendation of 23 July 2003 on guidelines for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming, Recital 3.

[2] Coexistence of Process Based Agricultural Production Systems - Conventional, Organic and Genetically Engineered (GE) Crops Reference Number: 029 Last Update: June, 2004, Croplife Canada: http://www.croplife.ca/english/resourcecentre/bio-positionpapers-coexistance.html

[3] Commission Recommendation of 23 July 2003 on guidelines for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming.

production practices. The document expects that countries will introduce measure for co-existence that are efficient and proportionate, avoiding unnecessary burdens for farmers and others producing the food or feed. Most importantly, the manner in which each state chooses to regulate should take account of national liability rules in the event of economic damage resulting from the ‘pollution’ needs to be carefully considered and all those involved in the production process should be fully informed of the criteria that will apply in their country in relation to liability and redress.

The European Parliament has taken a much stronger stance, in that it believes that risk assessment procedures are not infallible. “In contrast to the introduction of other technologies or substances in the agricultural and food economy, GMOs are capable of reproducing and exchanging genetic information with other crops and wild plants”… “The key factor in organising coexistence in the context of risk management is therefore the question of whether the placing on the market and the release of GMOs can be reversed.” It is seen as vital that inadequate and unclear rules make it impossible to properly implement authorisation, traceability and labelling rules.[4]

There is much to discuss in relation to transgenic crops, their impact on the environment, the concerns that they are really different from those produced by ‘traditional’ means, and the manner in which any liability for harm to other producers or to the environment can properly be addressed. All the countries in Europe are considering the approach they take to coexistence and the modifications needed to their statute law or voluntary agreements that might truly permit co-existence. The stance taken by the Netherlands and Denmark is very different, and this will be highlighted in the presentation.

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[4] Report on coexistence between genetically modified crops and conventional and organic crops 2003/2098 (INI) European Parliament, 4 December 2003.

Cross-fertilization in maize- experiences with the cultivation of Bt-maize in Spain

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On 1998 two Bt maize varieties were approved for commercialization, but only one of them, (Compa CB (Bt 176) from Syngenta Seeds) has been sold effectively. During 1998-2002 Bt maize adoption in Spain stagnated at about 25 000 ha because Syngenta's voluntary arrangement. In 2003 and 2004 the adoption of Bt maize had increased to 32000 and 58000 ha respectively, due to the fact that new varieties developed by several companies were approved. Bt maize has been grown for feed production and only in those areas such as the valley of river Ebre where corn borers produce significant losses. Several studies published recently have pointed out that till now, there has not been detected any significant problems related with the coexistence with conventional or organic crops. Nevertheless, according to the EU Legislation, an official regulation on coexistence based on reliable data, has to be established in all countries from EU, and so, several field studies have been performed recently in Spain, mainly focused to the evaluation of pollen-mediated gene flow in our particular conditions.

A field study was conducted by our Institute at Lleida (Catalunya). A 50 x 50 plot of Bt maize was planted in the middle of a field and surrounded by a the conventional variety. The total area of the trial was 7.5 ha. Speed and wind directions were recorded during the flowering period. By analyzing by RT-PCR technique the 250 samples collected, it was found that the level of GM adventitious presence decreased rapidly with distance from the GM emitter crop. Wind direction strongly influences the rate of GM adventitious presence. Thus, in the direction of the prevalent wind, the rate of GM in the non- GM crop was already less than 0.9% at a distance of 10 m whereas in that located upwind direction, the rate of GM was less than 0.9% at a distance of 2 m from the GM emitter crop. These results agree with those obtained in other field trials conducted during the same year. Nevertheless, the size of Bt maize plot used in this field trial was too small to properly predict what will happens with larger fields such as those commonly planted in the south of Spain.

So, another field trial was conducted on 2004. In this case, four Bt maize varieties (with yellow colored grain) were planted in such a way that each one of them occupied different area. Bt varieties were surrounded by a non- transgenic one (white grain). Total area of transgenic varieties was of 4 ha whereas the total area of the field was of 27 ha. In this trial a visual identification of yellow grains placed in the cobs of the white variety will allow us to evaluate the gene flow very accurately along the whole field. By analyzing these grains it will be possible to identify from what variety GM pollen comes from and to estimate the influence of the size of the field in cross pollination. This research is still in progress.

Another question that has to be taken into account, is what can happens when, GM, conventional and organic fields coexists in the same area; this is in a real situation of coexistence such as we have in Spain. In this sense a research program has been recently started in the frame of SIGMEA project. Data obtained in this research will be used to validate the gene flow simulation models and will contribute to design and implement an operational, practical and dynamic generic gene flow modeling platform a the landscape level.

Coexistence and maize – experiences and results from the German „Erprobungsanbau“ 2004

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Introduction

Worldwide transgenic maize is grown on 19.3 Mio. ha. This is about 15 per cent of the whole maize acreage, and it is expected that the acreage will increase in future and that Germany cannot ignore this development. The “Erprobungsanbau” 2004 should clarify on German conditions if simple rules for the coexistence between GMO and non-GMO can be derived for maize. For this purpose Bt-maize was grown at 28 sites in seven German countries, 19 with silage maize, 8 with kernel maize and one with LKS (crushed husks and cobs). At all sites the Bt-maize field – the size varied between 0.3 and 23 ha – was surrounded by the near isogenic conventional maize variety in all directions. All Bt-maize varieties carried the construct MON 810. The field depth of the conventional maize was planned to be at least 60 m in all directions. However, since the maize was grown on farmers’ fields in normal practical conditions, this could not be realized in some cases, so that missing cases had to be taken into account. Corresponding to this plan the farmers arranged the sowing, cultivation and harvest. The whole crop had to be stored and used within the farm.

Observations in the vegetation period

Within the project the flowering time of male Bt-maize and female conventional maize plants was controlled . To see if coexistence could be arranged by choosing different sowing dates for Bt-maize and conventional maize or by choosing earlier developing conventional varieties, one track width of conventional maize was sown within the Bt-maize field at some sites. However, the climatic conditions in 2004 with retarded growth in the early phase led to the situation, that there was an overlapping period between male and female flowering periods at all sites. Therefore Bt-maize pollen could fertilize the conventional maize plants.
The other important climatic factor is the wind. The corresponding data were collected from the nearest weather station. The direction and strength of the wind is most import during the hours of pollen flow. There was no main wind direction in 2004.

Harvest procedure

At harvest samples were taken from the surrounding conventional field in all 4 directions in 3 distances from the Bt-maize field (0-10m, 20-30m and 50-60m) yielding 12 samples per site. At some sites additional samples were taken, namely in the track of conventional maize within the Bt-maize field and in a few cases in larger distances. To get representative samples, each sample was a mixture of sampling on three points within every distance or direction. The samples were dried and grinded to a particle size of 4 mm. This was necessary to get enough particles for the analysis. The samples were divided into 5 sub samples of 1 kg each. Two selected certified labs got one sample for estimation of GMO-DNA content.

Selection of labs

We tested four certified labs to select those giving the most reliable data. For that purpose these labs got 4 samples of maize flour with a known content of GMO by mixing conventional and Bt-maize kernels. The contents were 0 %, 0.4 %, 0.8 % and 1.5 %. All labs found no GMO in the samples with 0% and detected the GMO content of the other samples in the correct order. One lab underestimated and one lab overestimated the content in both independent analyses. There was no systematic bias for the remaining labs which were therefore selected for the main analysis.

Results

The first remarkable result was, that the labs were able to detect errors of the design at one site with silage maize. At this site large GMO contents above 20 % were detected at the distance 50-60 m. This is only possible if Bt-maize plants were harvested at this distance. This site therefore was excluded from further evaluations.

As expected, the largest content of GMO was found within the short distance, followed by the other two distances. As mentioned above, there was no main wind direction. Therefore the values from every distance in all four directions were averaged. The coexistence depends on the keeping of threshold values. The EU threshold value for unwanted contamination is 0.9 % genetically modified DNA within the total DNA. Charges with a higher content have to be signed. This happened in some cases within the distance of 0-10 m. Six out of 19 sites with silage maize and four out of 8 sites with kernel maize and the site with LKS had a higher content, the maximum value was 3.74 %. But there were also four sites showing no more than 0.2 % within this distance. Since the Bt-maize fields varied in size from 0.3 to 23 ha, we tested if there was a correlation between field size and content of GMO, but a correlation could not be detected. Further, the size of the Bt-maize field is not only main cause for the large differences, but probably the different environmental conditions found in German maize growing areas.

For other distances (20-30 m and 50-60 m) no value was above the threshold value of 0.9 %. The maximum was 0.69 % and 0.36 % for the distance 20-30 m and 50-60 m, respectively. Therefore no charge from these distances would have been signed.

The tracks with conventional maize within the Bt-maize field yielded in some cases GMO contents above the threshold value. Two reasons for explanation are possible. The first one is the good overlapping period of the flowering time. Second it cannot be excluded that some Bt-maize plant of the neighbouring track were also harvested. Even a single plants can strongly bias the results. Therefore these results cannot be generalized.

Conclusions

1. In 2004 it was not possible to avoid overlapping flowering periods by choosing different sowing dates or varieties differing in the development. This year also showed that the direction and strength of the wind during flowering cannot be predicted in advance.
2. At a distance of 0-10 m from the field with Bt-maize the farmer has to expect that the GMO content in the conventional maize may be above the EU threshold value of 0.9 %. Such charges must be signed as long as the GMO content is not estimated.
3. If the distance was at least 20 m, no sample above the threshold value was found under the conditions in 2004. Since the trade takes large charges, it is expected that the GMO content of a charge is below the values found at the distance 20-30 m.

Acknowledgements

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Cross fertilization in maize – results of a Swiss study

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In 2003 and 2004 a study on cross pollination of maize was performed in three regions of Switzerland (Kanton Zurich, Uri and Zug) by a research group of ETH Zurich. Around 30.000 ears of 22 field trials with sizes of 0.5 to 3 ha were checked for cross pollination. The cross pollination study was done without using transgenic plants, but using maize varieties with different kernel colours. A white kernel hybrid maize of the company DSP Delley seeds and plants AG was used to check the cross pollination of yellow kernel maize fields, because yellow kernel colour is dominant to white kernel colour. If pollen of yellow kernel maize fertilizes on white kernel maize, there will be a yellow kernel on the white kernel ear for each successful pollination. By just counting the yellow kernels on white kernel plants, it is possible to calculate the cross pollination rate quickly. Because this method is simple and rapid, it was possible to take high sample numbers.

In 13 field experiments, the cross pollination between fields with long distances of 52 m to 4440 m in the Urner Reusstal (Switzerland, Kanton Uri), a region with a low density of maize fields but high density of grassland, was investigated. This region, surrounded by up to 3000 m high mountains and the Vierwaldstättersee lake, is representive for agricultural areas close to mountains with prevailing grassland use and a few fodder maize fields. Because of the high mountainous surrounding and the long distance to next maize fields we assume, that pollen that disperses around the valley was only coming from the fields of this valley. In all fields the total cross pollination rate was below 0,017%.

In 6 more field trials, cross pollination on short distance, in the case of two fields growing next together, was investigated. The field trials were performed in Lindau/Tagelswangen (Kanton Zurich), a region typical for hilly land of Switzerland with a mixture of housing areas, woods and agriculture like maize, wheat, rapeseed and grassland. If maize is growing next to another maize field within the first 20 m adjacent to the pollen donor field, the cross pollination rate declines quickly below the EU threshold of 0,9%. On short distances of 1-10 m, there is a big variability in cross pollination. For example, on one ear there are 179 cross pollination events and on the adjacent ear 44. The different developmental stage of single plants as well as differences in wind patterns within a maize field could be a reason for this big variance.

Cross pollination will only occur if fertilizable female flowers have emerged and they have not been fertilized yet. Pollen must not disperse too early or too late. Good timing of flowering is important. With an experiment it was examined if a field with just beginning flowering can cross pollinate in maize field, just finishing main flower period. Of 185 checked white kernel ears close to the pollen donor (distance 1 m), only 41% showed cross pollination kernels. Usually in this distance, with good flower synchrony, nearly 100% of the ears show cross pollination kernels. It was evident that particularly late developed plants (small plants and ears) show high cross pollination rates. 5,5% of the sampled ears were classified late because of their small size. 64% of these ears had high cross pollination rates of up to 80% and contributed by 47% to the cross pollinations of the whole field. 74% of the sampled ears were classified normally developed and only 8,5% of these ears had cross pollination kernels, with usually only a few cross pollination kernels per ear. The female flower of late developed plants was around two weeks later and so flowering synchrony to the next later flowering field was good. But the quota of the late developed plants was only 5,5% and therefore the cross pollination rate of the whole field was 0,46%.

The study was funded by BUWAL (Federal agency for environment, forest and countryside), Swisssem (Swiss Seed production association) and Fenaco (Association of Swiss Agriculture).

Outcrossing of oilseed rape

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Summary

Crop production and seed multiplication take place either in terms of integrated or organic farming. In both production systems crop cultivation and quality of harvesting products have to meet special demands. In particular, occurrence of admixtures of genetically modified plants and seeds is not accepted in organic farming.

Oilseed rape (*Brassica napus* L.) belonging to the genus *Brassica* is a member of the subtribe *Brassicinae*, tribe *Brassiceae*, Brassicaceae family. *Brassica* contains more than 100 species including *Brassica napus* and *Brassica rapa* L. with both oil-yielding and tuber-bearing types, and also the different groups of *B. oleracea*, *B. juncea*, *B. nigra* and *B. carinata*. In Europe, *Brassica napus* mainly is autumn sown. Common used cultivars refer to open pollinating lines, different types of hybrids, and composite material. To produce hybrids the cultivation of male sterile parental lines and an effective pollination control systems are essential in seed production. Oilseed rape (n=19) is an amphidiploid derivative of *B. oleracea* (n=9) and *B. rapa* (n= 10). Crop is pre-dominantly autogamous with different percentages of intraspecific cross-fertilization. Therefore, gene flow from crop to crop varies enormously (Eastham, Sweet 2002; Förster, Diepenbrock 2002) depending on cultivar, size and design of the donor field as well as the acceptor field, distance between donor and acceptor, flowering period, pollen concentration in the air above rape field, availability of insect pollinators, wind mediated pollen dispersal and pollen viability. Therefore results on outcrossing are extremely variable due to year, cultivar and location. In 1996/1997 and 1997/1998 field experiments with three genetically modified (GM) hybrids (PGS Gent), three open pollinated cultivars, one restored hybrid and one composite hybrid were carried out in Saxony-Anhalt, Germany. Cross-fertilization of conventional cultivars ranged from 5 to 18 % (8 to 12 %) if the plots bordered on the left and the right side of genetically modified hybrids. Without direct contact between conventional cultivars and herbicide-tolerant oilseed rape outcrossing rates decreased to 3...5 % (2...6 %). The acceptor potential of the conventional cultivars increased due to adjacent cultivars differing in cross-pollination and flowering period. Our results are closed to data of Hühn and Rakow (1979) using erucic acid content as cross-fertilization marker.

In a field experiment a blend of *B. rapa, B. juncea, Sinapis alba* and *Raphanus sativus* var. *oleiferus* was cultivated adjacent to herbicide tolerant (HT) oilseed rape plants. Flowering period and rape pollen quantity were artificially raised by cutting the racemes

of rape plants. Seed samples were taken at distances of 2.3 m and 12.2 m from HT plants. Spontanous outcrossing was observed in *B. juncea* (frequency: 0.38 % / 0.11 %) and *B. rapa* (frequency: average 0.23 %). 36 herbicide tolerant *B. juncea* plants occured developing some siliques which were seedless or contained abnormal small seeds. Chromosomal analysis subjected to *B. rapa* confirmed the hybrid character (*B. rapa* x *B. napus*) of the herbicide tolerant F1 plants.
In some regions of Germany, *Sisymbrium loeselii*, *S. officinale*, *Descurainia sophia* and *S. altissimum*, all cruciferous weeds of the tribe *Sisymbriae*, are associated to winter oilseed rape. The weeds synchronously flower with oilseed rape indicating to act as rape pollen acceptors. However, hybridization failed using artificial crosses, isolated flowering of *B. napus* and weeds as well as testing seeds harvested from weed plants growing in HT rape field. Thus, all these weeds do not represent a risk for gene flow from oilseed rape to the environment.
Outcrossing of oilseed rape in other cultivars or strong related species happens under field conditions. Nevertheless, *B. juncea* and *B. rapa* do not contribute significantly to German rapeseed areas. On the other hand, if genetically modified oilseed rape is cultivated, gene transfer through intraspecific cross-fertilization is most probable.

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Literature

Eastham, K., J. Sweet 2002: Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer. EEA

Förster, K., W. Diepenbrock 2002: Use of genetically modified plants - consequences for crop production. In: Schwellenwerte für Produkte aus gentechnisch veränderten Pflanzen. DFG Mitt. 7, 50-58

Hühn, M., G. Rakow 1979: Einige experimentelle Ergebnisse zur Fremdbefruchtungsrate bei Winterraps (*Brassica napus oleifera*) in Abhängigkeit von Sorte und Abstand. Z. Pflanzenz. 83, 289-307

Managing spatial and temporal gene flow in Oilseed rape

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Data from a vast number of gene dispersal studies of self-fertile varieties in several countries are being used in models in the SIGMEA project to calculate probabilities of adventitious presence and their statistical distribution for seeds harvested from fields of different width, at different distance from a GM field and with different separately harvested buffer zones. The models simulate agricultural areas with several farms over several years and include the following parameters and variables:

- The biology of winter oilseed rape: germination time, flowering time, germination capacity, pollen dispersal, seed loss.
- The genotype of the oilseed rape variety (GM herbicide tolerance). Both seed production of hybrid varieties as well as oilseed rape growing for production can be studied.
- The crop rotation in the individual fields.
- Cropping techniques in the crop rotation (soil tillage, sowing date and density, use of herbicides, harvest date,).
- The regional location of the fields with natural vegetation between these (ditch edges, hedgerows).

The results of the model calculations indicate that the average GM content in the field as a result of pollen dispersal can be kept below threshold levels at manageable separation distances, depending on the relative size of the GM and reipient fields.

The models indicate that it may be technically possible but economically difficult to comply with a 0.3% threshold for production of certified seed as well as a 0.9% threshold for oil seed rape crops due to the complexity in the changes needed. A ~0.1% threshold for an organic crop may be virtually impossible to achieve.

Seed dispersal

The seed banks in many cultivated areas contain oilseed rape, which can be seen from the frequency with which oilseed rape is found in all crops. Oilseed rape seeds can survive in the seed bank for many years. Studies in Scotland and England have shown that viable oilseed rape seeds can be found in the soil for 10 to 12 years after growing both spring and winter oilseed rape. On average, the seed pool was about 100 seeds / m2.

Crop rotation and soil tillage are important aspects of land management determining the composition and size of the seed bank. In Europe winter cereals are often grown after winter oilseed rape, and are often sown without ploughing beforehand. However, if the oilseed rape stubble is ploughed just after the oilseed rape has been harvested, shed seed is incorporated into the soil and becomes dormant, thus preserving its germinating capacity for a long time.

To avoid the incorporation of oilseed rape into the soil, it is important that there is no soil inversion immediately after harvest. Seeds of oilseed rape have no or very little dormancy at harvest and will germinate on the soil surface after harvest under humid conditions. This can be encouraged by light harrowing to mix the seed into the top 1-2 cm of soil. These germinated seedlings can be controlled by a later soil tillage and/or herbicide treatment. .

Farm machinery and transport of harvested materials across fields are a very important means by which seeds can be dispersed from field to field. To avoid that dispersal, it is important to clean the machines. As oilseed rape seeds are relatively small (1 kg of oilseed rape corresponds to approx. 200,000 seeds), it may be difficult to carry out a complete cleaning of combine harvesters.

The presence of GM seeds in seed lots is a very important source of adventitious presence of GM material in crops and fields and can be assessed to help determine the GM content in the harvested crop. Where farm-saved seed is used, hybrid seeds from volunteers and weeds, e.g. between oilseed rape and wild turnip, can be an extra source of GM content in the harvested seed crop. Even without growing GM oilseed rape in the EU, GM material can be dispersed via admixtures in seed introduced from other countries, such as seen with the Hyola 401 variety.

Measures to manage and control admixture

In areas where GM oilseed rape has been previously grown, it is estimated that seed banks and volunteers are the greatest problem.

- Control measures are: adjusting the soil management and herbicide programmes to make volunteer control more effective and extending or adjusting the cropping intervals in crop rotations

Where oilseed rape fields are adjacent to GM fields, measures are needed to ensure that it is possible to maintain the crops below the GM threshold. Pollen dispersal is the most important factor – especially for non-GM seed production crops or hybrid varieties.

- Control measures are: increased separation distances (isolation distance), separately harvesting the field border in the non-GM field and increased field size of the non-GM crop.

In order to maintain non-GM fields below GM thresholds in an area with GM fields for the foreseeable future, pollen dispersal, seed dispersal and seed handling all require to be managed.

- Control measures are: those mentioned above plus: i).Removal of all oilseed rape volunteers and related weeds every year in all fields on both GM and non-GM farms including non-cultivated areas. ii). ensuring that machinery is completely cleaned and that transport takes place in seed-tight containers. iii).Using only certified seed with a low GM content.

The actual requirements for control measures depend on the threshold values to be achieved and an evaluation of the particular management issues on each farm.

Seed persistence of oilseed rape and population-dynamics of its volunteers

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Introduction

Oilseed rape is a rather problematic crop for the co-existence of gm- and non gm-crops. This is due not only to its partial allogamy, but also to weedy traits which enable this crop to spread its genes independently in the agro-ecosystem. These are: an extended flowering period and corresponding long period of ripening, seed pods that easily shatter, and small seeds with a high potential to develop light sensitivity, enabling them to persist in soil for several years.

To minimise gene escape in time it is important to know the reasons for seed persistence, to understand fully the population-dynamics of volunteers and to know how management options impact on these aspects. In this paper data of 15 years of research on the issue are summarised.

Seed losses

Seed losses vary enormously, depending on climate, date and method of harvesting. They have been found to be as small as 20 kg ha-1(equivalent to c. 500 seeds m-2), but they also can reach 600 kg ha-1 (c. 15,000 seeds m-2). It appears that, currently 3,000 – 5,000 seeds m-2 have to be accepted as an average value, althoughthere is potential for reduced losses by breeding genotypes with increased shatter resistance.

Induction of secondary dormancy

Oilseed rape seeds can develop secondary dormancy as a result of stress conditions, mainly water stress, and darkness. Under laboratory conditions, this can affect up to 80 % of the seeds. The proportion of dormant seeds is a function of genotype and the time span the seeds are exposed to darkness and stress. The longer seeds are exposed to these conditions, the larger is the proportion of seeds that becomes dormant. Dormant seeds can be triggered to germinate by light and alternating temperatures.

The implication of time and darkness for the induction of secondary dormancy suggests that the time seeds are incorporated into the soil is relevant for the development of a soil seedbank. The implication of genotype shows that choice of cultivar and in the long run breeding of low-dormancy oilseed rape are options to reduce the probability of gene escape in time.

Adjusted tillage

When cultivating the stubble immediately after oilseed rape harvest the probability of a high proportion of seeds becoming dormant increases the drier the soil is after harvest. Taking a series of field experiments in England, Austria and Germany together c. 10 % of the seeds dispersed on the ground would persist when cultivating the stubble immediately. This proportion can be reduced down to 1 % by postponing the first tillage operation for 4 weeks after harvest. No matter what the type of following tillage operation is, time to first cultivation appears to be the major factor governing the development of a soil seedbank.

Despite this, zero-tillage can also result in seed persistence, as has been established from Canadian experiences, due to induction of dormancy and/or to a lack of germination factors. As the seeds are concentrated at the soil surface it results in a high probability of emergence and consequently enhanced seed production, whichcan increase the problem,.

Long-term persistence and emergence of volunteers

In pot experiments and experiments using nylon sachets with buried seeds it has been shown that oilseed rape has the potential to persist for at least 10 years. Under the conditions of an arable field this potential will be smaller. Currently, there are few data sets on this aspect. In two field experiments at Rothamsted, UK the seedbank declined by 95 % over the first 2 - 4 years and remained fairly constant after that.

Emergence of volunteers is a function of the size of the soil seedbank and the position of the seeds in the soil. In the majority of experiments it has been found to be very small: less than 0.1 % of the total seedbank giving rise to volunteers.

Rotation and volunteer control

Not all of the emerged volunteers flower and set seed. This is partly due to heavy attack by pests and diseases And partly to competition by the sown crop and crop protection measures affecting volunteers. A rotation maximising the proportion of crops where volunteer oilseed rape does not thrive is a very efficient means to minimise volunteer populations. Additionally, the efficiency of chemical and cultural control of volunteers can have a significant impact.

When changing from gm-rape to non-gm rape, however, it has to be considered that gm-volunteers will develop very well and cause unvisible contamination in a gm-crop. This can make it difficult to stay below the threshold of 0.9 % gm-seed.

Conclusions

The data presented in this paper show that there is a whole bunch of agronomic means which can be used to minimise gene escape in time by volunteer oilseed rape. This is certain for conventionally bred oilseed rape and presumably can be transferred to herbicide tolerant gm-oilseed rape. It may be different for gm-oilseed rape with other traits, e.g. enhanced resistance against pests and diseases or with an altered oil content.

Preventing outcrossing now by cultivation of male sterile cms-maize

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Concerns exist about the potential hazards of releasing pollen from genetically modified (GM) maize. Under suitable atmospheric conditions, GM maize pollen can travel some distance in a viable state and fertilize non-GM plants away from the pollen source. Agronomic measures such as spatial isolation and border rows cannot reliably prevent the dispersal of transgenes. We, therefore, propose growing 80:20% mixtures of cytoplasmic male-sterile (cms) GM hybrids and male-fertile non-GM hybrids, whereby the latter component acts as pollen donor for the entire stand. Since the cms GM plants release no pollen or, at least, no viable pollen, the transgenes cannot escape from the GM maize field. There are at least five advantages over most other strategies for transgene containment cited in the literature. First, there is experimental evidence that cms hybrids yield better than their male-fertile counterparts. Second, pollination of the cms hybrids by genetically distinct pollen donor hybrids (= non-isogenic pollination) can bring about additional grain yield benefits through xenia. Third, blends of male-sterile Bt maize and male-fertile non-GM maize may help delay the development of Bt toxin-resistant insect populations. Fourth, it is not mandatory to genetically engineer maize for cms, because several sources of cms, which can be divided into three major groups, are available. Fifth, our method can be implemented immediately, because inexpensive seed of cms versions of current high-yielding hybrids can be produced in large quantities using existing standard methods. This system represents a simple and efficient novel solution for policy makers, who must establish the legal requirements that regulate the parallel production of GM and non-GM maize. In principle, our method is applicable to all crops which produce a sufficient surplus of pollen.

Is cytoplasmic male sterile maize really sterile?

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The growth of mixtures of transgenic CMS (cytoplasmic male sterile) and conventional maize hybrids as pollen donors was proposed as a method for transgenic pollen flow containment. This system, which is based on state-of-the-art technology, has the great potential to contribute to a sane coexistence between GM and non-GM crops, given that the cytoplasmic male sterility is a stable trait. By stability we understand the lack or low rate of a reversion to fertility of the CMS plants, triggered either by environmental factors or by the presence of restorer of fertility genes in the genetic pool.

CMS types and Rf genes in maize:

In maize, there are three major male-sterile cytoplasms, CMS-T, CMS-S, and CMS-C, which are defined according to the specific nuclear restorer of fertility genes (Rf genes) capable of countermanding the CMS trait and restoring pollen fertility.

In the **CMS-C** type, for fertility restoration at least two to three restorer genes (Rf4, Rf5, Rf6) are responsible, exhibiting duplicate and complementary gene action.

In the **CMS-T** type, two unlinked restorers are required for full restoration, Rf1 and Rf2. Rf1 can, at least partially, be substituted by two other restorer genes, Rf8 or Rf*. The CMS T system is the most stable and reliable of the maize male-sterile cytoplasms.

One dominant nuclear gene, Rf3, restores fertility to plants carrying **CMS-S** cytoplasm. Apparently, for some CMS-S sources, genes other than Rf3 are necessary for restoration.

Unlike the CMS-T type of male-sterility, spontaneous reversions to fertility occur in field-grown CMS-C plants, and, in a greater extent, in CMS-S plants.

CMS and transgenic maize hybrids:

CMS has been used in maize since about 1950 as an aid in the commercial production of hybrid seed, because cross-pollination can be achieved without the laborious task of emasculation. Because the CMS-T cytoplasm was found to be the most stable and reliable male-sterile cytoplasm under different environmental conditions, it was adopted as the CMS type for the commercial seed industry. Nevertheless, in 1969 and 1970 an epidemic of southern leaf blight broke out in the USA, and resulted in a billion-dollar crop loss due to the susceptibility of CMS-T to southern leaf blight. Thereafter, the use of CMS-T maize was abruptly discontinued. CMS-C and CMS-S have since become the cytoplasms of choice for the large-scale production of hybrid seed by breeders who continued to use sterile cytoplasms.

For the adoption of this CMS-based strategy for the transgenic hybrid seed industry, the absence of Rf genes in the genetic pool, which may lead to transgenic pollen production during the breeding process and later on in the commercial field, must be guaranteed. The standard method for checking for Rf genes in a genetic pool consists of crossing the material with tester CMS lines and to look for a restoration to fertility in the next generation. As an alternative to this time-consuming method, we aim to map the major restorer of fertility genes for CMS-S and CMS-C, Rf3 and Rf4, and to develop a PCR-based protocol for the easy identification of sources of fertility restoration in the breeding pools.

It is known that the CMS-S and CMS-C types of sterility present different degrees of fertility restoration depending on the environment. It will be a major scope of our studies to determine in which extent different environmental factors trigger a reversion to fertility.

A final aim will be to provide a cautious estimation and prediction of the risk of outcrossing of transgenic pollen when growing transgenic CMS maize hybrids.

CLEISTOGAMY IN OILSEED RAPE : A TOOL TO MINIMISE POLLEN FLOW

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Oilseed rape is a hermaphrodite plant with a highly variable autogamy rate. The autogamy rate seems to mainly depend on the genotype but also on the environmental conditions. Intraspecific and interspecific cross pollination can occur and pollen transfer is partly due to wind or pollinating insects according to the situation.

The interest in developing cleistogamy (closed flowers) in this species lies in two ways:

- to favour self pollination
- to reduce gene flow by limiting pollen dispersal.

The cleistogamous trait has been selected from induced mutagenesis in oilseed rape and patented (Renard and Tanguy, 1997). This trait is mainly controlled by one gene (Clg1) and a positional cloning project is in progress to try to isolate this gene (Génoplante Project).
Since 1998, several field experiments have been carried on cleistogamous rapeseed lines to study the impact of this trait on pollination (PhD Thesis A. Fargue, 2003). Nevertheless, some results were impaired by the lack of stability of cleistogamy in the tested lines (Fargue et al., in prep).
Presently, new lines with a good stability exist and the aim of the project is to estimate the impact of cleistogamy on autogamy and pollen dispersal limitation under several climatic and agricultural conditions or cultivation techniques. This study can be made by using phenotypic markers (eg. erucic acid seed content) that can be easily detected in the offspring.
Another goal is also to verify the stability of the trait under various environmental variations in order to establish its interest in several situations. From the integration of these results into a spatio temporal model, the benefits of cleistogamy could be simulated.
Studies have also been undertaken on the impact of the closing of the flowers on the pollinating insects behaviour. It has been shown that honeybees visit closed flowers only to probe nectar (giving honey) (Pierre & Renard, 1999) but do not open them to collect pollen. Doing so, they have no contacts with the reproductive organs of the closed flower and have not a high impact on the pollen dispersal (Pierre et al., 2002).This typical behaviour is observed when they are foraging in a homogeneously cleistogamous field. But their behaviour is still unknown when some cleistogamous plants are placed in a conventional field and reciprocally, i. e. when volunteers have a floral morphology unlike

the major plants present in the field. This has to be taken in account to reliably evaluate the efficiency of cleistogamy on plant containment and coexistence.

Another aspect of the use of cleistogamous rapeseed concerns the production of honey which is of economical interest. The amounts of nectar produced are known to be very variable between rape varieties (Pierre et al., 1999). The amounts of nectar actually collected by honeybees (available nectar) on cleistogamous flowers need to be estimated, because the nectar probing behaviour is different on closed flowers. A reduced nectar availability could impair the quantities of nectar collected by honeybees and consequently the honey production.

References :

Fargue A., 2003. Maîtrise des flux de gènes chez le colza : Etude ex ante de l'impact de différentes innovations variétales. PhD INRA Paris Grignon, 168pp.

Fargue A, Colbach N., Pierre J., Picault H., Renard M., Meynard J.M. Predictive study of the advantages of cleistogamy in rapeseed. (in prep).

Pierre J., Mesquida J., Marilleau R., Pham-Delègue M.H., Renard M., 1999. Nectar secretion in winter oilseed rape, Brassica napus – quantitative and qualitative variability among 71 genotypes. Plant Breeding 118, 471-476.

Pierre J., Renard M., 1999. Plant development mutants : incidence on honey bees behaviour and pollination. Proc. Xth International Rapeseed congress , 26-29 September 199, Canberra, Australia (CD Rom, 5p).

Pierre J., Picault H., Tanguy X., Renard M., 2002. La cléistogamie chez la fleur de colza empêche-t-elle la dispersion du pollen par les insectes pollinisateurs. C.R. AIP OGM et environnement, INRA ed., 104-107

Renard, M.; Tanguy, X. (1997). Obtention de mutants cléistogames de crucifères. Brevet FR 97 15768.

A Gametohpytic cross-sterility Gene in maize and its use as pollen barrier against GMO contamination

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Summary:
The use of genetically modified maize hybrids increases from year to year. At the same time there are corn growers requiring GMO free production possibilities.
This coexistence poses a problem when fields of GMO and GMO free hybrids are in vicinity (~less than 300m), as maize pollen can travel a long distance, and as maize is primarily and out-crossing species.
Several gametophytic incompatibility genes have been described. They cause the silks to be receptive only for pollen of matching types.
The objective of our project is to introgress the GA1-s Gene (Schwarz D. 1950. Proc. Natl. Acad. Sci. USA 36:719-724) into parental lines, to make hybrids with incompatibility against foreign pollen. Such hybrids will be save from GMO cross contamination.
The GA System: almost all temperate corns contain the inactive ga-allele at the gametophyte factor locus GA. (Nelson EO. 1960. MNL 34:114-116)
Some exotic strains like popcorns or Central American flints have the Ga-allele at the GA locus, and they show cross incompatibility.
Ga silks inhibit growth of ga pollen, and GA plants are more or less sterile when pollinated with ga pollen. (House, L.R. and O.E. Nelson, Jr. 1958 J.Hered. 49:18-21)
The Ga1-s gene is one of the gametophytic factors. It is located on chromosome 4S-32. It shows stronger expression, than other GA genes.
Converting parental inbred lines to GA1-s: Introgressing the GA1-s allele into inbreds follows the normal backcross procedure, except that the male has to be the GA1-s strain. (The reciprocal cross will not set seed).
The conversion has to be done on both parental lines of a hybrid, in order to achieve near complete cross incompatibility.
After 2 backcross generations a Selfing generation will be necessary to obtain seeds homozygous for Ga1-s.
A second self-pollination follows, this time with red kernel marker pollen, blended in the Selfing pollen. All ears resulting from these selves that show traces of red kernels don't have the GA1-allele homozygous, and are discarded. Only ears with 100% non pigmented kernels are saved.
The homozygous ears go to the next backcross generation. After 2 more backcrosses and 2 subsequent selfings, the second one again with added red-marker pollen, the line should be isogenic to the original inbred and ready for hybrid seed production.

Remarks of caution:

- GA conversions are labour intensive because the trait is not visible on the phenotype; a progeny test with red-marker pollen is necessary.
- For the pollen shield to be reliable it has to be assumed that GMO-corn breeders will not use GA1 in their breeding sources. This risk is low, as GA-stocks have little breeding value.
- It will be the responsibility of the breeder of cross incompatible hybrids to monitor the shielding ability of his hybrids. This is an easy test: All it needs is planting a row of such a hybrid, detassel it before flowering, and observe if there is seed set.
- In the conversion process quantitative selection for shielding ability is necessary, as trait expression is background dependant, and there could be modifier genes influencing the trait.

Potential Applications of Apomixis in Plant Breeding and Transgene Containment

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Apomixis refers to the asexual reproduction through seeds and bears great potential for agricultural applications, due to the clonal nature of the offspring produced. We will review the genetic basis of apomixis and highlight potential applications for plant breeding, seed production, and transgene containment (Spillane et al. 2004). For many years, apomixis and sexuality have been viewed as two distinct reproductive pathways. Based on a developmental point-of-view, however, apomixis and sexual reproduction seem closely interrelated (Grossniklaus, 2001; Koltunow and Grossniklaus, 2003). Thus, a better understanding of the genetic and molecular basis of sexual reproduction will also provide new tools towards the engineering of apomixis. We use Arabidopsis thaliana and Zea mays as model systems to identify genes that play a role in key steps of sexual reproduction that may also function during apomixis. We concentrate on three developmental processes that are highly relevant to apomixis: (1) megasporogenesis, (2) double fertilization, and (3) functional endosperm formation. Making use of the enhancer detection system developed by Sundaresan et al. (1995) we identified promoters that are useful to mis-regulate gene expression during plant reproduction. Enhancer detection allows the identification of genes based on their patterns of expression as well as the isolation of insertional mutants disrupting specific developmental events. Enhancer detection is a powerful tool to identify genes expressed in single, poorly accessible cells such as the cells of the embryo sac (Vielle-Calzada et al. 1998; Grossniklaus et al., 2003). By screening through a few thousand enhancer detector lines we identified several genes expressed in specific cell types. These include the nucellar region where aposporic initials form in apomictic species, the megaspore mother cell, as well as the egg and central cell where parthenogenetic or autonomous development of embryo and endosperm are initiated, respectively. We are isolating cell type-specific promoters for use in targeted mis-expression experiments that may lead to the engineering of some components of apomixis in a sexual model species.

In both sexually and apomicitically reproducing plants successful seed development depends on the formation of functional endosperm, an issue that has been largely ignored in the past. Apomicitc plants evolved specific adaptations to ensure functional endosperm formation that often rely on developmental changes in the female gametophyte. One aspect of endosperm formation in apomicts is mirrored by the phenotype of medea (Grossniklaus et al. 1998) and other fis mutants: autonomous endosperm development in the absence of fertilization. However, autonomous endosperm formation is rare among apomicts, especially the grasses, such that alternative reproductive modes have to be considered. The importance of functional endosperm formation will be highlighted and a mutant screen in maize, aimed at the isolation of mutants displaying certain elements of apomixis, will be presented.

References:

Grossniklaus U. (2001) From sexuality to apomixis: molecular and genetic approaches. In Advances in Apomixis Research (Savidan, Y., Carman, J., and Dresselhaus, T. eds). CYMMIT Press, Mexico. pp. 168-211.

Grossniklaus U, Moore JM, Brukhin V, Gheyselinck J, Baskar R, Vielle-Calzada J-P, Baroux C, Page DR, Spillane C. (2003) Engineering of apomixis in crop plants: what can we learn from sexual model systems. In: Plant Biotechnology 2002 and Beyond (Vasil, I. ed), Kluver Academic Pulishers, Dordrecht, pp. 309-314.

Grossniklaus U, Vielle-Calzada, J-P, Hoeppner MA, Gagliano WB. (1998) Maternal control of embryogenesis by MEDEA, a Polycomb-group gene in Arabidopsis. Science, 280: 446-450.

Spillane C, Curtis MD, Grossniklaus U. (2004) Apomixis technology development – virgin births in farmer's fields? Nature Biotechnology, 22:687-91.

Sundaresan V, Springer P, Volpe T, Haward S, Jones JD, Dean C, Ma H, Martienssen R. (1995) Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. Genes Dev. 9:1797-810.

Vielle-Calzada J-P, Moore JM, Gagliano WB, Grossniklaus U. (1998) Altering sexual development in Arabidopsis. J. Plant Biol., 41: 71-83.

The concept of the Danish working group on coexistence

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The task of the working group

In the spring of 2002, the Danish Minister for Food, Agriculture and Fisheries initiated strategy work on the cultivation of authorised GM crops in Danish agriculture. The aim was to produce a Danish model for the co-existence of GM, conventional and organic crops in support of the free choice of consumers and to ensure development possibilities for new and existing agricultural production forms. Three groups were set up, that were assigned the task of presenting the scientific and legal problems as well as drawing up a proposal for a Danish co-existence model. These groups were the analysis group (working group), the legal group and the contact group.

The analysis group was assigned the task of

- Conducting a scientific analysis of the sources of dispersal from GM to conventional and organic production forms
- Assessing the scope of dispersal as well as the need for control measures to be taken
- Identifying as well as assessing potential measures to secure the co-existence of GM, conventional and organic production forms.

After several meetings a draft report was presented to the contact group, where a broad range of stakeholders were represented. In January 2003 the report was presented in the Danish parliament, and in August 2003 the final report was published. The conclusion is, that for the majority of crops grown in Danish agriculture co-existence is possible, at the stipulated or presupposed threshold values, when the recommended control measures are applied, however, a few out-crossing crops and/or crops with long seed persistence in soil, the analysis group were not able to recommend control measures to ensure co-existence.

Identified control measures

Crop by crop control measures have been identified, measures that need to be adopted in crop production and management practice to ensure co-existence.

Pollen disperses in space – between fields, farms and regions. The identified control measures are

- Isolation distance, border row management

- Control of volunteers, hybrids
- Modeling and monitoring

Seed disperse over time – between fields, but also between farms and regions (propagated seed). The identified control measures are

- GM-free propagated seed
- Cropping intervals – control of volunteers, hybrids
- Cleaning of farm equipment, storage facilities and during handling.

The effect of the specific control measure will depend on the crop (breeding system), field size, farming structure, landscape, production area of the specific crop in the region etc. It is concluded that the control measures should be crop, site and farm specific.

The Danish regulation

The Danish Parliament has adopted "Act on the Growing etc. of Genetically Modified Crops" Act No. 436 of 9 June 2004 (http://www.fvm.dk/fvm_uk). The Act applies to commercial growing, handling, sale and transport of genetically modified crops as far as the first buyer with a view to limiting the possibility of dispersal of pollen, seeds and vegetative propagation material to other fields and crops there from.

On 31 March 2005 the ministerial order was published. It regulates the growing of authorised varieties of GM beet, maize and potatoes by defining isolation distances, cropping intervals etc. Persons holding a license to prove that they fulfil certain education requirements within the field of coexistence may only perform the growing, handling and transport of genetically modified crops. In addition the ministerial order describes the reporting of fields with genetically modified crops, information to neighbours etc.

The regulation is based on the co-existence report from the Danish working group on co-existence (Tolstrup et al., 2003), discussions with stakeholders in the contact group, presentations by international experts at hearings in the Danish Parliament and conclusions from the co-existence conference, GMCC-03 (Boelt, 2003).

Conclusion

Regulation for the growing of GM-crops of beet, maize and potatoes in Denmark is in place. However, there is a need to evaluate the regulation when 'real life' data and large-scale studies, which include the effect of field size, farming structure, landscape etc. to ensure coexistence between organic, conventional and GM-crops are obtained.

References

Boelt, B. (2003). Co-existence of Genetically Modified Crops with Conventional and Organic Crops. Proceedings from the 1st European conference on the Co-existence of Genetically

Modified Crops with Conventional and Organic Crops. GMCC-03. Ed.: Boelt, B. Snekkersten, Denmark. 228 pp.

Tolstrup, K., Andersen, S.B., Boelt, B., Buus, M., Gylling, M., Holm, P.B., Kjellson, G., Pedersen, S., Østergaard, H., Mikkelsen, S. (2003). Report from the Danish Working Group on the co-existence of genetically modified crops with conventional and organic crops. DIAS-report 94, 275 pp.

Dutch Farm Coexistence Measures

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Beginning November 2004 dr.ir. C. Veerman, minister of Agriculture, Nature and Food Quality and mr. P. van Geel, deputy minister for Housing, Spatial Planning and the Environment received a report containing an agreement on measures for coexistence of genetically modified, organic and conventional crops in the Netherlands. The report - produced by a committee of farmer and chain organizations involved in Dutch agriculture- stipulates measures, which minimize mixing products from the different production types and possible economic damage at the farm level and includes recommendations for government action. The committee was instituted at the invitation of the minister, who stated he intends to follow the recommendations.

Introduction

Starting point for the committee has been the recommendation by the European Commission (2003/556/EC) that member governments should develop national strategies and best practices for coexistence. The government of the Netherlands has been reluctant to design new laws in a time when it tries to curtail the administrative burdens for producers.

Instead farmer and relevant chain organizations were invited to come to an agreement amongst themselves. The organizations involved were LTO Nederland (Netherlands Union of Farmers and Horticulturists), Biologica (representing the chain of organic producers), Plantum NL (representing producers of plant propagation material) and Platform ABC (an alliance of agricultural producers and special interest groups). The minister of Agriculture, Nature and Food Quality appointed an independent chairman and secretariat after consulting the organizations involved.

The work of the committee was focused on coexistence at the farm level. The committee considered crops that have been found safe for humans, animals and the environment following EU regulation. For gm-crops this is Directive 2001/18/EU. Therefore only the potential economic consequences of the mixing of production systems and issues of liability hereof were considered.

Liability and fund

Liability in the case of hindrance between farmers is covered in the Dutch ‘’Burgerlijk Wetboek’’ (Civil Code). It is foreseen that the agreement by the committee, which is based on the most up to date relevant scientific information, will function as principal reference in future liability cases.

The committee agreed that freedom of choice for consumers is largest if the mixing is as low as possible. After taking the proposed coexistence measures the chance of mixing and consequent economic damage has been reduced to an utmost minimum. The agreement stipulates that if a grower does not adhere to the coexistence measures he can be held liable for crop damage that springs from the mixing between gm and non-gm crops. If he does adhere to the measures he will be freed from claims for economic damage that is caused because of mixing. In that case a fund will restitute the economic damage suffered. This fund will be drawing on all relevant parties: seed firms, growers and processing companies.

Coexistence measures

The agreement stipulates measures for potatoes, sugar beets and maize because these crops are most relevant considering the availability of gm-varieties. Within the timeframe of 7 months of the committee it was not possible to prepare measures for rapeseed.

A farmer intending to grow gm-crops must communicate his intentions to his neighbours at an early date. He should also register his intent in the national register before the first of February.

In all stages of cultivation, planting, growing, harvesting, on farm transport and storage, measures must be taken to prevent the mixing of gm-cops and non-gm crops. This means thorough cleaning of machinery, separation distances, prevention of volunteer crops and spilling, and separate storage.

Separation distances during cultivation are most effective to prevent mixing.

For potatoes a distance to non-gm potato of 10 meters for production chains which have been defined as ‘GM-free’ and 3 meters to other non-gm potato chains. Furthermore volunteers must be eradicated. For sugar beets the distance is 3 meter to sugar beets in ‘GM-free’ chains and 1.5 meter to sugar beets of other non-gm chains. There will be strict controls on the prevention of flowering and the eradication of volunteers. For maize the respective distances are 250 meter to ‘’GM-free’’ production chains and 25 meter to other non-gm production chains.

Compliance

The measures will be included in certified Good Agricultural Practice (GAP) schemes. Any calamity (such as spilling during transportation from the field to the farm) must be reported to the certifying agency. Furthermore the measures will be included in a Coexistence Regulation from the Main Board for Arable Products.

Compliance is guaranteed through certification and control of the Regulation. Failure to comply will make a producer loose his GAP certificate (and consequently his license to deliver), liable to damage claims and to a fine by the Main Board.

Monitoring, research and evaluation

The effectiveness of the coexistence measures will be monitored intensively. If results of monitoring show that the measures are too strict or need tightening the coexistence measures can be adjusted.

Recommendations to the government

The committee has recommended the government to set up a research program to evaluate mixing as caused by cross-pollination, especially for maize with destination animal feed (whole plant silage). Besides research the committee has recommended the government to contribute to the fund, the monitoring program and to the dissemination of information on the agreement as well as to extension on gm-crops to the wider public. The committee advises the government to urge for harmonization of strategies in Europe and to follow the development of strategies in the neighbouring countries so that these do not cause problems in border areas.

Evaluation and time span agreement

The current agreement is for a three-year period. Before the ending of this period the functioning of the agreement will be evaluated and, if need be, adjustments to the agreement will be made. Adjustments may also be made during the three-year period on the basis of new information (for example from research or monitoring).

A concept for coexistence of GM and non-GM crops in Switzerland

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Today, the coexistence of agricultural systems with genetically modified (GM) and non-GM crops is highly debated, both in politics and in public. Similar to the European Union (EU), Swiss legislation stipulates that protection of GM-free production and consumers' freedom of choice must be guaranteed if GM crops are to be commercially cultivated. There is an urgent need to discuss and define the conditions and measures required to ensure coexistence on a scientific and legal basis. The aim of the present study (Sanvido et al., 2005) was to analyze if coexistence of both GM and non-GM crops was technically feasible in Swiss agricultural production based on current Swiss legislation. Using maize, oilseed rape and wheat as examples, technical and organisational measures have been listed that ensure that adventitious GM-levels in food and feed do not exceed the thresh-old of 0.9% specified by EU and Swiss legislation. While the study was confined to agri-cultural production, from the crop-planning phase to delivery of the harvest by the farmer, costs of co-existence and the potential for separating the flow of goods during processing and marketing were not covered. The evaluation was based on the principles and meth-ods of existing systems for identity preservation (Sundstrom et al., 2002), and on a co-existence study conducted by the European Commission (Bock et al., 2002), as well as on a recent Danish study (Tolstrup et al., 2003).

Six important mechanisms were identified in the agricultural production chain and during on-farm handling that can potentially result in mixing of non-GM with GM products. Sev-eral technical and organisational measures can help to minimize mixing during each of these six steps. Dispersal from seed impurities can be minimised by using certified seeds. Volunteers can be controlled by ensuring optimal soil preparation techniques after harvest and before sowing, as well as by using cropping intervals. The extent of out-crossing be-tween fields of GM and non-GM crops can effectively be reduced by respecting isolation distances. The risk of mixing in machinery can be reduced by a thorough cleaning practice of machines after use on GM crop fields. A clear segregation of the harvested material and the documentation of procedures during storage, processing and transport from field to collection point can also minimize the risk of mixing.

In order to determine the required isolation distances between fields of GM and non-GM crops, an analysis of available gene flow data was performed using twelve recent studies in maize and eleven studies in oilseed rape. This analysis indicated that 25 metres for silage maize and of 50 metres for grain maize is sufficient to keep adventitious GM-levels below 0.5% at the border of non-GM crop fields (without taking into account the additional dilution that takes place during harvest of the whole field). For fertile oilseed rape varieties (conventional varieties and hybrids with restored fertility) the respective isolation distance was 50 metres. For oilseed rape with male sterile components (varietal associations), an isolation distance of 400 metres was recommended, as in the case of basic seed produc-tion with comparable proportions of male sterile components.

Two different approaches were used to assess the feasibility of spatial co-existence of GM and non-GM based farming in Switzerland. The first approach was based on an agricul-tural farming data survey from 2003, yielding data on the acreage of maize and oilseed rape cultivation in Switzerland. Taking into account the proposed isolation distances, the area required to allow for spatial isolation of 10% GM crop cultivation was calculated for every Swiss commune. These calculations showed that the available arable-land areas are sufficient for an isolation of 10 % GM-maize and 10% GM-oilseed rape in the majority of Swiss communes. The second approach was based on an assessment of aerial pic-tures covering a 164-square-kilometre area in eastern canton Zurich. Geographic informa-tion systems (GIS) were used to calculate the shortest distance between two maize fields at a resolution of 50 metres. The results of the GIS analysis showed that the density of maize cultivation and the distances between the maize fields varied considerably within a very small area in relation to the landscape structure. In the area investigated, half of the fields were more than 90 metres apart. The analysis suggested that establishment of iso-lated GM crop fields with the proposed distance of 50 metres should be possible for the majority of maize fields in this area.

The here presented evaluations of scientific information and legal frameworks indicate that the coexistence of GM and non-GM based agriculture is possible in Switzerland within the current legal threshold of 0.9%. However, technical and organizational meas-ures as well as the exchange of information and agreements among farmers are neces-sary. The approach developed in this study may be of assistance for evaluations in other countries or for evaluations based on lower threshold definitions.

Literature:

Bock AK, Lheureux K, Libeau-Dulos M, Nilsagård H and Rodriguez-Cerezo E (2002) Scenar-ios for co-existence of genetically modified, conventional and organic crops in European agriculture. Institute for prospective technological studies, Sevilla

Sanvido O, Widmer F, Winzeler M, Streit B, Szerencsits E and 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

Sundstrom FJ, Williams J, van Deynze A and Bradford K (2002) Identity preservation of agricul-tural commodities. University of California - Division of Agriculture and Natural Resources, Davis, published at: http://anrcatalog.ucdavis.edu/pdf/8077.pdf

Tolstrup K, Andersen S, Boelt B, Buus M, Gylling M, Bach Hom P, Kjellson G, Pedersen S, Østergård H and Mikkelsen S (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, Kopenhagen

Poster session

Conventional and genetically modified corn co-existence management from field to silo - The French initiative

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A 3-year experiment on co-existence of transgenic Bt corn and non Bt corn has been conducted in France under controlled field conditions from seed to storage. It studied the traceability of crops, and leads to the analysis of the different conditions governing co-existence. At each stage of corn chain, procedures were implemented to insure traceability of corn grain production, with all operations and key points listed, and by using an adapted documentary system, as well as suitable controls. Through this program we managed to identify all parameters to be considered to insure crops segregation, to control key points to minimize adventitious mixture, and to build specific procedures for harvest, transport, drying and storage. We now have reliable data which confirm that co-existence is indeed practicable. The implementation of good farming practice and normal harvesting practices is usually sufficient to ensure that GM adventitious presence levels in non GM corn are below the 0.9% EU labelling threshold. The program was conducted by a scientific committee made of experts from research and industrial organizations.

Conventional waxy production, an experience to evaluate co-existence of GM and conventional maize

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Waxy maize has been produced for a long time in the South of France (40 000 ha). Purity results in waxy maize can give good information in the context of coexistence between genetically modified and conventional maize.

Cross pollination study between different consumption maize fields is based upon differentiation of waxy from other type of maize. Waxy character is genetically recessive. Cross pollinated grains by conventional pollen are easily recognizable by a simple coloured test practised directly on the ear.

Field situations have been selected to be the most contaminated in order to study the worse real conditions. Main selection criteria were the following: flowering concordance, minimal isolation distance, single contamination source (conventional maize), a long border between the two fields. To take into account a maximum of parameters influencing maize pollen dispersal, real cultivated situations have been chosen (2001, 15 locations and 2002, 12 locations).

The first exposed rows (border) were the most contaminated. Therefore, the removal of these rows would bring a purity increase of only 0.07%.

On 2002 situations, cross pollination between conventional and waxy maize fluctuated from 0.05% to 0.72%. The 2001 results were similar with cross pollination rates from 0.24% to 1.17% (waxy seed purity not included).

The waxy maize chain is a good example of coexistence. Cross pollination analysis results can be transposed to evaluate cross pollination level between GM and conventional maize.

Cytoplasmic male sterility (cms) in Maize (*Zea mays* L.)

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Cytoplasmic male sterility (cms) has been identified in maize at the beginning of the 20th century. Cms plants are characterized by their inability to produce viable pollen, while the female fertility is unaffected. Cms is a maternally inherited trait.

Today, the interest in maize cms relived with two new potential applications:
1) The Plus-Hybrid system, which represents a promising approach for increasing maize grain yield by up to 20% by the combination of the cms- and the Xenia- effects.
2) The Plus-Hybrid-System as a method of transgenic pollen flow containment.
The cultivation of GM crops is continuously growing (10% every year since 1998), even though many countries still maintain their reserve about this biotechnological application. Actually, one of the main arguments brought by the non-GM crops partisans is the release of transgenic pollen in the environment. A way to prevent this type of contamination would be through the integration of the transgene in cytoplasmic male sterile hybrids which produce no pollen. As pollen donors, conventional male-fertile plants in a ratio of 80:20 would be used.
This strategy has the potential to enable coexistence between GM and non-GM crops.

Transgenic pollen flow in maize – a Swiss study

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The advent of the cultivation of GM-crops in the EU and also in Switzerland is, at least in a long-term, foreseeable. Intense discussions are taking place in Switzerland regarding the risk of cultivating GM-crops under its specific topographic and meteorological characteristics. A parameter with direct consequences on the feasibility of coexistence between GM and non-GM crops is the pollen flow distance and the rate of outcrossing.

In previous years, we focused our research on long distance pollen dispersal in maize (*Zea mays* L.). We performed vast field trials in typical Swiss landscapes where maize cultivation is widespread. The results of our studies were in accordance with the results presented by other researchers in the EU, indicating that the required isolation distance between GM and non-GM maize should be of 10 to 28 m in order to keep the contaminations below the 0.9 % threshold.

In future field trials, we want to perform studies focusing on the less studied short distance pollen dispersal. In these studies, we want to pay special attention to factors affecting the rate of outcrossing, such as the influence of meteorology (thermal lift, wind speeds and directions, etc.), competition effects, flowering synchronization and topographical factors on pollen flow. With these data, we aim at predicting the risk of outcrossing and at defining guidelines for the coexistence between GM and non-GM maize fields in Swiss agriculture.

For the simulation of the transgenic pollen dispersal, we will continue applying the well proven method of using a white (recessive) maize hybrid (DSP 17007) and a yellow (dominant) maize hybrid simulating the transgenic hybrid. The advantages of this method are no need of transgenic maize, the simple, cheap and quick realization, and the ease of handling a large number of samples.

Apomixis technology development: a tool for transgene containment

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Pollen is a natural vehicle by which genes flow between individuals. This flow of genes is limited by interspecific incompatibility between species, reducing the possibility of hybridization. These strategies have not evolved to prevent the flow of genes between related plants and so, to benefit from the great potential offered by plant biotechnology, containment technologies that prevent gene flow between crop plants, or their wild relatives, must be developed. These technologies must safeguard food purity and prevent any negative influence GM technology may have on the environment. A possible solution to this problem occurs naturally through the clonal production of seed in apomictic plants, providing a possible mechanism by which to maintain seed production in the absence of pollen. However, while over 400 apomictic plant species are known, apomixis is very rare among crop plants, and the transfer of functional apomixis to crop varieties by conventional breeding has been largely unsuccessful. An alternative approach is to *de novo* engineer apomixis in sexually reproducing crops, taking advantage of the close developmental relationship between apomixis and sexuality. Key elements of apomixis are apomeiosis (the avoidance of meiosis to produce an unreduced egg), parthenogenesis (the autonomous development of an embryo) and the production of viable endosperm. These elements are thought to have evolved from the deregulation of sexual developmental processes. Here we concentrate our efforts on the first two elements, identifying genes that may allow apomeiosis or parthenogenesis. In sexual plants, within the nucellus tissue, a cell is identified that undergoes meiosis, resulting in the production of (among others) a reduced egg cell. The nucellus tissue, therefore, is an obvious candidate tissue-type in which to mis-express genes that may result the avoidance of meiosis. In sexual reproduction, the egg cell initiates embryogenesis upon its fusion with a sperm cell. The egg is, therefore, an obvious candidate for the targeted mis-expression of genes, which may allow parthenogenesis. Our strategy is to identify candidate genes in the model sexual plant Arabidopsis, as proof of concept, and transfer this technology to crop plants. Candidates will be identified in screens that conditionally express activation tagged genes in the nucellus and the egg cell. We use an activator construct that provides cell-specific expression of the chimeric transcription factor XVE and a tagging construct, which is able to trans-activate genes adjacent to a lexA-binding site.

Tagged genes are conditionally mis-expressed in these cell-types and screened for elements of apomeiosis or parthenogenesis. In apomeiotic screens, tagged genes can be rescued in plants showing sterility or lethality, by collecting viable seed from non-induced sectors of a plant. In parthenogenetic screens a conditional male sterile mutant is used to identify mutants that show elements of seed development in the absence of fertilization.

Attempts to minimize the outcrossing of transgenic oilseed rape

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Due to changes in legislation the disposal of conventionally bred crops with a minimal content of transgenes that emerged by pollen transfer from transgenic Part B plants (emitted for field experiments but not for the market), is prohibited. Since scientists are not in a position to finance the destruction of the harvest and to substitute the loss of income of neighbors, the deliberate release of transgenic plants becomes nearly impracticable in Europe. This is especially true for field trials with canola or oil seed rape. Therefore we aim to develop, combine and improve planting strategies that should minimize the pollen transfer from these crops in order to reduce out crossing below the detection level. This might help to carry out essential part B field trials even with Brassicaceae and under the current conditions.

Ten years of coexistence across the globe

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This year, farmers will plant and harvest their 10th crop enhanced with biotechnology traits. Since the first biotech seeds went into the ground in 1996, biotech crops will have been grown by more than 8 million farmers in 20 countries on over 1 billion acres, accounting for a 47 fold increase in the planted acreage over the past 10 years.

As biotech crops are not approved simultaneously in all world areas but international grain trade continues regardless, coexistence between biotech and conventional or organic crops is being successfully addressed in many countries.
On-farm experience in North America but also other world regions including Europe has demonstrated that practical solutions can be put in place to allow farmers the option of using different production systems, often without government involvement. Coexistence can be obtained with the help of good communication with neighbors, the separation of crops by space and time and the use of good husbandry practices for example. Working hand in hand with local grain handling facilities willing to accept biotech products has also proved to be a successful model.

This poster will present some of the concrete solutions that have ensured a well-functioning coexistence in various world areas throughout the 10 years of commercialization and trade of biotech crops.

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